Ecotypic variation in *Lotus corniculatus* L. and implications for grassland restoration: Interaction of ecotypes with soil type and management, in relation to herbivory

Erica Jane Mortimer

A thesis submitted in partial fulfilment of the requirements of

Bath Spa University for the degree of Doctor of Philosophy

School of Society, Enterprise & Environment, Bath Spa University

2016

Word count: 37515

Ancillary Data: 23116 (Contents/glossary/acknowledgements: 3311; tables/figures/captions: 10438; references: 9367)
Appendices: 16417

ABSTRACT

This research assesses the importance of using ecologically-similar rather than geographically-local seed in grassland restoration projects, with particular reference to herbivorous invertebrates, including pollinators.

Seed from *Lotus corniculatus* L. (bird's-foot trefoil) populations at nine sites across south-west England were collected to represent ecotypes potentially adapted to a range of soil types (calcareous loam, neutral loam and calcareous sand (referred to as 'sand')) and management regimes (grazed, cut with aftermath grazing (referred to as 'cut') and unmanaged).

From October 2011 the ecotypes were planted within three different treatment soils (calcareous loam, neutral loam and calcareous sand ("sand"), and two management treatments (grazed [simulated] or unmanaged [neither cut or grazed]). Differences in plant morphology and phenology under these treatments were recorded at four-weekly intervals and immediately prior to harvest on 16th July 2012. Fresh and dry biomass were recorded and leaf-nitrogen and leaf-hydrogen cyanide (HCN) levels determined. Treatments were maintained and plants grown for a further 15 months [after harvest]. Bee preference for ecotypes grown under treatment combinations was also recorded during peak flowering periods of 2012 and 2013. Data were tested using Kruskall-wallis and ANOVA. A Generalized Linear Mixed Model (GLMM) was built to test all ecotype and treatment differences including interactions. A separate Non-linear Mixed Effects model (NLME) was created to investigate spatial autocorrelation between ecotype sites. The standard chosen in the models was Cockey Down (a calcareous loam, grazed ecotype) grown in matching treatments.

The phenotypic traits retained were most pronounced in ecotypes from home-sites of sand soil type and cut management, which were considered to be the more stressed environments of the study, requiring rapid adaptation. By harvest, ecotypes from sand home-sites produced significantly greater number of stems per plant, greater leaflet number per main stem and lower

HCN. Model results for sand ecotypes additionally identified delayed seed pod formation, increased hirsuteness and higher leaf-HCN compared to the standard. Significant differences found within ecotypes from cut home-sites included fewer stems per plant, fewer leaflets per main stem, more seeds per pod, greater leaf-HCN and shorter time to first flower. The model also found this ecotype to be less hirsute with fewer seed pods (in unmanaged treatment) than the standard. Ecotypic traits shown in plants from the less stressed home-sites included calcareous loam ecotypes having two clear flowering peaks in both years and highest leaf-HCN, and unmanaged ecotypes having lower leaf-HCN.

Three significant interactions indicated additive character factor effects: neutral loam ecotypes grown in neutral loam treatment soil had earlier pod formation than the standard; and, sand ecotypes grown in sand treatment soil and unmanaged ecotypes receiving unmanaged treatment had greater flower number (over both years) than all other ecotypes, treatments and combinations.

Results from the bee ecotype preference study showed no preference for ecotypes geographically close to the test foraging area. However significant differences were shown by bees in terms of ecotype preference, with avoidance of plants containing highest leaf-HCN. Plants grown in calcareous soil treatment were preferred which suggests nectar of plants are of most value to bees when grown in optimum [for *L. corniculatus* growth] soil.

Eotypic differences in herbivory defence [leaf-HCN and hirsuteness induced by home-site soil and management], would be of importance to receptor site invertebrate herbivores/pollinators. Pollinators could also find difficulties with the ecotypic differences in flowering asynchronicity.

Both home-site soil type and management could also influence the viability of the plant population through reducing fecundity. Delayed seed pod formation in sand ecotypes (compared to the standard) indicates an adaptation to summer temperatures or low-nutrient availability. Calcareous loam ecotypes lack such adaptation and if introduced to a sand sites and could fail due to poorly timed germination, deep seed burial [from shifting sand] or poorly allocated energy. Cut ecotypes also produced significantly fewer seed pods (than the standard) with significantly greater seed numbers per pod suggesting an energy allocation adaptation to produce fewer, larger pods before defoliation rather than continuous pod formation throughout the season, a potentially critical adaptation for seed return.

Findings from this study are of national relevance, and Natural England should adopt new recommendations on seed provenance in agri-environment schemes. Instead of recommending strictly geographically local seed, the management regime (particularly details of intensity and timing of management operations) should ideally be similar between the donor and receptor sites. Soil types, especially pH and clay/organic matter content should also be matched as far as possible as these were the greatest limiting factors within this study. Suitable donor sites may be local sites of similar habitat. If no such sites are available then recommendations from this study should be followed in seeking suitable sowing material. If seed suppliers are used, then they should provide greater detail on donor site conditions to aid land managers.

CONTENTS

Table of Figures

Table of Tables

GLOSSARY

ECOTYPE SITES		
Used sites:		
bd	-	Berrow Dunes
cd	-	Cockey Down
dw	-	Dawlish Warren
ff	-	Folly Farm
hh	-	Hellenge Hill
sp	-	Salisbury Plain
ss	-	Southstoke (cut)
wb	-	Woodborough Hill
ww	-	Woolacombe Warren
Additional sites where collections were made:		
bh	-	Burledge Hill
fm	-	Fontmell Down
gc	-	Goblin Combe
ssg	-	Southstoke (grazed)
stc	-	St. Catherine's
vs	-	Valley of Stones
Sites visited but not collected from or used:		
am	-	Avis Meadow
bb	-	Braunton Burrows
bg	-	Bigbury
ch	-	Chittoe Heath
cp	-	Crook Peak
dm	-	Distillery Meadow
dv	-	Devenish
ed	-	Everleigh Down
hhf	-	Heath hill Farm
mb	-	Melbury
TREATMENT SITES		
Used treatment soil sites		
C	-	Win Green (Calcareous loam treatment)
N	-	Avis Meadow (Neutral loam treatment)
S	-	Woolacombe Warren (Calcareous sand treatment)
Rejected treatment soil site		
CB	-	Silk Hill (Calcareous loam)

Abbreviations and acronyms used in text:

AES	Agri-environment Schemes
AIC	Akaike Information Criterion
ANOVA	Analysis of Variance
AONB	Area of Outstanding National Beauty
BAP	Biodiversity Action Plan
CAP	Common Agricultural Policy
CBD	Convention on Biological Diversity
CCA	Canonical Correspondence Analysis
CEC	Cation Exchange Capacity
CSS	Countryside Stewardship Scheme
DEFRA	Department of Food and Rural Affairs
ELS	Entry level stewardship
ESA	Environmentally Sensitive Areas
GLMM	Generalized Linear Mixed Model
HCN	Hydrogen Cyanide
HLS	Higher Level Stewardship
HRZ	High Rainfall Zone
JNCC	Joint Nature Conservation Committee
LME	Linear Mixed Effects Model
MVSP	Multivariate Statistical Package
NLME	Non-linear Mixed Effects model
PCA	Principle Components Analysis
ppm	Parts Per Million
RDS	Rural Development Service
ROW	Right of Way
SAC	Special Area of Conservation
SPA	Special Protection Area
SSSI	Site of Special Scientific Interest

ACKNOWLEDGEMENTS

I have a lot of people to acknowledge in completing this thesis, there are as follows:

My family, particularly my parents Audrey and Bryan Mortimer and gran Mary Freeman for putting up with me throughout this research. My parents were also a great help on soil/seed collections, treatment soil collections (very heavy work!) and bee preference surveys. Also thanks to my boyfriend, Matthew Skewes who luckily for him only met me in the last year but still needed to be patient and understanding!

Bath Spa university staff, special thanks here to my supervisor David Watson for all his advice throughout, as well as help on some of the soil/seed collections, proof reading and moral support! My second supervisor, Nigel Chaffey for proof reading and suggestions on writing technique. Darrel Watts for glasshouse technical support, statistical advice and bee identification. Graham Smith for statistical advice. Laura Dodge and Derek Beard for technical laboratory support.

Support of various friends and colleagues, particular thanks go to; Emily Smith and Debbie Lanc for seed and soil collection help and company. Emily Chalke for an evening helping count seed pods. Chris Smith and Tony Parsons for aid in bee identification, and Liz Greenaway for final proof-reading.

Various landowners and Natural England for permission to use their land as seed and soil donor sites, also to those who helped with identifying suitable sites.

Last but definitely not least I thank my loyal dog Nutmeg who kept me company throughout many long hours of writing, site surveys and collections. The last months of writing without her were the most difficult.

1 INTRODUCTION

1.1 Biodiversity Loss and Habitat Restoration

Intensification of agricultural practice and land use change have been blamed for a substantial decline of species-rich grassland and associated biodiversity (Rackham, 2003; Hopkins & Clements, 2004; Walker *et al*., 2004[c]; Flower, 2008; Pywell *et al.,* 2012). Between 1930 and 1984, semi-natural, lowland pasture declined by 97% in England and Wales (Fuller, 1987). Due to the increased need for food production during World War II (WWII), arable farming increased with large amounts of grassland ploughed (Shrubb, 2003). The replacement of the farm horse with the tractor further augmented this move to intensive arable farming after WWII (Flower, 2010). In 1973 Britain joined the European Economic Community (EEC), and consequently the Common Agricultural Policy (CAP), which aimed to provide EU citizens with affordable food and farmers with a fair standard of living (European Commission, 2012[a]). However, the Policy's early years rewarded higher production farms and this policy shift resulted in further large areas of land being put into production, grassland was converted to arable and other habitats such as hedgerows and field margins were also lost to the plough (Bignal *et al.,* 2001; Flower, 2008). In addition to conversion of grassland, the key change in grassland management since WWII has been regular large-scale re-seeding and therefore the further loss of unimproved grasslands (Shrubb, 2003; Goulson *et al*., 2005).

Habitat restoration has increased in recent years (Walker *et al.,* 2004[a;] Walker *et al.,* 2004[c]). A substantial driver for restoration was the UK becoming a signatory of the Convention on Biological Diversity (CBD) at the first meeting in Rio De Janeiro in 1994. A subsequent CBD meeting in Johannesburg in 2002 resulted in an agreement on the urgent need to reduce the rate of biodiversity loss by 2010, this brought about further habitat restoration (JNCC, 2012). Due to these CBD agreements, Natura 2000 sites were set up to produce a network of nature protection areas under the Birds' Directive 1979 (Special Protection Areas) and the Habitats' Directive 1992 (Special Areas of

Conservation) (European Commission, 2012[b]). This was an important influence in retaining many habitats. Other programmes such as the EU LIFE project, funded habitat restoration throughout the EU, including the direct targeting of grassland habitats (European Commission, 2008).

Agricultural practice was also amended in the mid 1980's (Natural England, 2012[a]) to achieve biodiversity goals. The CAP, which had been blamed for loss of large amounts of 'high nature value' farmland in preceding years (Brignal *et al.,* 2001; McCracken, 2011) was reformed, becoming the driver for introduction of Agri-environment schemes (AES) to the UK, the first large scale one of which were Environmentally Sensitive Areas (ESA) in 1987 followed by the Countryside Stewardship Scheme (CSS) in 1991. Under these schemes farmers were rewarded financial aid for using environmentally-positive approaches to farming (Critchley *et al.*, 2003; Natural England, 2013[a]). Views on the contribution of European AESs during this period were mixed. Shrubb (2003) was sceptical of their value, questioning the practicability and effectiveness of large scale environmental subsidy, suggesting essential monitoring and management of such schemes was often too difficult to achieve. Others considered AESs as one of the main incentives to deliver biodiversity objectives (Ovenden *et al.,* 1998; Critchley *et al.,* 2003). A study by Kleijn *et al.* (2001) in the Netherlands comparing 78 paired conventionally-managed fields and fields with agri-environment management agreements concluded that there were no positive effects on plants or birds (Kleijn *et al.*, 2001). Conversely a later study, again by Kleijn *et al. (*2006), on agri-environment success across Europe, revealed the density of plant species and one arthropod group was significantly higher in agri-environment scheme fields compared to conventional controls in four of the five countries studied. Critchley *et al.* (2003) also found encouraging environmental results when surveying 38 UK fields in agri-environment schemes over durations of 3 to 8 years.

The CAP was reformed in 2003 when it was recognised by the European Commission as a potential aid to decrease abandonment and intensification of biodiversity poor areas (European Environment Agency, 2010). Additional reforms of the CAP as well as other EU agricultural policies were implemented in the UK by the Department for Environment, Food and Rural Affairs (Defra). In 2003 the mixed opinions and results of AES were taken into account as a review was undertaken in the UK, with a view to launch a new scheme combining the most successful elements of ESA and CSS (Mountford *et al.,* 2013). This new programme, (Environmental Stewardship), comprised of grants awarded to farms meeting specific sustainability criteria, in a two-tiered system; Entry Level Stewardship (ELS) and Higher Level Stewardship (HLS). Practices rewarded under the initiative included arable reversion and species-rich grassland restoration (Defra, 2012). Results from a review of UK agri-environment scheme benefits (Boatman *et al*., 2008), suggest all have seemingly gone a long way to achieve UK Biodiversity Action Plan (BAP) targets such as BAP grasslands kept in favourable condition and farmland bird population increases. However, Pywell *et al*. (2012) highlighted the success only of 'evidence-based' approaches and argued that ELS, (accounting for 630,000ha (99%) of created habitat in England), is relatively unsuccessful with consistently lower species-richness of plants, bees and birds in their study; compared to the evidence based habitat enhancement of HLS (which covers just 8100ha (1%) of habitat created in England).

Since Environmental Stewardship ended to new applicants in 2013, a new land management subsidy has been developed, again named Countryside Stewardship, first agreements of this scheme are scheduled to start in January 2016 (Natural England, 2015). It is thought that the new Countryside Stewardship will further develop biodiversity advances, based upon scientific evidence of the most successful options from past years (Silcock *et al*., 2012). Consequently, the scheme includes additional options for pollinators within its Wild Pollinator and Farm Wildlife Package (Natural England, 2015).

During development of the new stewardship scheme, in 2012 the Joint Nature Conservation Committee (JNCC) and Defra published the 'UK Post-2010 Biodiversity Framework' (JNCC & Defra, 2012), successor to the UK BAP. This was developed under the Biodiversity 2020 strategy for England's wildlife, in response to the publication of the CBD's Strategic Plan for

Biodiversity 2011-2020 and the launch of the new EU Biodiversity Strategy (EUBS) in 2011 stating six main targets, two of which include; ‘Maintain and restore ecosystems and their services’ and ‘Increase the contribution of agriculture and forestry to maintaining and enhancing biodiversity’ (Defra, 2011; JNCC & Defra, 2012 p.13).

1.2 Restoration Ecology and Seed Provenance

Habitat resoration has been subject to considerable research over an extended period (Ormerod, 2003). Critical reviews such as that by Parker (1995) have prompted an evidence-based approach to developing best practice. In grassland restoration this research has been wide-ranging: the effects of soil nutrients (Gough and Marrs, 1990; Walker *et al.,* 2004[b]); the long-term influences of fertiliser application and grassland management (Smith *et al.*, 2000); cost effectiveness of restoration techniques (Manchester *et al.,* 1999); recreation of target National Vegetation Classification communities (Pywell *et al*., 2002); using plant species traits to predict restoration performance (Pywell *et al.,* 2003); and, more recently linking biodiversity restoration with an ecosystem-based approach (Bullock *et al.,* 2011) with particular emphasis on using plants from functional groups (such as legumes) to increase resources for insect polinators in agricultural grasslands (Woodcock *et al.,* 2014).

The increase in habitat restoration and enhancement since the 1990s as driven by the UKBAP (JNCC, 2012), in particular, prompted considerable debate regarding seed provenance (Lesica & Allendorf, 1999; Manchester & Bullock, 2001; Sackville Hamilton, 2001; Hufford & Mazer 2003; Krauss & Koch, 2004; Walker *et al.,* 2004[a]; Bischoff *et al.,* 2006; Smith *et al*., 2009). Seed banks can become diminished so severely (Graham and Hutchings, 1998) that in many grassland restoration projects, seed enhancement is necessary for the successful recreation of the sward (Hopkins & Clements, 2004; Bumblebee Conservation, 2012). In the UK it is recognised to be good practice to use native seed for restoration and enhancement projects (Flora Locale, 2008). Differences in phenology or morphology of non-native ecotypes can result in dependant invertebrate species missing, or being unable to use the plant altogether (Flora Locale, 2008; Natural England, 2008[a]). One such herbivore is larva of the Clouded Yellow *(Colias croceus* Geoffroy*)* (Hopkins & Murray, 1998). Research into the feeding habits of this butterfly on cultivated and wild *Lotus corniculatus* revealed that although equal proportions were consumed of each ecotype, slight differences in plant physiology resulted in the butterfly's greater ability of carbon absorption from the wild ecotype (Hopkins & Murray, 1998). Other arguments behind the native strategy is the reduction of 'wildness' as native gene pools are diluted by artificial outcrossing giving rise to increased disease risk, biodiversity loss and alterations to community competition [where cultivated varieties are often bred for qualities such as fast vegetative growth] (Lesica & Allendorf, 1999; Flora locale, 2008; Schröder & Prasse, 2013). Thus use of British native-origin seed has been a requirement by Natural England for semi-natural grassland restoration payments in agri-environment funding (Natural England, 2008[a]). Further to this issue concerns have been raised as to how important 'locally' obtained seed is (Sackville Hamilton, 2001; Krauss & Koch, 2004). Concerns include possible ecotypic variation of non-local seed causing difficulties in their use by dependant invertebrates (Hufford & Mazer, 2003).

In plant restoration ecology 'native origin' is the location of naturally occurring native plants or, when buying seed/plants it is the location where a native plant was collected (for grassland species this introduction would need to have been within six generations removed from the parent material, as long as no artificial selection had taken place) (Flora Locale, 2012). The term 'local provenance seed' in grassland restoration applies to seed whose native origin is geographically close to where it will be planted (Flora Locale, 2012). The importance of obtaining such seed in restoration projects has been developed from the concept of 'ecotype' denoting that local populations will have become genetically adapted to their local environment and will therefore be more successful and ecologically acceptable than non-local genotypes (Begon *et al*., 1990). 'Ecologically acceptable' here would derive from the 'niche' concept, where a species needs suitable environmental variables to persist in any particular habitat and with those environmental variables including physical, chemical and biotic elements, the species too becomes an environmental variable to in turn affect other niches within that habitat (Tivy, 1993).

The ‘local seed’ practice has raised concerns that geographical proximity is not always better than provenance of an ecological similarity (Bischoff *et al.,* 2006; Warren, 2012). For example, research has shown there is a range of adaptability in plant genotypes from those surviving over a wide variety of environments to more localised ecotypes with low adaptability to change (Crawford, 1990).

1.3 **Wider Ecology of Habitat Restoration**

Although seedling establishment, vegetation diversity and establishing a viable population are key objectives in ecological restoration (Montalvo *et al*., 1997; Ruiz-Jaen & Mitchell Aide, 2005; Martin & Wilsey, 2006), major concerns have also been raised regarding plant ecotypic variation, adversely affecting dependant biodiversity. These concerns included differences in plant physiology and morphology making them less suitable for use by local dependant herbivores (Hufford & Mazer, 2003; Walker *et al.,* 2004[a]). Previous studies have found strong plant-invertebrate relationships within grasslands; some have shown plant populations can evolve differences in phenotype to benefit their pollinators (Pérez-Barrales *et al.,* 2007). Others have found herbivore adaptation to chemical defences of plants, often concluding they may utilise these chemicals to their advantage (Jones *et al.,* 1962; Ehrlich & Raven, 1964). This has been shown in a study which found burnet moths (*Zygaena sp.*) take-in the cyanogenic glycosides from their food plants (members of the Fabaceae), including *L. corniculatus,* and are then able to use the compounds afresh for predator defence (Zagrobelny & Møller, 2011).

Keesing & Wratten (1998) stress the importance of higher trophic level tailoring in habitat restoration research; the requirements of those animal species higher up the food chain need to be examined in relation to the primary sources, in order to achieve whole community rather than plant component-only success. This idea was further investigated by Young (2000) who determined that most botanical-based research in restoration ecology would directly benefit from more precise zoological consideration.

1.4 Need for Further Research

Although there has been much research into seed provenance and habitat creation, little has been undertaken to investigate ecotypic variation caused by specific donor site environmental differences. In addition, restoration projects often focus on the plant or community success without considering other trophic levels, however, understanding such interactions are obviously important (Keesing & Wratten, 1998; Neal 1998; Hufford & Mazer, 2003; Walker *et al*., 2004[a]; Smith *et al*., 2005).

In current habitat restoration, best practice methodology often uses the perceived idea that seed of geographically local provenance [regardless of management history and soil type], is the best choice (Smith *et al*., 2009). This research will test the current 'local provenance' hypothesis, by analysing growth of *L. corniculatus* ecotypes when planted in different soil types with different management regimes, with a focus on plant parameters of greatest nutritional importance to invertebrate herbivores. The research will additionally evaluate preferences of bees [already present in the glasshouse] between the ecotypes (grown in treatments). Results of this should inform future seed choice of grassland managers, in achieving greater conservation success of habitat restoration on a whole community basis.

This thesis outlines the objectives and methodology overview used in the research undertaken, it introduces each element studied individually with a final concluding discussion tying the results together.

2 AIM AND OBJECTIVES

This research focusses primarily on grassland restoration within agri-environment schemes. Such grassland restoration is important due to the large-scale coverage of agriculture, which currently covers approximately 71% of total land area in the UK (RICS, 2015). However, it is aimed that results from this research can have a wider application for use, in addition encompassing mitigation schemes for infrastructure development.

2.1 Aim

The main aim of this research was to assess the importance of using ecologically-similar seed (from sites of similar environmental conditions) rather than geographically-local seed in grassland restoration projects, with particular reference to herbivorous invertebrates, including pollinators.

2.2 Objectives

Three interaction studies were completed to examine potential translocation effects on different ecotypes of *Lotus corniculatus.* Study 1; 'Soil type treatment' investigated ecotype:soil interaction and Study 2; 'Management regimes treatment' examining ecotype:management interaction. Relationship between these two studies in conjunction with each other was also investigated as the glasshouse study was prepared in a full factorial design. Ecotypic variation studied throughout was largely focused on parameters of greatest relevance to herbivore and pollinator requirements, this culminated in the final study 'Bee Interaction' (Study 3) where behaviour of bees using the ecotypes was also undertaken.

Results from each study was examined, combined and discussed in relation to the research aim, which has been broken down into questions relating to the three objectives (revisited in the results and concluding discussion):

Objective A: Plant Fitness

- Will ecotypes of edaphically-distant provenance show reduced fitness (survival, growth and fecundity) when grown in soil types different to that of their donor site, and will the ecotypes retain traits related to donor-site soil type?

- Will a different management regime to the donor-site reduce fitness of ecotypes, and will the ecotypes maintain characteristics related to donor-site management?
- Will plants of closer geographical distance show similar growth traits (suggesting similar genotypic profiles)?

Objective B: Herbivore Requirement

- Will ecotypes of edaphically different sites maintain home-site characteristics of potential benefit or detriment to generalist herbivores/pollinators, and could generalist herbivores/pollinators receive greater dietary benefit from plants grown in soil types similar to the donor site?
- Will ecotypes receiving different management regime to their donor site maintain characteristics of potential benefit or detriment to generalist herbivores/polinators; and will generalist invertebrate herbivores/pollinators receive greater dietary benefit from ecotypes grown under the same management regime?
- Will plants of increasing geographical distance show characters of decreased potential benefit to generalist herbivores/pollinators?

Objective C: Bee Preference Study

- Will bees prefer ecotypes growing in a soil type similar to the ecotype’s donor site?
- Will bees prefer ecotypes receiving the same management regime as the ecotype donor site?
- Will bees prefer ecotypes of closer geographical distance to the test foraging area?

3 OVERVIEW OF METHODS USED

Seed from each site [populations] are referred to as ‘ecotypes’ for this study. ‘Grazing treatment’ refers to simulated grazing from cutting by hand. Please note that within this chapter, calcareous sand is referred to as ‘sand’ and cut with aftermath grazing is referred to as ‘cut’.

Studies 1 and 2 were conducted in a full factorial design with seed collected from selected soil type and management conditions, then grown-on in one of three different treatment soils, receiving one of two separate management treatments. This was carried out in a controlled glasshouse environment to determine whether seed source and treatments alone or in combination affected the morphology, physiology and phenology of the plants. Parameters recorded to determine variance between plants were chosen with herbivore requirement of the plants of foremost importance. Therefore methods included data collection of nutritional quantity and quality of the plants as well as observing those plants used and pollinated more frequently by bees visiting the glasshouse.

3.1 Ecotypes

Nine populations were chosen from a range of National Character Areas (NCA), (Natural England, undated) in south-west England. Populations chosen were from a range of soil types (calcareous loam, neutral loam and sand) and a range of management regimes (unmanaged, grazed and cut).

The populations were representative of different seed donor sites. Plants at each donor site were presumed to be ecotypically adapted to the site’s climate, soil type, management etc., and as such, the seed from each site [the populations] are referred to as ‘ecotypes’ for this study.

Seed of 20 *Lotus corniculatus* plants together with companion soil samples were collected from each site and stored separately. Extensive chemical analysis of soil samples was carried out to identify site-specific soil attributes. Information was collected from site owners/managers to ascertain historical management of each site.

3.2 Soil Treatment (Study 1)

Three treatment soils; calcareous loam, neutral loam, and sand were selected and analysed, for comparison with ecotype (donor site) soils. These three soils were selected to be used as growing media for the glasshouse experiment. The soils were chosen to represent typical receptor site soil with respect to nutrient levels and physical composition. These soils are referred to as the 'treatment soils' throughout this thesis.

Ecotype seed was germinated and planted singly into each treatment soil type (October 2011), and grown for nine months.

3.3 Management Treatment (Study 2)

A reciprocal experiment to Study 1 was carried out at the same time, with the glasshouse study extended to be a full factorial design. All seed was propagated and grown within soil types as in 'Study 1' to eliminate confounding factors between interaction effects.

Management was designed to mimic that of grassland during establishment (the first year in grassland restoration) due to the time restrictions of the study.

When more than 50% of plants were over 10cm in length (March 2012) treatments commenced on samples, cutting to a height of 7cm to simulate the 'grazed treatment'. The rest of the samples represented the 'unmanaged treatment'.

Cut plant material was removed, weighed and recorded at four-weekly intervals, immediately prior to the four weekly monitoring for each individual plant.

3.4 Monitoring and Harvest

Main stem length, flowering phenology and fecundity were recorded at four-weekly intervals (from October 2011) over a period of ten months to establish extent of variance in plant growth, form and development.

After ten months detailed growth data were collated, (main stem length, longest branch length, branch number per main stem, stem number per plant, leaflet number per main stem, flower number, floret number, seed pod number, plant hirsuteness, flower scent) immediately prior to harvest where all plants were cut to 5cm above ground level (July 2012).

Fresh and dry weights were taken to establish variance in biomass and moisture content between plants.

Leaf-hydrogen cyanide (HCN) was measured during the harvest on freshly cut plant material. Leaf-nitrogen (N) was established later with dried plant material.

These plant growth parameters and chemical analysis from Studies 1 and 2 determined the extent of variance between ecotypes/treatments in plant fitness (**Objective A**) and nutritional value of potential detriment/benefit to herbivores/pollinators (**Objective B**).

3.5 **Post-Harvest Monitoring**

After plants were harvested (July 2012), they were left to recover for five weeks, after which main stem length and mortality were recorded.

Seven weeks after harvest, the grazed treatment was applied once more before the winter. They were then overwintered in controlled conditions in the glasshouse. The following spring, main stem lengths and mortality were recorded.

It was decided that treatments should continue for a second year to gain additional opportunities to observe bee activity (4.6 Bee Interaction). Therefore grazed treatment was re-commenced in April 2013 and again repeated every four weeks. Flower number was recorded during each grazed treatment visit and any bees observed were recorded as the previous year.

A final grazed treatment was applied approximately 22.5 months after the study commenced, a later application was not made as more than 50% of grazed plants failed to reach 5cm.

Main stem length, mortality, seed pod number and mean seeds per pod were calculated at the end of the study in October 2013.

3.6 Bee Interaction (Study 3)

The full factorial design used for Studies 1 and 2 was also used for this work.

Bee monitoring was regularly carried out, commencing during the peak flowering times in 2012 (Year 1) and 2013 (Year 2), to establish the ecotype/treatment most preferred by bees visiting in the glasshouse.

For this study, individual bee’s were followed for five minute periods. Bee species and time spent on each plant were recorded, with any particular behaviour noted.

Results from this were analysed and discussed in relation to results from Studies 1 and 2, to achieve **Objective C**.

4 STUDY SPECIES LOTUS CORNICULATUS L.

Lotus corniculatus L. was chosen for this investigation for a range of reasons associated with its ecology. In brief, it has been selected due to being an important component of many grassland communities (Rodwell ed., 1991; 1992; 2000) and its popularity of use in grassland seed mixtures (Smith *et al.,* 2009). In addition, its conservation (*Walker et al.,* 2004[a]; *Pywell et al.,* 2006) and agronomic qualities (Majak *et al.*, 1995; Carter *et al.*, 1997; Wilkins, 2001) as well as the potential effects of its complex chemical make-up on herbivores (Jones & Turkington, 1986; Howe & Westley, 1988).

4.1 Natural History

L. corniculatus is a herbaceous perennial legume, widespread and indigenous to Britain (Grime *et al*., 1992; Rose 2006) with a wider natural range spanning most of Europe as well as northern and eastern Africa and parts of Asia (Jones & Turkington, 1986). This is a polycarpic species, mainly budding from June to September, the flowers 1-1.5cm in length, are yellow, often with a deep orange to red tinge (Chinery, 1982; Grime *et al*., 1992), (Figure 1). Leaves are arranged with three distal leaflets and two basal leaflets on each (Jones & Turkington, 1986).

Figure 1. *Lotus corniculatus* in flower
Source: Author, 2012

Regeneration is mainly from seed which mature in pods. The seeds are hard-coated (Figure 2) and can persist in the seed bank for at least five years (Grime *et al*., 1992; Thompson *et al*., 1993), clonal regeneration is possible but uncommon (Grime *et al*., 1992; Ollerton & Lack, 1998). Pollination is entomophilous (Kozuharova, 2000), mainly by bumblebees (*Bombus sp*., Apidae: Hymenoptera) (Ollerton, 1993). Self-pollination can occur, though rarely (Jones & Turkington, 1988; Ollerton, 1993). The most common habitats for *L. corniculatus* are limestone pastures as well as open habitats such as wasteland, quarries and spoil heaps (Grime *et al*., 1992), however it can also be found in maritime and montane habitats, and on soils with moderately low pH (5-6) such as mesotrophic grasslands and heath (Jones & Turkington, 1986; Rodwell ed., 1991; 1992; 2000). The plant normally forms nitrogen-fixing nodules in association with the soil bacteria, *Rhizobium lupini* (Grime *et al*., 1992).

Figure 2. *Lotus corniculatus* seed
Source: Author, 2007

4.2 Agriculture

L. corniculatus is now used in agriculture thoughout the world as an important forage legume (Wilkins, 2001; Graham & Vance, 2003), with various cultivars available. The plant is a valuable addition to grassland mixes due to its nitrogen fixing nodule bacteria, reducing fertiliser requirements (Wilkins *et al*., 2001). Other agronomic attributes of the plant which have been harnessed in the many varieties include the plants long seasons of palatability (Redmon *et al*., undated) and the non-bloating properties [for livestock] associated with proanthocyanidins (PA) found in the plant leaves (Majak *et al*., 1995; Aerts *et al*., 1999). These condensed tannins are also shown to reduce gastrointestinal parasites in cattle by reducing plant protein breakdown to ammonia in the animal's rumen (Aerts *et al*., 1999), and they contribute to the plants ability to increase milk production in dairy cows (Woodward *et al.,* 2000). A UK study using *L. corniculatus* in combination with chicory (Cichorium intybus) as a forage for Lleyn lambs with naturally occuring helminth infections, showed the animals had fewer helminth parasites than those grazing rye grass (*Lolium perenne* L.)/ white clover (*Trifolium repens* L.) forage (Marley *et al*., 2003), the impact of which is found to be related to the the condensed tannins found in the plant (Molan *et al*., 2000). Research carried out by Warda (2002) found this particular legume to be better adapted to less fertile soil than *Trifolium repens*, and persistent in grassland managed by regular cutting. Varieties are bred to expand on the adaptation already present in *L. corniculatus,* to drier and lower altitudes. Such varieties have increased legume production in highly stressed locations such as the high rainfall zone (HRZ) of southern Australia (Real *et al*., 2012). It has also been reported that the plant's deep tap root of over 25cm in length can penetrate a lower layer of soil than many grassland species, making additional minerals available to livestock through their leaves (Flower, 2008).

4.3 **Conservation**

L. corniculatus is commonly used in grassland restoration projects; its wide edaphic plasticity and varied habitat occurrence (as shown in the National Vegetation Classification communities within which it is found) (Rodwell ed., 1991; 1992; 2000) have made it a plant often used in restoration of areas difficult to establish vegetation cover, including contaminated soils (Grime *et al.,* 1992; Escaray *et al.,* 2012). Its long woody tap-root (Jones & Turkington, 1986; Flower, 2008) helps control soil erosion and can establish the plant on sand dunes and other sites in need of stabilisation (Carter *et al.,* 1997). However, work on plant traits by Pywell *et al*. (2003) suggests that it is a species that doesn't establish or persist particularly well on enriched former agricultural soils.

The plant's importance and appeal to many bee species (Bees, Wasps & Ants Recording Society, 2013) has made it a favourite for inclusion in pollen and nectar mixes in agri-environment conservation (Emorsgate Seeds, 2013). Although most of the bee species which use *L. corniculatus* are polylectic species, there are exceptions such as the three species of Red Data Book Listed solitary bee; *Osmia parietina* Cutis is oligolectic for *L. corniculatus* pollen, *Osmia uncinata* Gerstäcker and *Osmia xanthomelana* Kirby also depend mainly on *L. corniculatus* (Bees, Wasps & Ants Recording Society, 2013).

L. corniculatus is also important for many other insect species, including several in the orders Coleoptera, Collembola, Dermaptera, Diptera, Heteroptera and Homoptera (Jones & Turkington, 1986; Virteiu *et al*., 2014). It is the larval food plant for many Lepidoptera species including the common blue (*Polyommatus icarus* Rottemburg*)* and wood white (*Leptidea sinapsis* L.) (Hopkins & Murray, 1998), six-spot burnet (*Zygaena filipendulae* Dupont) and dingy skipper (*Erynnis tages* L.) (Chinery, 2004). Field voles (*Microtus agrestis* L.) and rabbits (*Oryctolagus cuniculus* L.) are among some of its larger herbivorous consumers (Jones & Turkington, 1986).

4.4 **Plant Defences**

Herbivory, whether above or below ground has been found to alter plant performance (Jing *et al*., 2014). Host plant physiological and morphological reactions can be caused by herbivory in *L. corniculatus* such as changes in tannin and hydrogen cyanide level, and root:shoot ratios (Briggs, 1991; Bazin *et al.*, 2002).

L. corniculatus produces both quantitative and qualitative chemical defences against herbivory. The qualitative 'secondary metabolites' produced are the cyanogenic glycosides 'Linamarin' and 'Lotaustralin' (Jones & Turkington, 1986). These cyanogenic glycosides are classified as phytoanticipins, and release Hydrogen Cyanide (HCN) when the plant is broken or crushed by herbivorous activity, due to bio-activation from *β*-glucosidases (Morant *et al.,* 2008). When activated, these toxins obstruct certain enzyme reactions within the animal and can be poisonous in small doses (Howe & Westley, 1999). Cyanogenic glycosides are polymorphic in *L. corniculatus* (Ellis *et al*., 1977) which is where genetically alternative forms occur within one population in a provisionally ambiguous environment (Harper, 1990; Kakes, 1991). it has been suggested wind and wind-borne salt exposure are the main factors in determining frequency of cyanogenic and acyanogenic *L. corniculatus* plants within a population (Ellis *et al*., 1977). This form of adaptive polymorphism in plants is controlled by two independent genes: *Ac/ac* for the production of cyanoglycosides and *Li/li* for the production of *β*-glucosidases (linamarase) [for hydrolysis of the cyanoglycosides] (Hughes, 1991; Kakes, 1991; Olsen *et al*., 2008). Acyanogenic *L.corniculatus* plants or organs may have an absence of cyanoglycosides, the compatible *β*-glucosidase, or both (Hughes, 1991; Kakes, 1991). Although cyanogenesis is genetically controlled, its expression is often influenced by environmental factors (Ellis *et al*., 1977; Vickery *et al*., 1987; Gebrehiwot & Beuselinck, 2001).

The quantitative 'digestibility reducers' are condensed tannins (polyphenols) (Price *et al.,* 2011). These act in several ways to hinder the herbivore's digestion, often impeding protein usage and affect the animal depending on the quantities eaten (Howe & Westley, 1988; Freeman & Beattie, 2008). It could also be argued that tannins are additionally a qualitative defence due to the bitter taste they give which therefore make them an undesirable food source (Freeman & Beattie, 2008).

Legumes such as *L. corniculatus* may have more defences than is presently known and the allelochemical protection method is still not fully understood (Howe & Westley, 1988). Although these chemical compounds are generally associated with protection against herbivorous predation (Crawley, 1986), the examination of two *Zygaena* species eggs, larvae and pupae has shown the release of hydrocyanic acid when crushed. Further studies showed the larvae of this moth to voluntarily feed on cyanogenic ecotypes of *L. corniculatus* (Jones *et al.,* 1962). This demonstrates the moth's apparent resistance to the plant's secondary compounds and suggests it could intentionally select the cyanogenic ecotype to build up a chemical defence of its own or even just to widen its food plant range. This evolutionary 'arms race' theory was first suggested by Ehrlich & Raven (1964). At present, many invertebrates cannot detoxify the cyanogenic compound; molluscs are one such family preferring to select the acyanogenic ecotype (Ellis *et al*., 1977).

HCN, like many plant defences, is an environmental adaptation maintained by natural selection (Till-Bottraud & Gouyon, 1992), where there is a fine balance between herbivory risk and the cost of producing the toxin for the plant (Bloom *et al.,* 1985). HCN production would therefore be of greater cost when weighed against survival in more exposed or extreme conditions. At such sites it has been found that herbivore frequency is reduced and presence of acyanogenic *L. corniculatus* are more common (Ellis *et al*., 1977).

5 ECOTYPE SITES – INITIAL COLLECTIONS

Please note in this chapter there are no shortened references to calcareous sand or cut with aftermath grazed ecotypes due to additional discussion of other similar sand based soils and cutting regimes.

5.1 Ecotypic Variation in Seed

Many environmental factors contribute to ecotypic variation and phenotypic plasticity. Work already carried out on ecotypic differentiation of *Lotus corniculatus* includes a study by Kelman *et al.* (1997) who found main stem length, yield and condensed tannin concentration differed between populations, for example, condensed tannin was highest (8.73% of dry weight) from the Portugese group and lowest (2.96% of dry weight) from the Turkish group of those studied. Associations were also found in Kelman's (*et al.,* 1997) research where high condensed tannin was associated with prostrate growth habit in populations from Spain and vice-versa in populations from Russia. Sareen & Dev (2003) reported biomass, branch number and branch size varied significantly between genotypes. Air temperature and carbon dioxide concentrations have also been shown to cause variation in *L.corniculatus* genotypes (Carter *et al*., 1997). Cyanogenesis is polymorphic in this species (Ellis *et al*., 1977; Crawley, 1986), however, as already discussed (Chapter 3), research indicates predominantly acyanogenic *L. corniculatus* often occurs in environmental conditions where cost of producing the chemical is outweighed by other factors (Ellis *et al.,* 1977; Till-Bottraud & Gouyon, 1992).

Examples of ecotypic variation within similar grassland species has been found in *Armeria maritima* Willd. with zinc tolerance (Jiménez-Ambriz *et al.,* 2007), altitude tolerance altering flowering behaviour of *Sonchus arvensis* L. (Neuffer, 1990), competition and management adaptations of *Poa annua* L. (McNeilly, 1980), and differing growth responses of *Trifolium repens* L. when grown with different 'companion' species (Collins *et al.*, 2003). This indicates the widely varying conditions that can induce specialised genetic adaptations within a species.

5.2 Factors in Choice

Sites chosen to collect populations needed to represent a range of soil types, management regimes and geographic distances where *L. corniculatus* is commonly recorded in the plant community. Some sites needed to be geographically local to each other but with differing soil types and/or management history and some had to be geographically non-local to each other but with matching soil type and/or management history.

Also important was ensuring that sufficient historical knowledge of each site existed to be sure the plants were of natural origin rather than an agricultural sown variety.

There is no definitive timescale for plants to become ecotypically adapted, it can be anything from 'a few years to several hundred years' (Millien *et al.,* 2006). Flora Locale (2012) defines 'native origin' as having originated from the wild where it is highly probable that they had not been sown recently (e.g. *c.*1930 for grassland species). Therefore where possible, this timescale was sought but was found difficult to specify as owners knowledge and records were often limited to later accounts of the sites' history. However, as 'native origin' plants introduced elsewhere would lose this status after six generations removed from the parent material (Flora Locale, 2012) this was also taken into account and history of approximately 10 years was sought for established management and natural growth.

The occurrence of *L. corniculatus* at sites was required to be 'occasional'. Any lower than this and the population at the site may have been compromised by the seed collection, and any higher frequency may have caused irregularities in the study.

5.3 Ecotype Site Choices

To assist with choice of appropriate geographical distances involved in site selection, county boundaries and Natural England's 'Natural Character Areas' (previously known as Joint Character Areas) (Natural England, undated) were used. These geographical segments sharing similar characteristics, are used for natural environment decision-making and to inform land management choices (Natural England, 2013[b]) they are recommended by Natural England guidelines to be used in sourcing ‘local origin seed’ (Natural England, 2008[a]).

Sites were predominantly chosen using Biological Record Centre (Bristol Regional Biological Records Centre, 2009) and National Biodiversity Network (NBN, 2009) data on *L. corniculatus* locations and this information was combined with that derived through consultation with Wildlife Trusts, Plant Life, National Trust, Local Council, Ministry of Defence and Natural England, as well as website information on wildlife reserves and SSSIs.

5.4 Sites with Permissions

For all sites, permission was sought and received from owners, some of the statutory designated sites also required Natural England consents which were obtained. Potential sites were honed down to a final list (Table 1).

Permissions were obtained for 39 sites during 2009, 25 of which were visited at least once with collections of 14 sites made between August and October 2009. A further site (Dawlish Warren) was collected the following September (2010).

The visited sites where collections weren’t made, were unsuccessful due to various reasons; Avis and Distillery meadows were visited twice but no seed was found due to slow growth following the hay cut which was carried out at the end of August. Melbury, Bigbury, Crook Peak, Devenish, Heath Hill Farm, and Everleigh Down also had either no seed or unripe seed during the visits. Braunton Burrows and Chittoe Heath were only visited to establish initial potential but as SSSI permissions for these sites were difficult to obtain and the pre-survey found very small amounts of *L. corniculatus* further pursuit for collection was abandoned.

Table 1. Details of sites where permissions were granted, in alphabetical order of site name. Highlighted rows indicate those sites where seed and soil sample collections were made. NCA is Natural England's 'Natural Character Areas', See Appendix I for explanation of these and see 'Glossary' for statutory designation acronyms.

Name Location	Owner/ Contact	OS Grid ref.	NCA	Stat. Designation	Soil	Management	Visit Date
Avis Meadow North Wiltshire	Wiltshire Wildlife Trust	SU02 1878	108	N/A	Neutral	Hay cut & aftermath grazing (2009 cut towards end of Aug)	11/09/09
Bencroft Hill Meadows Calne, Wiltshire	Mr E Jones	ST96 2731	117	SSSI	Neutral	Cattle-grazed	
Berrow Dunes Somerset coast	Sedgemoor District Council	ST29 2532	142	SSSI	Fixed calcareous dune	Unmanaged	15/09/09
Braunton Burrows N. Devon coast	The Tapeley Life Interest Trust	SS45 1365	145	SSSI	Fixed calcareous dune	Mixed grazing	02/10/09
Britford Water Meadows Salisbury, Wilts	James Whittle	SU16 6274	132	non SSSI areas	Water meadows	Cattle-grazed (June)	
Brooklands Farm Dorchester	Dorset Wildlife Trust	SY66 6952	134	N/A	Neutral	Usually hay cut & aftermath grazed	
Burledge Hill Bishop Sutton, Somerset	Avon Wildlife Trust	ST58 6586	118	SSSI	Limestone Plateau & 3 neutral fields (NVC MG5)	Avon Wildlife Trust areas are grazed by cattle	08/09/09
Chancellors Farm Priddy, Wells, Somerset	Somerset Wildlife Trust	ST52 5525	141	SSSI	Neutral	Cut with aftermath grazing	
Chittoe Heath Spye Park, Calne, Wilts	P Lewis Spye Park	ST95 9673	117	SSSI	Lower greensand heath	Grazed	14/09/09
Cockey Down Salisbury, South Wiltshire	Wiltshire Wildlife Trust	SU17 3320	132	SSSI	Calcareous	Long history of various grazing. Before 1842 parts were ploughed.	10/09/09
Corfe Mullen Meadow Dorset	Dorset Wildlife Trust	SY98 0967	135	SSSI	Neutral	Cut with aftermath grazing	

Name Location	Owner/ Contact	OS Grid ref.	NCA	Stat. Designation	Soil	Management	Visit Date
Crook Peak Mendip hills, Somerset	National Trust	ST38 5555	141	SSSI	Calcareous (NVC: CG1,2,4)	Tightly grazed (some topping of bracken & scrub)	09/09/09
Dawlish Warren South Devon coast	Teignbridge County Council	SX98 3789	148	SAC SPA SSSI NNR	Fixed calcareous dune	Grazed since 2004 by Dartmoor ponies	24/09/10
Devenish Salisbury, South Wiltshire	Wiltshire Wildlife Trust	SU12 6345	132	N/A	Calcareous	Mixed grazing	10/09/09
Distillery Meadow North Wiltshire	Wiltshire Wildlife Trust	SU02 7892	108	SSSI	Neutral	Hay cut & aftermath grazed (2009 cut towards end of August)	11/09/09
Dolebury Warren Mendip Hills, Somerset	National Trust	ST45 5589	141	SSSI	Calcareous & limestone- heath (NVC: CG1, 2, 3)	Managed by Avon Wildlife Trust. Sheep-grazed	
Draycott Sleights Mendip Hills, Somerset	Somerset Wildlife Trust	ST48 3516	141	SSSI	Calcareous (NVC mainly CG2)	Cattle-grazed	
Folly Farm Stowey, Somerset	Avon Wildlife Trust	ST61 1606	118	SSSI	Neutral (NVC MG5)	Cut with aftermath grazing by cattle	25/09/09
Bigbury Fontmell & Melbury Down, Dorset	National Trust	ST87 6176	134	SSSI	Calcareous	Cut with aftermath grazing	23/09/09
Fontmell Hill Fontmell & Melbury Down, Dorset	National Trust	ST87 8182	134	SSSI	Calcareous	Receives hay cut, just cattle-grazed on steeper areas	03/09/13 23/09/13
Melbury Hill Fontmell & Melbury Down, Dorset	National Trust	ST88 3194	133	SSSI	Calcareous	Receives hay cut	23/09/09
Goblin Combe Bristol, North Somerset	Avon Wildlife Trust	ST47 3652	118	SSSI	Calcareous & limestone- heath	Grazed by small number of sheep and more numerous rabbits	09/09/09
Goren Farm Honiton, Devon	Julian Pady	ST23 3022	147	N/A	Neutral (soil pH approx.. 5.5)	Cut with aftermath grazing (receives farm yard manure).	

Name Location	Owner/ Contact	OS Grid ref.	NCA	Stat. Designation	Soil	Management	Visit Date
Haydon Hills Dorchester, Dorset	Dorset Wildlife Trust	SY67 0945	134	N/A	Calcareous downland	Grazed	
Heath Hill Farm Stourhead, Wiltshire	Steve Harris (Land Manager)	ST75 7336	133	SSSI	Neutral patchy (NVC MG5)	Some cut for hay and aftermath grazed early August, some extensively cattle-grazed.	23/09/09
Hellenge Hill Bleadon, North Somerset	Avon Wildlife Trust	ST34 5571	141	N/A	Calcareous Mendip scarp, north is semi- improved neutral	Grazed by cattle, sometimes sheep too	15/09/09
Lawrence Weston Moor North Somerset Levels	Avon Wildlife Trust	ST54 8790	118	SSSI	Dry & wet neutral meadows	Receives hay cut but no aftermath grazing	
Middleton Down West Salisbury Plain	Wiltshire Wildlife Trust	SU04 3242	134	SSSI	Calcareous	Grazed	
Salisbury Plain (Bulford Down) South Wiltshire	Ministry of Defence	SU19 2481	132	SSSI/SAC /SPA	Calcareous and neutral	Cut with aftermath grazing	29/09/09
Salisbury Plain (Everleigh Down) South Wiltshire	Ministry of Defence	SU19 4522	132	SSSI/SAC /SPA	Calcareous and neutral	Cut with aftermath grazing	29/09/09
Southstoke Bath, B&NES	Agent - Stephen Thompstone	ST73 7610	107	None	Limestone Brash	Cut with aftermath grazing by cattle for at least the last 10 years (2009)	25/08/13 04/09/09 21/09/09
Southstoke Bath, B&NES	Agent - Stephen Thompstone	ST74 2608	108	None	Limestone Brash	Grazed by cattle from June- Oct/Nov for at least 10 years	25/08/13 04/09/09 21/09/09
St. Catherine's Valley **C**hippenham, Wiltshire	Edward Lippiatt	ST76 0725	107	SSSI	Limestone Brash, wet grassland	Cattle-grazed (Long horn cattle)	25/08/13 14/09/09

Name Location	Owner/ Contact	OS Grid ref.	NCA	Stat. Designation	Soil	Management	Visit Date
Stonehenge - Seven Barrows South Wiltshire	National Trust	SU13 7425	132	World Heritage Site	Calcareous	Annually brush-harvested for seed to 2007. Rested in 2008 & 2009. It was grazed in 2009 summer.	
Twerton Roundhill Bath	Bath & NE Somerset Council	ST72 4632	107	LNR	Limestone grassland	Cut, not always every year, not aftermath grazed	
Upton Heath Poole, Dorset	Dorset Wildlife Trust (DWT)	SY98 9951	135	SAC, SSSI	Heathland	Grazed by a donkey and two Shetland ponies	
Valley of stones Abbotsbury, Dorset Coast	Natural England (NE)	SY59 8874	134	SSSI, NNR	Various NVC's: CG2, MG5, U20. The MG5 area was collected.	Mixed grazing	18/09/09
Woodborough Hill Alton Barnes, Wiltshire	Tim Carson	SU11 7614	116	N/A	Calcareous	Cut (July) and aftermath grazed by cattle	06/09/09
Woolacombe Warren, North Devon Coast	National Trust (NT)	SS45 5426	145	AONB Biosphere reserve	Fixed calcareous dune	Unmanaged	02/10/09

References: OS Grid ref/ NCA/ Designation: Magic, 2013. ***Lotus corniculatus* presence /Soil type / Management:** WWT, 2003[a]; WWT, 2003[b]; DTE SP, 2008; Banks, 2009; Bristol Regional Environmental Records Centre, 2009; Carson, 2009; Chambers, 2009; Christie Estates, undated; Corner, 2009; Cox, 2009; Edgington, 2009; Feneley, 2009; Glazebrook 2009[a]; Grazing Animals Project, 2009; Lawrence, 2009; Marshall, 2009; Martin, 2009[a]; Martin, 2009[b]; Martin, 2009[c]; Morrison, 2009; Pady, 2009; Sedgemoor District Council, undated; Smart, 2009; Thompstone, 2009; Whitbourn, 2009; Whittle, 2009.

There were also various reasons why the other sites were not visited or used; Bencroft Hill, Dolebury, Draycott Sleights, Haydon Hills and Middleton Down were all disregarded as there had already been enough successful collections made at sites either within these areas or with the same soil types/ managements. Britford Water Meadows became too difficult to collect from around shooting times. Corfe Mullen Meadow was pursued for two years but on each occasion the grazier (independent of Dorset Wildlife Trust) cut the field the day before the scheduled visit. With further investigation Goren Farm was resolved as unsuitable due to possibility of agricultural varieties of *L. corniculatus* present (Pady, 2009), as was Lawrence Weston Moor (Glazebrook, 2013), Twerton Round Hill (Corner, 2009) and Stonehenge (Morrison, 2009) due to irregularities to the specification with management regimes. Chancellors Farm was deemed unsuitable due to increased lead levels of the soil mentioned in the permission letter received (Hancock, 2009). Brooklands turned out to have been enhanced by seed in 1998 (Banks, 2013), and Upton heath was suggested as unsuitable due to the small amount of *L. corniculatus* present (Banks, 2009).

It was difficult to obtain sand ecotypes that had either cutting or grazing management. Therefore only one such site was collected (Dawlish Warren) and as management here had only commenced in 2004, seven years before the collection was made this wasn't ideal, however in the absence of more suitable sites, it was included.

5.5 Seed Collection Methodology

Seed was hand harvested (Figure 3) from each site between August and October 2009 with one last site (Dawlish Warren) taken in September 2010. Collections were carried out following guidelines used in the Millennium Seed Bank Project (Royal Botanical Gardens, Kew, 2002). Under these guidelines, landowner permissions and SSSI consents were obtained, collections were only taken from populations with a large enough (>49) number of individuals, and no more than 20% of the viable seed on the collection day was obtained to protect the population. Seed pods were taken from 20 plant clumps where possible, only those plants which had at least five seed pods were sampled. This was carried out randomly with at least five metres separation distance between each sample point (Bischoff *et al*., 2006) to be sure of obtaining different individuals in a population. Care was taken in identification of the plant to ensure it was not an agricultural variety or a similar *Lotus* species.

Figure 3. Collection of ripe seed

Post-harvest handling followed Millennium Seed Bank Project guidelines (Royal Botanical Gardens, Kew, 2008); immature and wet seed pods were slowly dried over 1-2 weeks with branches still connected and ripe seed was stored in paper bags and envelopes at a minimum temperature of 15°C. In addition, during winter, seed was stored in temperatures similar to outdoor conditions to give the seed the cold stratification they would have naturally received. The seed bags were checked regularly for weevils with any infestations quarantined.

[Image redacted in this digitized version due to potential copyright issues]

Figure 4. Map to show locations of ecotype sites visited with Natural England's Natural Character Area map in background. Colour circles indicate those sites where successful collections were made, black squares are sites visited but not used for collections. Ecotype key; am - Avis Meadow, bb - Braunton Burrows, bd - Burrow Dunes, bg – Bigbury, bh - Burledge Hill, cd - Cockey Down, ch - Chittoe Heath, cp - Crook Peak, dm - Distillery Meadow, dv - Devenish, ed - Everleigh Down, ff - Folly Farm, fm - Fontmell, gc - Goblin Combe, hh - Hellenge Hill, hhf - Heath Hill Farm, mb - Melbury, sp - Salisbury Plain, ssg/c – Southstoke, stc - St. Catherine's, vs - Valley of Stones, wb - Woodborough Hill, ww - Woolacombe Warren. Map source: Magic, 2013

6 ECOTYPE SOILS (DONOR SITES)

Please note in this chapter cut with aftermath grazing is referred to as ‘cut’. There is no shortened reference from calcareous sand to ‘sand’ in this chapter due to additional discussion of other sand based soil sites.

6.1 Soil Type Introduction

There are over 1800 soil types in Great Britain (British Society of Soil Science, 2013) which can change over short distances due to Britain’s diverse range of underlying geology, land use and climate (Natural England, 2013[k]). More localised differences can occur through instances such as history of mining and waste/pollutant disposal causing toxicities and deficiencies, with increased competition between species for the lower levels of nutrients (Gudin & Syratt, 1970; Green & Renalt, 2007). Some plants have become adapted to variations in soil type characteristics, others have certain plasticity to differences. Gudin & Syratt (1970) observed the success of legume species in oil contaminated soils and suggested the nitrogen fixing qualities of these species help with the impoverishment of available soil-nitrogen at these sites. Those plants that have neither adaptation nor plasticity to certain extremes of nutrient availability either do not survive in such locations or exist in a weakened state, with depressed vigour and/or growth (Tivy, 1993).

Previous studies with legumes have shown soil moisture content can cause considerable variation. Brewer (1947) recorded increased performance in the soil with greatest moisture content and Foulds (1978) correlated poor growth with severe drought. In *L. corniculatus* such differences resulting from soil moisture levels include alteration in growth rate, root and shoot biomass, and fecundity (Carter *et al.,* 1997). Mismatched Rhizobia, have also been found to modify *Lotus* germination, root nodulation and yields when a species is not translocated to a soil containing its corresponding symbiotic *Rhizobium* sp. (Gwynne & Beckett, 1980).

An understanding into the cause of morphologic differences is necessary to draw conclusions from modified behaviour to soil types, for example, Phosphorus is an essential macronutrient, plants not adapted to low levels, planted in a phosphorus impoverised soil would display initial symptoms of older leaves darkening in colour (Taiz & Zeiger, 1991). However, high levels of phosphorus can favour more competitive species in a sward, and be a limiting factor to species-rich grassland establishment (Gough & Marrs, 1990).

Effects of different soil type can often be identified from plant communities, as these groupings are constructed of species with similar tolerances and over time, genotypic adaptations to abiotic and biotic elements including soil type. National Vegetation Classification attempts to categorise these communities and soil types can often be shown by the precursor letters to the NVC code, such as CG for calcicolous grasslands found on high pH soils, SD for sand dune grasslands found on soils of various coastal sand dune soils, H heathland found in acid soils, and MG for mesotrophic grasslands found on neutral soils (Rodwell, 1991; 1992; 2000). However it can be difficult to separate some communities to one particular soil type and therefore there are occurrences such as MG1 *Arrhenatherum elatius* grassland and MG5 (*Cynosurus cristatus - Centaurea nigra* grassland) which can be found on both neutral and high pH soils.

L. corniculatus doesn't have strict tolerances to soil pH, and can therefore be found in communities of various soil type origins such as MG4, CG2, U15, SM16, SD8, OV27, and H7 (Rodwell, 1991; 1992; 2000) where it will have site specific adaptations, so may show discreet ecotypic variation. It has been suggested this species can survive such pH extremes due to a high ability to exploit phosphate and calcium from even the most nutrient-poor of soils (Brewer, 1947).

Out of the 16 elements fundamental to plant survival, 13 come from the medium they grow in (Arteca, 2015). There are also substances absorbed from the soil by plants which can be harmful (Rorison & Robinson, 1986), these are equally as important for plant adaptation. Even essential nutrients are toxic in large quantities (Moore & Chapman, 1986).

Descriptions of soil elements measured in the analysis are in Appendix II.

6.2 **Ecotype Soil Collection and Analysis Methodology**

6.2.1 Soil Collection

Topsoil samples were taken during the seed collections (Figure 5). The top 1cm of surface soil with vegetation was carefully removed and set aside for later replacement then a hand-trowel of soil was extracted from where the plant roots were most common, to a depth of 7.5cm (Natural England, 1999). Shallow soils were sampled to the depth each allowed with any limited measurements recorded. Surface soil was then replaced. The soil samples were taken from all areas where seed was collected, and kept in separate bags for later use. Samples were taken in accordance with Seed Collection Guidelines (Royal Botanical Gardens Kew, 2002).

Figure 5. Topsoil sampling under *Lotus corniculatus* growth

6.2.2 Soil Chemical and Physical Analysis

Six of the 20 soil sample replicates covering each site were sieved and air dried. To ensure chemical analysis was represented over the whole range where the 20 samples per site were collected the same soil sample range was used for each ecotype (sample nos. 2, 5, 8, 11, 14, 17), this numbering ensured even and comparable coverage. The only exceptions to this were Folly Farm and Berrow Dunes where fewer seed samples were found for collection (due to low amount of separate populations found at these sites) and therefore the corresponding soil samples used were no’s. 2, 4, 6, 8, 10,

12. The prepared soils were then used for chemical (Figure 6) and particle analysis (Figure 7) as outlined in Table 2.

Figure 6. Filtering soils through Whatman No.2 filter paper to decolourise with charcoal for phosphate analysis

Figure 7. Soils after addition of hydrogen peroxide during particle analysis

Table 2. Methods of soil chemical and particle analysis used to investigate soil samples

Soil Variable	Method	Reference
Organic Matter	By calculating percent weight loss, after heating in a Muffle furnace at 500°C for 6 hours.	O'Hare, 1990; Rowell, 1994
Conductivity	Extracted with deionised water using a calibrated conductivity meter probe.	MAFF, 1986
pH	Determined by deionised water extraction, using a calibrated pH meter probe.	MAFF, 1986
Phosphate ***(Used on soil with pH <7.4)***	Extracted using Morgan's Solution and decolourised using charcoal. Phosphate determined using a 400nm wavelength spectrophotometer to calculate light absorbance of the solution mixed with vanadomolybdate reagent. Absorbencies are compared on a curve of phosphate standards prepared using the same method.	MAFF, 1986; Tintometer Ltd, undated
Phosphate ***(Used on soil with pH 7.4 and above)***	Extracted using sodium bicarbonate extraction (Olsen Method) and decolourised using charcoal. Phosphate is determined by using a 400nm wavelength spectrophotometer to calculate light absorbance of the solution mixed with vanadomolybdate reagent. Absorbencies are compared on a curve made from standards of phosphate prepared using the same method.	MAFF, 1986
Nitrate	Extracted with saturated calcium sulphate solution and determined by adding ionic strength adjusting buffer then using a selective nitrate ion electrode. The electrode is first calibrated using standards of known nitrate concentrations prepared in the same way. Results are multiplied by 2.5.	MAFF, 1986
Sodium	Extracted with ammonium nitrate. Concentrations are then determined using an Atomic Absorption Spectrophotometer, first calibrated using known concentrations of sodium. Results are multiplied by five.	MAFF, 1986
Potassium	Extracted with ammonium nitrate. Concentrations are then determined using an Atomic Absorption Spectrophotometer, first calibrated using known concentrations of potassium. Results are multiplied by five.	MAFF, 1986
Calcium	Extracted using Hydrochloric Acid extraction with calcareous soils diluted. Concentrations are determined using an Atomic Absorption Spectrophotometer, first calibrated using known concentrations of calcium. Results are multiplied by the original dilution amount.	MAFF, 1986; Rowell, 1994
Particle Size	Analysis by wet sieving through a series of mesh sizes (>500µm, 250µm, 63µm) after removing organic matter with hydrogen peroxide, and dispersing soil with sodium hexametaphosphate. Percent's are calculated from dry weights of sieved soil fractions compared to original dry soil weight (of sediment and organic matter).	Rowell, 1994

6.2.3 Data Analysis

All parameters were first analysed with box charts and line graphs in Excel version 15.0.4551.1005 (Microsoft, 2013) to highlight particular patterns. R statistical software version 3.1.0 (R Core Team, 2013) was used to generate histograms for determining the dataset distributions.

Differences in soil results were non-parametric so were examined for significance using a critical U value table (Hole, 2011) with figures generated using the Mann-Whitney U formula, in Excel (Microsoft, 2013 version 15.0.4551.1005).

Data was entered into 'Multi-Variate Statistical Package' (Kovach, 2006), ordinated in Principle Components Analysis (PCA) and a scatter graph generated from this.

6.3 Ecotype Soil Results

6.3.1 All Ecotype Soils

Soil sample results from the 15 ecotype sites were analysed, raw data for these are shown in Appendix III. Mean soil results from the nine ecotype sites used are summarised in Table 3. Most obvious distinctions include; Highest pH found in the calcareous sand and calcareous loam sites, with the highest at Woolacombe Warren (pH 8.01), and lowest at Folly Farm (pH 6.24). Highest nitrate and potassium levels were found in the neutral and calcareous loam sites with exception of Woolacombe Warren which had highest nitrate result throughout (29.08ppm). Neutral sites generally contained the lowest amounts of phosphate and calcium, and calcareous sand sites had the lowest percentage of organic matter. The low pH values for Hellenge Hill and Salisbury Plain were contrary to the limestone and chalk bedrocks which these lie on.

Table 3. Summary of soil chemical properties of ecotype samples (*n=6*). Grouped by main soil types. Ecotype key; cd - Cockey Down, ssc - Southstoke cut, wb - Woodborough Hill, ssg - Southstoke grazed, fm - Fontmell, ff - Folly Farm, hh - Hellenge Hill, sp - Salisbury Plain, bh - Burledge Hill, stc - St. Catherine's, vs - Valley of Stones, bd - Berrow Dunes, ww - Woolacombe Warren, dw - Dawlish Warren, gc - Goblin Combe.

		Calcareous loam					Neutral loam					Neutral sandy loam	Calcareous sand			Limestone heath
		cd	**ssc**	**wb**	**ssg**	**fm**	**ff**	**hh**	**sp**	**bh**	**stc**	**vs**	**bd**	**ww**	**dw**	**gc**
pH	**Median**	**7.47**	**7.43**	**7.41**	**7.41**	**7.54**	**6.24**	**6.50**	**6.74**	**5.56**	**5.57**	**5.27**	**7.42**	**8.01**	**7.69**	**7.55**
	Min	7.39	7.33	7.37	7.35	7.38	5.50	5.96	6.34	5.45	5.31	4.96	7.29	7.61	7.42	7.40
	Max	7.60	7.49	7.48	7.49	7.61	7.18	6.87	7.38	6.65	5.88	5.41	7.49	8.08	7.96	8.08
Cond. mS m^{-1}	**Mean**	**0.76**	**0.53**	**0.69**	**0.67**	**0.57**	**0.24**	**0.64**	**0.41**	**0.47**	**0.29**	**0.14**	**0.42**	**0.40**	**0.13**	**0.67**
	SE	0.02	0.01	0.02	0.02	0.01	0.03	0.05	0.02	0.04	0.02	0.01	0.02	0.05	0.01	0.02
N ppm	**Mean**	**18.05**	**19.96**	**18.06**	**8.02**	**25.07**	**19.99**	**17.74**	**14.89**	**17.91**	**14.31**	**7.05**	**10.80**	**29.08**	**6.12**	**18.20**
	SE	2.89	3.83	3.02	1.14	7.35	3.32	1.40	2.18	2.82	3.96	0.36	1.47	3.58	1.69	1.76
P ppm	**Mean**	**21.82**	**21.75**	**31.13**	**28.12**	**16.65**	**4.23**	**11.81**	**3.75**	**11.74**	**5.32**	**2.86**	**30.85**	**10.80**	**3.02**	**22.43**
	SE	4.38	12.22	4.27	4.42	2.69	0.60	0.90	0.19	1.23	0.54	0.19	3.54	5.87	0.76	3.67
OM %	**Mean**	**20.47**	**22.55**	**18.92**	**24.28**	**23.10**	**13.82**	**23.68**	**21.20**	**25.20**	**31.57**	**12.38**	**6.75**	**2.78**	**5.04**	**19.23**
	SE	1.15	0.72	1.05	0.44	1.26	0.75	0.93	1.08	0.56	2.48	0.30	0.69	0.79	1.31	0.66
K ppm	**Mean**	**49.33**	**191.77**	**73.42**	**131.03**	**45.78**	**128.39**	**218.47**	**99.14**	**251.93**	**121.93**	**58.33**	**54.84**	**37.36**	**52.73**	**118.33**
	SE	3.29	24.54	6.13	11.16	4.26	11.47	24.56	17.42	37.96	8.13	6.45	11.49	3.82	9.22	18.26
Na ppm	**Mean**	**20.37**	**22.78**	**13.77**	**26.31**	**44.50**	**27.07**	**21.50**	**31.11**	**39.58**	**45.28**	**37.61**	**17.71**	**28.19**	**38.75**	**20.91**
	SE	2.82	1.56	0.83	2.27	6.50	1.32	0.72	2.09	1.80	4.30	2.39	0.89	2.48	4.78	1.19
Ca ppm	**Mean**	**134544.27**	**44543.84**	**158887.48**	**75057.26**	**85855.26**	**3965.45**	**3812.48**	**7476.04**	**4407.02**	**3306.84**	**634.96**	**26494.41**	**36536.30**	**65261.50**	**76161.63**
	SE	20608.87	6887.39	12069.30	4203.68	14667.88	1632.70	869.67	2991.41	1111.87	522.57	170.06	1285.75	1742.27	26524.95	24701.30

Particle analysis results were plotted into a 100% stacked column barchart to illustrate each soil texture profile (Figure 8). Organic matter percent from hydrochloric acid removal was replaced by the original organic matter results (Table 3) due to inconsistencies noticed with the results from the removal method.

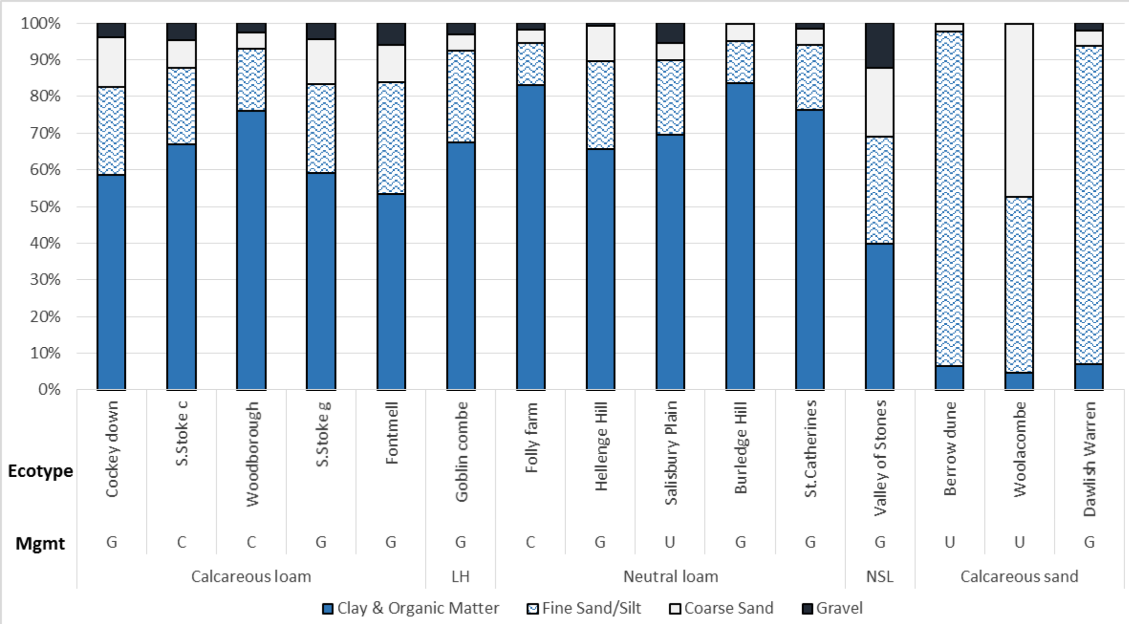


Figure 8. Particle analysis of ecotype soils shown in 100% stacked column, grouped by main soil type order. Taken from means of six replicates per sample. Management key: G - grazing, C - cut, U - unmanaged. In the soil type split, LH is limestone heath is and NSL is neutral sandy loam.

The calcareous loams (Cockey Down to Fontmell) in Figure 8 showed similar characteristics. The neutral sites (Folly Farm to Valley of Stones) were also similar but contained more clay and organic matter content. Goblin Combe (limestone heath) showed most similarity to the neutral loams. The calcareous sands (Berrow Dunes to Dawlish Warren) showed large dissimilarity to the others with very low clay and organic matter content and high amounts of fine sand and silt content. Woolacombe Warren sand portion was split almost in half with 47.05µm of coarse sand and 47.95µm fine sand/silt.

Soil chemical analysis of all ecotypes was entered into MVSP and Principle Components Analysis (PCA) generated. Data had to be standardised and centred to prevent an x-axis linear effect.

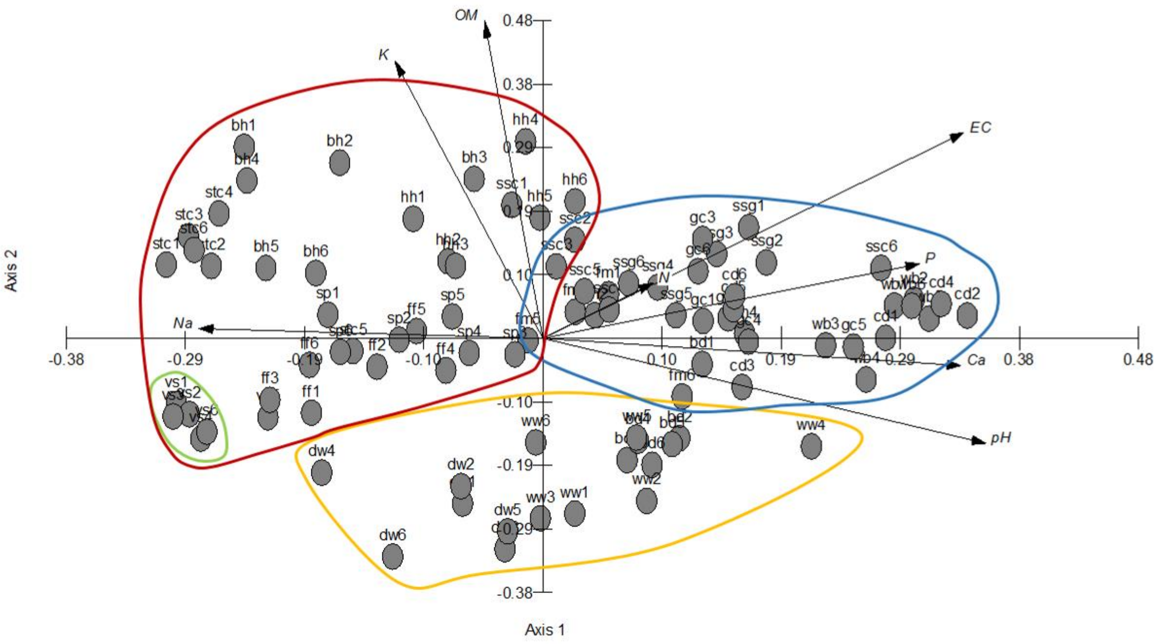


Figure 9. PCA scatter graph of all ecotype soil chemical analysis. Red outline around neutral ecotypes, blue outline around calcareous loam/ calcareous heath ecotypes and yellow outline around calcareous sand ecotypes. The green outline is to highlight Valley of Stones which is discussed. Ecotype Key: bd - Burrow Dunes, bh - Burledge Hill, cd - Cockey Down, ff - Folly Farm, fm - Fontmell, gc - Goblin Combe, hh - Hellenge Hill, sp - Salisbury Plain, ssg/c - Southstoke grazed/cut, stc - St.Catherine's, vs - Valley of Stones, wb - Woodborough Hill, ww - Woolacombe Warren

The PCA grouped the main soil types together as shown by the coloured outlines. Valley of Stones (neutral sand), outlined in green was located close to the calcareous sand ecotypes. There was also an overlap between Southstoke (cut) and Hellenge Hill. Only nitrate had a short vector, indicating this variable had the lowest influence over the ecotypes. Conductivity and pH had the longest vectors, as pH was one of the main influences in initial choosing of different soil type sites it was thought that the removal of this would be of interest to the subsequent grouping of sites therefore this was removed and the PCA repeated as shown in Figure 10.

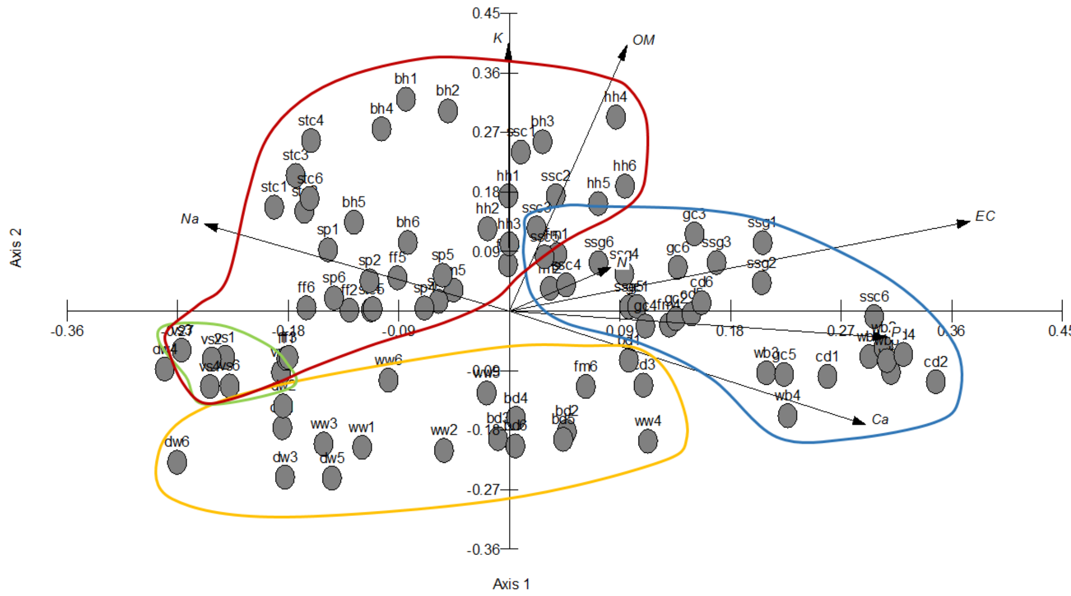


Figure 10. PCA scatter graph of all ecotype soil chemical analysis with pH removed. Red outline around neutral ecotypes, blue outline around calcareous loam/ calcareous heath ecotypes and yellow outline around calcareous sand ecotypes. The green outline is to highlight Valley of Stones which is discussed. Ecotype Key: bd - Burrow Dunes, bh - Burledge Hill, cd - Cockey Down, ff - Folly Farm, fm - Fontmell, gc - Goblin Combe, hh - Hellenge Hill, sp - Salisbury Plain, ssg/c - Southstoke grazed/cut, stc - St.Catherine's, vs - Valley of Stones, wb - Woodborough Hill, ww - Woolacombe Warren

Even with the pH removed in Figure 10, the main soil types of neutral, calcareous loam/calcareous heath and calcareous sand were still grouped together. Valley of Stones (neutral sand) was again located close to the calcareous sand ecotypes, this could be due to particle analysis similarities as shown in Figure 8. Goblin Combe (calcareous heath) was still located within the calcareous loams. Most vectors are of similar length and therefore influence, with conductivity slightly longer. Nitrate is again the shortest vector and thought to have been the element of least influence.

6.3.2 Chosen Ecotype Soils

From soil results and seed germination (Chapter 7) 9 of the 15 ecotypes were chosen for the main study. Differences between the chosen ecotype soils were compared to establish whether any of those identified from Table 3 were significant. As the data were not normally distributed non-parametric Mann-Whitney U tests (Table 4) were performed. Each of the key soil types varied significantly in pH and calcium. Additionally, the sand soils had significantly lower organic matter and potassium than the loams. Only nitrate levels were found to be not significantly different.

Table 4. Mann-Whitney U-Test of chosen ecotype soil results. These were generated from results of six replicates from each sample used in the glasshouse study. Key soil types were compared against each other. Ecotype key: bd - Burrow Dunes, cd - Cockey Down, ff - Folly Farm, hh - Hellenge Hill, sp - Salisbury Plain, ss - Southstoke, wb - Woodborough Hill, ww - Woolacombe Warren. As multiple tests were carried out, the significance level was decreased to <0.01. A sample size of *n*=18 was used, therefore the critical U-value that significant results (<0.05) will be equal or lower than is 81 (Hole, 2011), such values have been shown in bold underlined.

Compared	Neutral loam ff,hh,sp Compared	Calcareous loam cd,ss,wb	Neutral loam ff,hh,sp	Calcareous sand bd,ww,dw	Calcareous loam cd,ss, wb	Calcareous sand bd,ww,dw
pH	320	**<u>4</u>**	323	**<u>1</u>**	249	**<u>75</u>**
Conductivity	273	**<u>51</u>**	110	214	**<u>15</u>**	210
Nitrate	163	161	114	210	114	210
Phosphate	289	**<u>35</u>**	176	148	95	229
OM	179	145	**<u>1</u>**	323	**<u>0</u>**	324
Potassium	91	233	**<u>29</u>**	295	**<u>52</u>**	272
Sodium	**<u>60</u>**	264	155	169	231	93
Calcium	323	**<u>1</u>**	293	**<u>31</u>**	**<u>50</u>**	274

Data was re-entered into MVSP with PCA conducted for only those nine ecotypes chosen as shown in Figure 11. This clearly groups the three main soil types, however, there is an overlap shown between Southstoke and Hellenge Hill. The most influential element appears to have been conductivity with the calcareous sand soils at right angles to the negative end of this vector. Organic matter and sodium are the next in length and the rest of the vectors are of similar length except nitrate, again being the shortest and therefore thought to have been least influential

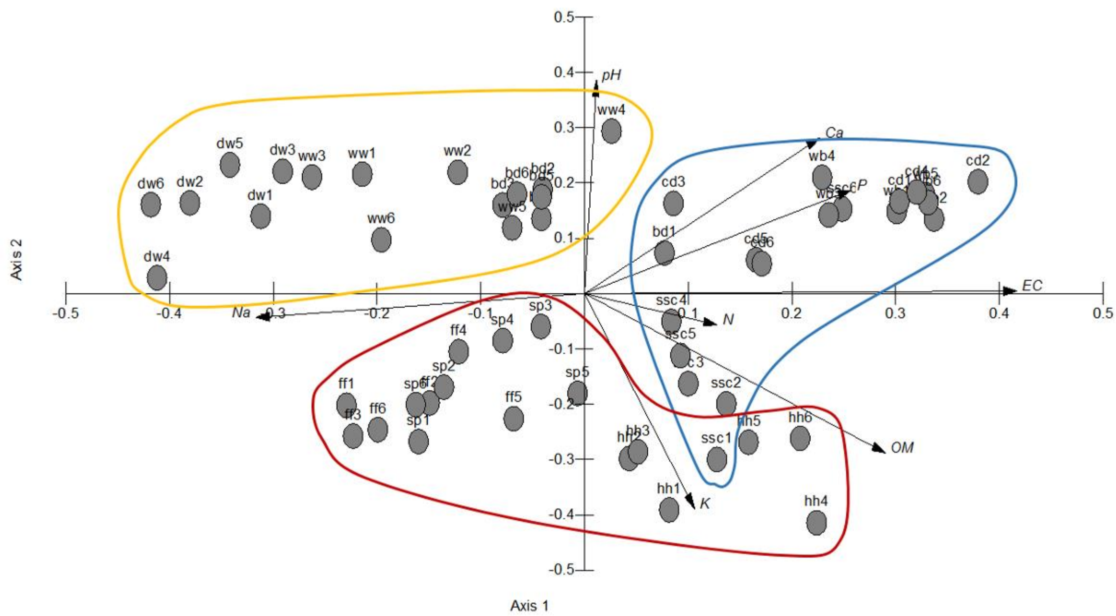


Figure 11. PCA scatter graph of chosen ecotype soils. Red outline around neutral loam ecotypes, blue outline around calcareous loam ecotypes and yellow outline around calcareous sand ecotypes. Ecotype Key: bd - Burrow Dunes, cd - Cockey Down, dw – Dawlish Warren, ff - Folly Farm, hh - Hellenge Hill, sp - Salisbury Plain, ssc – Southstoke cut, wb - Woodborough Hill, ww - Woolacombe Warren

6.4 Ecotype Soil Discussion

Analysis of soils from ecotype sites (Table 3) showed consistent results in key parameters with calcium levels generally rising with that of pH (Spectrum Analytic, undated) and higher organic matter correlating with higher potassium and nitrate, often the two macro-nutrients most easily leached (Rosen *et al.,* 2008). There was some unexpected variation however, for example the higher nitrate content of the Woolacombe Warren ecotype calcareous sand soil (mean=29.08ppm). One consideration due to inconsistent nitrate levels within Woolacombe Warren soil (smallest=16.59ppm) (Appendix III), is the replicates with highest nitrate (38.79ppm and 40.82ppm) may have received recent urea, [from urine] due to the beach's popularity with dog walkers (Self & Waskon, 2011). Urea quickly changes into ammonium in the soil. Unlike nitrate (NO_3 -), nitrogen in the soil in the form of ammonium (NH_4^+) does not leach easily, and acts as other cations, being adsorbed onto soil particles (Killpack & Buchholz, 1993).

The low pH of Salisbury Plain and Hellenge Hill was inconsistent with expectations of underlying chalk and limestone bedrock respectively. The area sampled of Salisbury Plain was in a valley bottom and once possibly part of a river system, therefore alluvial drift may have neutralized the soil (Ahmad, 2011). Parts of Hellenge Hill have been partially agriculturally-improved historically (Avon Wildlife Trust, 2001) which therefore may have altered the natural pH.

Particle analysis means showed similar patterns throughout the calcareous loam and neutral loam sites (Table 8 and Figure 2) due to their clay-loam composition, with higher amounts of <63µm organic matter and clay, the highest percent of which were found in the neutral sites. The three calcareous sand ecotypes differed in that almost half of Woolacombe Warren (47%) was coarse sand whereas 91% of both Berrow and Dawlish consisted of finer sand particles with the addition of a slightly higher amount of organic matter, showing these two sites may be at a further successional stage (McBride & Stone, 1977).

Although the PCA scatter graphs grouped soil types clearly, there was some overlap, in Figure 9 and Figure 10 this was shown with Valley of Stones 'neutral sandy loam' spanning between neutral sites and calcareous sand sites, closest to Dawlish Warren. Conductivity was thought to be a high influence on arrangement of these scatter graphs, the level of which was seen to have been the lowest for these two sites (vs = 0.14 mS m^{-1}, dw= 0.13 mS m^{-1}). There have been links made in past studies with soil conductivity and Cation Exchange Capacity (CEC) (Ouhadia & Goodarzi, 2007), which can be considered with these results; The low amount of smaller particles (clay and organic matter) in Valley of Stones and the calcareous sands, [seen from particle analysis results] would influence a reduced CEC of these soils, by providing less negatively charged particles (anions), thereby reducing the attraction of positively charged ions (cations) (Ketterings *et al.,* 2007), as reflected in the similarly low potassium content of Valley of Stones (58.33ppm) and calcareous sand sites, and noticeably low calcium content of Valley of Stones (634.96ppm). It could be argued that Valley of Stones had a higher organic matter content than the calcareous sand sites and therefore conductivity similarity is not linked, however CEC associated with organic matter is pH dependent (Ketterings *et al.,* 2007). Therefore, as Valley of Stones had the lowest pH and organic matter content of the neutral sites, this would have contributed to a lower CEC, possibly more similar to capacity of calcareous sands rather than the neutral loams. It has been found that anion type in soils with low CEC is an important factor in soil conductivity (Ouhadia & Goodarzi, 2007), low amounts of the anions nitrate and phosphate in Dawlish Warren and Valley of Stones may have therefore contributed to their similar conductivity (Ouhadia & Goodarzi, 2007).

The other overlap in the PCA scatter graphs was between the calcareous loam site (Hellenge Hill) and the neutral loam site (Southstoke). Both of these sites held the highest organic matter contents of their soil groups, Hellenge Hill, had a conductivity amount more relative to the other calcareous loam sites than neutral and Southstoke had a higher potassium, similar to the neutral loam sites. Figure 8 also illustrated the similarity between these two sites in their particle analysis.

7 ECOTYPE SEED (Donor Site)

Please note in this chapter cut with aftermath grazing is referred to as ‘cut’. There is no shortened reference to calcareous sand in this chapter due to additional discussion of other sand based soil sites.

7.1 **Seed Preparation and Sowing Methodology**

7.1.1 Seed Preparation

An autumn sowing was decided upon as *Lotus corniculatus* can germinate in both spring and autumn (Jones & Turkington, 1986). It has been found that spring germination is more successful due to the cold stratification seed receives over winter followed by alternating temperatures, allowing water to permeate the hard seed coat (Van Assche *et al.*, 2003). However, an autumn sowing was chosen for two reasons. The collected seeds had already received a cold stratification, it was thought this would help seeds germinate successfully in the autumn planting. In addition, a slow growth in the glasshouse over winter was thought to be beneficial to giving a longer season within the pots and controlled minimum temperatures would ensure no mortality from winter kill, found to vary throughout *L.corniculatus* genotypes (Conje, (PhD thesis) 1971 *cited in* Jones & Turkington, 1986).

It was initially decided that seed from 13 of the 15 ecotype sites would be sown (later in the study this choice was refined to 9 sites). Two ecotypes were excluded from the experiment. The first exclusion was Goblin Combe due to this site having an unusual soil type compared to other sites sampled. Fontmell was also disregarded due to its close proximity to one of the treatment soil sites (Chapter 9) as well as being the site with lowest number of viable seed collected.

Seed was scoured between fine (Grade 1) sandpaper (Figure 12) to aid germination (Brewer, 1947; Cornelissen *et al*., 2011). Scarification was achieved prior to weighing of the seed (Figure 13) to eliminate risk of hollow seeds causing anomalies in resulting weights. Weighing was standardised by a collective weight measure of 10 seeds per sample and replicated by carrying out the procedure on 10 samples per ecotype.

Figure 12. Scarification of seed with sandpaper

Figure 13. Scarified seeds ready to be weighed

7.1.2 Seed Sowing

The glasshouse was prepared by cleaning all vegetation from the floor and erecting staging. Capillary matting was cut down to fit the staging tops with excess left to feed into water reservoirs ensuring matting would stay damp. On 16th September 2011 seed from the 13 ecotype sites were sown. From each ecotype 10 samples (from 10 plant clumps) out of the 20 samples collected were chosen to generate 10 ecotype replicates. Chosen samples needed to have at least 18 viable seeds. To get a representation across the whole area sampled for each ecotype, it was decided that most desirable samples to pick would be no’s 2, 4, 6, 8, 10, 12, 14, 16, 18, 20. However some of these samples had less than 18 seeds and therefore an alternative sample would be picked. Table 5 outlines which sample numbers were used in the initial sowing for each ecotype out of the 20 collected. At least 18 seeds per sample were used, giving a total of over 2340 seeds in the initial sowing.

Table 5. Ecotype samples used in initial seed sowing, in alphabetical order of ecotype.

	Ecotype sample number used to make Ten seed sowing replicates (r.)									
	r.1	**r.2**	**r.3**	**r.4**	**r.5**	**r.6**	**r.7**	**r.8**	**r.9**	**r.10**
Berrow Dunes	2	4	6	7	8	10	11	12	13	14
Burledge Hill	2	4	5	8	12	14	20	22	24	26
Cockey Down	2	4	6	8	10	12	14	16	18	20
Dawlish Warren	1	2	3	6	8	9	10	12	13	17
Folly Farm	1	2	3	4	5	6	7	8	10	12
Hellenge Hill	4	6	8	9	10	12	14	16	18	20
Salisbury Plain	2	4	6	8	10	12	14	16	18	20
Southstoke cut	2	4	5	8	10	12	14	16	18	20
Southstoke grazed	2	4	6	8	10	12	14	16	18	20
St. Catherine's	2	4	6	8	10	12	14	16	18	20
Valley of Stones	2	4	6	8	10	12	13	14	16	17
Woodborough Hill	2	4	6	8	10	12	14	16	18	20
Woolacombe Warren	2	4	6	8	10	12	14	16	18	20

Sample Seeds were sown in modular potting trays of 1:1 Compost (70% bark; 20% peat; 10% loam) (Dean, undated): perlite (aluminosilicate mineral) (Figure 14). Pot-grown plants can encounter problems with soil collapsing, causing poor aeration and drainage problems (Schnelle & Henderson 2007), perlite was therefore used to reduce this risk by increasing air filled porosity (Bragg & Chamber, 1988) and maintaining good water retention, without altering chemical composition of the soils (Perlite.info, 2004).

Although most viable non-hard seed was found to germinate in four days by Stickler & Wassom (1963 *in* Jones & Turkington, 1986), it can take between two to four weeks in correct temperatures (seedaholic.com, undated). To ensure germination was counted only for *L. corniculatus* and not any volunteer species [from seed inadvertently collected in treatment soils], seedlings of at least two weeks old were counted as these could be identified from botanical descriptions (Muller, 1978 *in* Jones & Turkington, 1986; Stace, 1997; Rose, 2006) therefore germination was recorded 18 days after sowing (Figure 15). More seed was added at this time (4th October 2011) in order to ensure full complements of seedlings for each ecotype used.

Figure 14. Sown seeds in prepared seed trays, with vermiculite covering in the glasshouse 16th September 2011

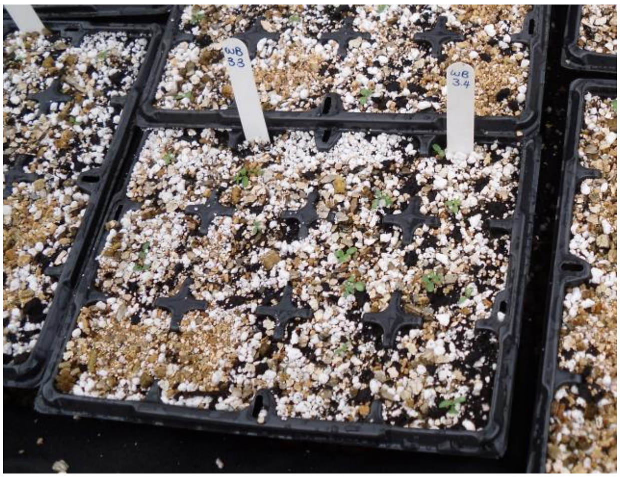


Figure 15. First seedlings of Woodborough Hill 4th October 2011

7.1.3 Data Analysis

All parameters were first tabulated and analysed with box charts and histograms in Excel (Microsoft, 2013 version 15.0.4551.1005) to highlight particular correlations and differences to further compare and establish whether data was normally distributed.

Seed weight was then plotted with germination on a scatter plot with the correlation co-efficient measured to identify if there were correlations between the two.

From results of the histograms in excel, differences in both sets of results were found to be non-parametric so were examined for significance using a critical U value table (Hole, 2011) with figures generated using the Mann-Whitney U formula, in Excel (Microsoft, 2013 version 15.0.4551.1005).

7.2 **Seed Weight and Germination Results**

Dry seed from 13 of the 15 Ecotypes were weighed after scarification, weights were obtained from 10 seeds per replicate (Appendix IV). Figure 16 illustrates seed weights were similar throughout with no particular management routine or soil type indicating effects on weight. However, Cockey Down, Southstoke grazed and Burledge Hill seed are all significantly heavier than the other neutral and calcareous loam ecotypes. Both the highest and lowest means were found to be in the neutral soil ecotypes, the three highest weights were ecotypes with grazing management.

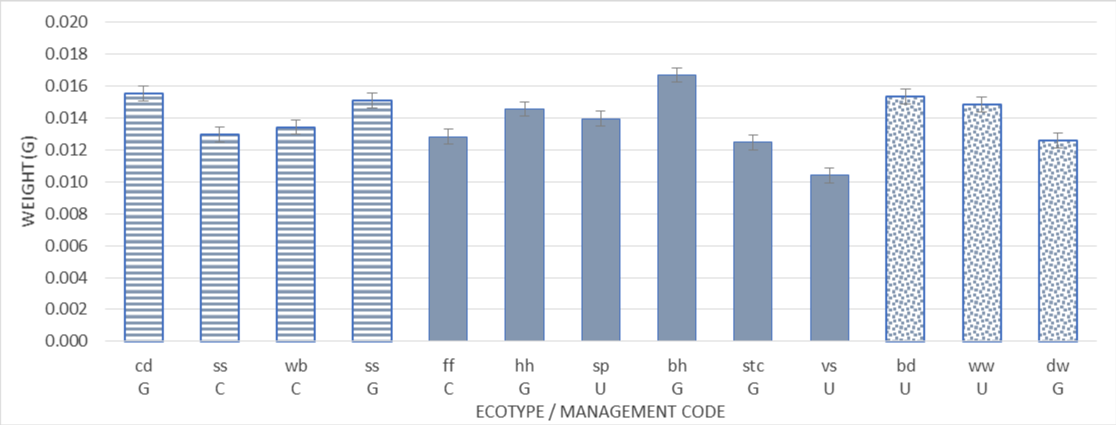


Figure 16. Mean seed weights (± 1 SE), (n = 10), grouped by soil type. Ecotype key: cd - Cockey Down, ssc - Southstoke cut, wb - Woodborough Hill, ssg - Southstoke grazed, ff - Folly Farm, hh - Hellenge Hill, sp - Salisbury Plain, bah - Burledge hill, stc - St.Catherine's, vs - Valley of Stones, bd - Berrow Dunes, ww - Woolacombe Warren, dw - Dawlish Warren. Soil key: horizontal lines - calcareous loam, solid fill - neutral loam, dots - calcareous sand. Management key: G – grazed, C - cut, U - unmanaged.

Germination of 18 seeds per replicate are tabulated in Appendix IV, the total number of seeds sown per ecotype was therefore 180.

Figure 17 illustrates the large variation between ecotype germination success with Hellenge Hill coming out significantly higher (mean of 11) than all except St. Catherine's, and Valley of Stones having a significantly lower germination (mean of 2) than the rest. Calcareous sand ecotypes all have lower germination compared to the rest of the ecotypes and the unmanaged ecotypes also all have lower figures. Interestingly, the calcareous sand ecotype with grazed management has slightly higher germination than the unmanaged calcareous sand ecotypes.

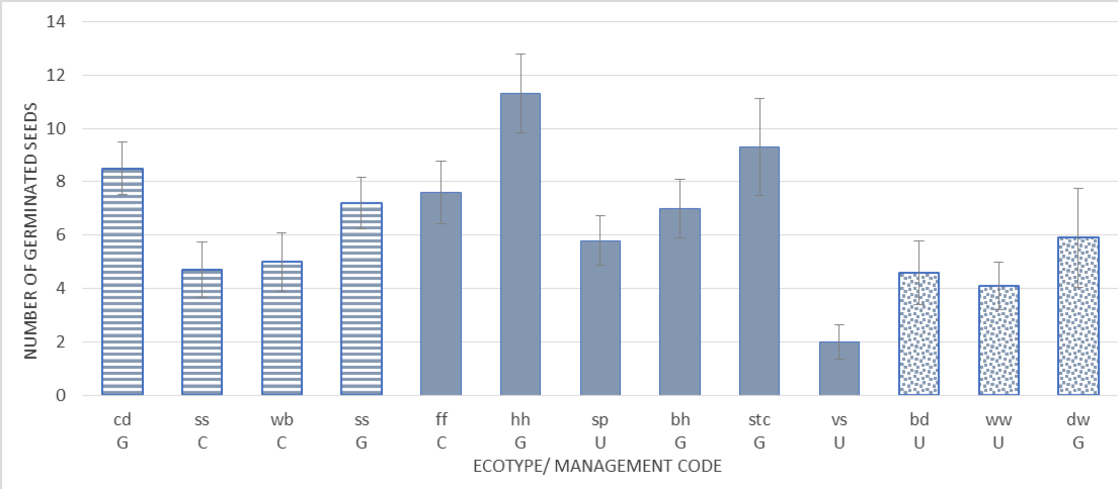


Figure 17. Mean germination counts (+ 1 SE) after 18 days, from 18 seeds sown per sample, grouped by soil type. Ecotype key: cd - Cockey Down, ssc - Southstoke cut, wb - Woodborough Hill, ssg - Southstoke grazed, ff - Folly Farm, hh - Hellenge Hill, sp - Salisbury Plain, bah - Burledge hill, stc - St.Catherine's, vs - Valley of Stones, bd - Berrow Dunes, ww - Woolacombe Warren, dw - Dawlish Warren. Soil key: horizontal lines - calcareous loam, solid fill - neutral loam, dots - calcareous sand. Management key: G – grazed, C - cut, U - unmanaged.

A very weak correlation ($R^{2} = 0.1235$) was found when seed weight and germination were plotted together in a scatter graph (Appendix IV), suggesting there is no relationship between seed weight and germination for samples in this study. To find out if this was the same for all individual ecotypes the analysis was carried out for each. R^2 values are tabulated in Table 6.

Table 6. Correlation R^2 value of mean dry seed weights (g) (taken from 10 seeds per ecotype replicate) with germination mean (taken from 18 seeds per ecotype replicate), for each individual ecotype. Soil codes refer to C=calcareous loam, N=neutral, S=calcareous sand. management refers to G=grazed, U=unmanaged.

Ecotype	Soil	Management	R^2
Cockey Down	C	G	0.2914
Southstoke cut	C	C	0.1226
Woodborough Hill	C	C	0.1541
Southstoke grazed	C	G	0.0100
Folly Farm	N	C	0.3612
Hellenge Hill	N	G	0.0124
Salisbury Plain	N	U	0.0256
Burledge Hill	N	G	0.1343
St.Catherine's	N	G	0.7197
Valley of Stones	N	G	0.0066
Berrow Dunes	S	U	0.0144
Woolacombe Warren	S	U	0.0009
Dawlish Warren	S	G	0.1003

Table 6 indicated that only St.Catherine's ecotype showed a positive correlation between mean dry seed weight and germination (R=0.7197), the other ecotypes have very low R^2 values indicating there was no relationship.

7.3 Seed Weight and Germination Discussion

There were similarities between ecotypes; Cockey Down and Hellenge Hill were both within the highest seed weights (Figure 17) and germination rates (Figure 16). The highest mean germination over-all (germination means from 18 seeds sown per sample) was Hellenge Hill (11.3 seeds). The only ecotype which had a positive correlation between seed weight and germination was St.Catherine's (R^2=0.7197), however, the very weak regression of the other sites (Table 6) and weak regression over-all (**Error! Reference source not found.**) (R^2=0.1235) eliminated the idea of a correlation.

The most evident observation was for that of lowest mean seed weight (0.010g) and mean number of seed germination (2 out of 18) for Valley of Stones which could have been due to poorer quality or unripe seed. Mean germination was shown to be generally lower for the calcareous sand ecotype samples (4.6, 4.1, 5.9), possibly a factor of harder seed coatings as an adaption to harsher environmental conditions at these sites such as desiccation and abrasion (Yasseen *et al.,* 1994; Maun, 2009; Zhu *et al*., 2014).

8 FINAL ECOTYPE CHOICE

Please note in this chapter cut with aftermath grazing is referred to as ‘cut’. There is no shortened reference to calcareous sand in this chapter due to additional discussion of other sand based soil sites.

8.1 Overview of Ecotype Selection

Following analysis of donor site soils and other ecotype site characteristics for all ecotypes sampled, three ecotypes were selected to represent each major soil type and a mix of the three different management regimes. Ecotypes were selected such that similar soil types and/or management regimes would be as geographically distant from each other as possible. Therefore the ecotypes initially selected for experimentation were Cockey Down (grazed), Southstoke (cut) and Woodborough Hill (cut) for calcareous loam, Folly Farm (cut), Salisbury Plain (unmanaged) and Valley of Stones (grazed) for neutral, and Berrow Dunes (unmanaged), Woolacombe Warren (unmanaged) and Dawlish Warren (grazed) for calcareous sand. It was thought that although the results of Principle Components Analysis (PCA) placed Southstoke (calcareous loam) close to neutral loam ecotypes and Valley of Stones (neutral sand) close to the calcareous sand ecotypes, results from these ecotypes would be of interest, especially if particular soil parameters showed more influence than the overall soil-type groupings.

The experimental design (Chapter 11) required 48 seedlings per ecotype (site), and within these ecotypes, 6 seedlings from each of the 8 samples (within the ecotypes) collected were necessary for replication. Due to a high failure rate of germination for the Valley of Stones ecotype (neutral sand) this was replaced by Hellenge Hill, the composition of the neutral soil at this site led to the whole neutral soil category being renamed as ‘neutral loam’. This site was less desirable due to a shortened geographical distance to the other neutral sites. However, it was thought adequate and as this ecotype was also grazed there were no further changes needed in the selections. Details of final selection ecotypes are shown in Table 7, along with soil chemical analysis summary (Chapter 6) as a quick reference guide, detailed information and discussion for each chosen site are in Appendix V.

Table 7. Quick reference guide to chosen ecotypes, according to donor site soil type. This will be the order in which they are shown throughout the remainder of the document. NCA is National Character Area. BR is Biosphere Reserve.

Name Location	Ref. Used in text	OS Grid ref.[1]	Metres above sea-level[1]	Statutory Desig-nation[1]	NCA[1]	Vegetation Characteristic[2]	Topsoil	Parent Bedrock Group[3]	Management[4]	Seed & Soil Collection Date	Soil chemical summary statistics (quick ref. guide only). Standard Errors in Table 3. Values are means except pH which are medians.							
											pH	Cond. Ms	N ppm	P ppm	OM %	K ppm	Na pm	Ca pm
Cockey Down Salisbury, Wilts	**cd**	SU17 3320	120	SSSI	132	Calcareous unimproved short sward (CG2/3)	Calcareous loam	Chalk	Grazed	10/09/09	7.47	0.76	18.05	21.82	20.47	49.33	20.37	134544
Southstoke Bath, B&NES	**ss**	ST73 7610	54	None	107	Calcareous semi-improved grassy sward	Limestone loam	Great Iolite	Cut [& aftermath grazing]	04/09/09 21/09/09	7.43	0.53	19.96	21.75	22.55	191.77	22.78	44543
Woodborough Hill Alton Barnes, Wiltshire	**wb**	SU11 7614	190	N/A	116	Calcareous semi-improved grassy sward	Calcareous loam	Chalk	Cut [& aftermath grazing]	06/09/09	7.41	0.69	18.06	31.13	18.92	73.42	13.77	158887
Folly Farm Somerset	**ff**	ST61 1606	140	SSSI	118	Mesotrophic semi-improved gassy sward	Neutral loam	New Red Sandstone	Cut [& aftermath grazing]	25/09/09	6.24	0.24	19.99	4.23	13.82	128.39	27.07	3965
Hellenge Hill North Somerset	**hh**	ST34 5571	160	SNCI	141	Mesotrophic semi-improved grassy sward	Neutral loam	Pembroke Limestone	Grazed	15/09/09	6.50	0.64	17.74	11.81	23.68	218.47	21.50	3812
Salisbury Plain (Bulford Down) South Wiltshire	**sp**	SU19 2481	100	SSSI/ SAC/ SPA	132	Mesotrophic unimproved tall sward (MG1)	Neutral loam	Chalk	Unmanaged	29/09/09	6.74	0.41	14.89	3.75	21.20	99.14	31.11	7476
Berrow Dunes Somerset coast	**bd**	ST29 2532	0	LNR/ SSSI	142	Calcareous unimproved *Festuca* dom. sward	Sand [Fixed calcareous dune]	Lisa	Unmanaged (since early C20[th])	15/09/09	7.42	0.42	10.80	30.85	6.75	54.84	17.71	26494
Woolacombe Warren Devon Coast	**ww**	SS45 5426	20	AONB/ BR	145	Calcareous unimproved *Festuca/ Ammo hila* dom. sward	Sand [Fixed calcareous dune]	Aeolian deposits	Unmanaged	02/10/09	8.01	0.40	29.08	10.80	2.78	37.36	28.19	36536
Dawlish Warren South Devon coast	**dw**	SX98 3789	5	SAC/SPA/SSSI NNR	148	Calcareous *Juncos* dominated dune slack	Sand [Fixed calcareous dune]	New Red Sandstone	Grazed	24/09/10	7.69	0.13	6.12	3.02	5.04	52.73	38.75	65261

[1] Magic, 2013. [2]Personal observations, 2009, 2010. [3] NERC, 2013. [4] **cd**; WWT, 2003[b]., **ss**; Thompson, 2009., **wb**; Smart, 2009 & Carson, 2009., **ff**; AWT, 1999., **hh**: AWT, 2001., **sp**; DTE, 2008., **bd**: Sedgemoor District Council, undated., **ww**; North Devon Biosphere, 2011; North Devon Coast AONB, 2013; **dw**; Chambers, 2009., & Grazing Animals Project, 2009.

8.2 **Geographical Distance between Chosen Ecotype Sites**

Locations of the chosen ecotype sites are shown in Figure 18.

[Image redacted in this digitized version due to potential copyright issues]

Figure 18. Map overlaying scatter graph with grid references of chosen ecotype sites plotted. National Character Area boundaries are shown in the background. Shapes indicate management: triangles - unmanaged, circle's - cut, square - grazed. Colours indicate soil type: yellow - calcareous sand, red - neutral loam, blue - calcareous loam. Ecotype Key: cd - Cockey Down, ss - Southstoke, wb - Woodborough Hill, ff - Folly Farm, hh - Hellenge Hill, sp - Salisbury Plain, bd - Berrow Dunes, ww - Woolacombe Warren, dw - Dawlish Warren.

The neutral loam and calcareous loam ecotypes are located closer to an ecotype of dissimilar soil type than they are to a matching soil type. This was more difficult to achieve when selecting calcareous sand ecotypes (Woolacombe Warren and Berrow are located closer to matching rather than unmatching soil sites), however this aim was achieved with the Berrow Dunes ecotype. The same was ideally required for management regimes of ecotypes as well, only Southstoke and Folly Farm differ from this.

Geographic distances have been calculated by Euclidian distances, measured in kilometres between ecotype OS grid references (Smith *et al.,* 2005) (Table 8).

Table 8. Distances between sites (km). Bold text distances indicate the greatest for that ecotype site and blue highlighted indicate the shortest distance.

	OS grid ref	cd	ss	wb	ff	hh	sp	bd	ww	dw
Cockey Down (cd)	SU173320	0	52.36	29.93	63.06	**86.55**	16.21	**90.61**	**172.13**	**130.31**
Southstoke (ss)	ST737610	52.36	0	38	12.61	39.38	47.29	45.18	**129.51**	**111.47**
Woodborough (wb)	SU117614	29.93	38	0	50.61	77.31	15.27	82.91	**167.26**	**140.23**
Folly Farm (ff)	ST611606	63.06	12.61	50.61	0	26.82	59.43	32.75	**116.99**	**103.05**
Hellenge Hill (hh)	ST345572	**86.55**	39.38	77.31	26.82	0	85.19	6.64	**90.19**	**86.26**
Salisbury Plain (sp)	SU192481	16.21	47.29	15.27	59.43	85.19	0	**90.14**	**173.79**	**139.3**
Berrow Dunes (bd)	ST292532	**90.61**	45.18	82.91	32.75	6.64	**90.14**	0	84.37	80.47
Woolacombe (ww)	SS455426	**172.13**	**129.51**	**167.26**	**116.99**	**90.19**	**173.79**	84.37	0	82.74
Dawlish Warren (dw)	SX983789	**130.31**	**111.47**	**140.23**	**103.05**	**86.26**	**139.3**	80.47	82.74	0

As Table 8 shows, although Woolacombe Warren and Dawlish calcareous sand ecotypes could not be located closer to a different soil type ecotype than their own, distance between them is still among one of the furthest (82.74km).

9 SOIL TYPE TREATMENTS (RECEPTOR SITES)

Please note that within this chapter, calcareous sand is referred to as ‘sand’ and cut with aftermath grazing is referred to as ‘cut’.

9.1 Soil Types in Grassland Restoration

Soil type is an important factor for grassland restoration. Generally, low soil fertility is needed for restoration to be successful to reduce the chance of more aggressive species and grasses from out-competing slower to establish herbs (Flower, 2008). As already mentioned in Chapter 6, fast growth of grasses is especially associated with high soil phosphate level (Janssens *et al*., 1998; Buglife, 2012). High phosphates can be a limiting factor in grassland restoration (Gough & Marrs, 1990), as they are slow to decrease after fertilizer application with phosphate ions attaching to the surface of other compounds, such as calcium in alkaline soils (Schulte & Kelling, 1996). Conversely, a study by Smith *et al.* (2000) found differences in species richness on soils which had similarly high phosphate levels. Walker *et al*. (2004[c]) suggested this could mean high phosphate levels only indicate that there has been past fertilizer application rather than it being a limiting factor in itself. Nitrogen is also a limiting factor to species richness but can be seen as less of a problem in restoration due to its ability to leach quickly from soils (Janssens *et al.,* 1998).

Reducing soil nutrients before grassland restoration can involve taking extensive hay cuts and ceasing any fertilizer application (Pegtel *et al.,* 1996). But this is still likely to take a number of years (Walker *et al*., 2004[c]), other techniques include topsoil stripping and deep ploughing to invert the soil profile (Jones, 2010), though differences in success have been found with the latter: Jones (2010) found erosion problems, yet Ödman (2011) found the technique successful in restoring soil chemicals to that of species-rich grassland within two years.

9.2 Treatment Soil Initial Choice

The three basic soil types chosen as treatments were a calcareous loam, neutral loam and sand. In selecting soil donor sites, it was important that these soils corresponded to the range of soil properties recorded for ecotype donor sites. In the initial stages of choosing treatment soil, 'making' soils was considered to achieve a particular pH. However, although this was attempted it proved ineffective [as detailed Chapter 16]. The sites chosen also needed to be representative of typical receptor sites. Table 9 outlines brief details of the soil donor sites initially selected, with a more detailed description of final chosen sites in sub-chapter 9.6.

Table 9. Details of sites initially chosen as soil donor sites

Name Location	Owner/ Contact	OS Grid ref.	NCA	Stat. Desig-nation	Soil type	Management	Visit Date
Win Green Cranbourne Chase, Dorset	National Trust	ST 924 205	134	N/A	Calcareous loam	Cut [with aftermath grazing by cattle]	Sample & collection 04/03/2011
Avis Meadow North Wiltshire	Wiltshire Wildlife Trust	SU 021 878	108	N/A	Neutral loam	Cut [with aftermath grazing]	Sample & collection 10/03/2011
Woolacombe Warren North Devon Coast	National Trust	SS 454 419	145	AONB/ Biosp-here reserve	[Calcareous] sand	Unmanaged	Sample & collection 06/03/2011
Silk Hill Salisbury Plain Wiltshire	Ministry of Defence	SU 1784 64	132	N/A	Calcareous loam	Unmanaged	Sample only 20/02/2013

9.3 Treatment Soil Collections and Analysis Methodology

9.3.1 Pre-Collection Decisions

Calculations from the experimental design (Chapter 11) indicated that at least 100L of each soil type would be needed. Difficulties were found in locating suitable sites where permission would be granted to remove such large amounts of soil. Therefore it was decided that for the neutral loam and calcareous loam sites, mole hills would be a useful way of collecting [already extracted] soil without damaging or disturbing the sampled fields.

9.3.2 Mole Hill Soil Properties

Although mole hill soil can be the only available substrate to use in biotic studies when needed from protected sites (and any site where digging is prohibited), they are generally avoided in routine soil testing due to possible irregularities in their analysis (PDA, 2011). Past work by Canals & Sebastia (2000) found that the molehills they tested had an elevated amount of inorganic nitrogen compared to surrounding soil. Furthermore, they suggested that the burrowing disturbance of the moles, together with the increased nitrogen, may have depressed mycorrihzal activity. Although Canals & Sebastia's (2000) field-work study found no plant colonisers exclusive to mole hills it was identified that ruderal and non-mycorrhizal species were facilitated on this soil. These findings support the concept that a competitor-free space, known ecologically as a 'gap', will have a differing assemblage of colonizing species from the surrounding vegetation (Fenner, 2000). This differing species assemblage is thought to be due to the initial succession of ruderals and also by arbuscular mycorrhizal associations becoming of greater importance as plant communities become progressively more competetive (Olsson & Tyler, 2004). In a similar study on anthills (King, 1977) it found that *Lotus corniculatus* was one of the species which was found in equal frequency on the anthills to the surrounding grassland, a suggestion for this was that this species can grow up through heaped soil. King (1977) also noted similarities in his anthill study with molehills, stating that although the molehill soil could be stony, it was no different in organic matter to the surrounding soil.

9.3.3 Soil Collection

During February and March 2011 120L-150L of soil was collected from each of the sites listed in Table 9. This was a larger amount than originally calculated in order to cater for any unexpected experimental design changes, spillages or soil volume discrepancies with stones and other materials accidentally collected and unable to be used. Soil was collected in approximately four empty animal feed sacks per site (sacks were turned inside-out to prevent contamination with feed residue). Where vehicular access was impractical, a wheel barrow was used to carry the soil, or in the case of Woolacombe Warren, several journeys with buckets were used in the steep climb up the dunes to the car park.

Six small soil samples (approx 250g per sample) were also collected at the same time and bagged separately for chemical analysis.

9.3.4 Soil Chemical and Physical Analysis and Final Treatment Choice

The soil samples were dried and sieved ready for examination. Chemical analysis was conducted using the same methodology as for the Ecotype site soils (Table 2).

After chemical analysis results were studied and compared with the ecotype soils, one out of the two calcareous loam sites was then rejected for the final treatment soil choice. It should be noted that comparison had to be made against all of the ecotype soil samples at this point rather than just those ecotypes used. Logistically treatment soils had to be selected and analysed before seeds were sown (and therefore before any unsuccessful sowings), consequently, final ecotype choice was not yet known.

Once the treatment soil choice was made, particle analysis of the three soils was conducted as before (Table 2). Figure 19 shows one replicate from each of the three treatment soils after the wet sieving stage of particle analysis.

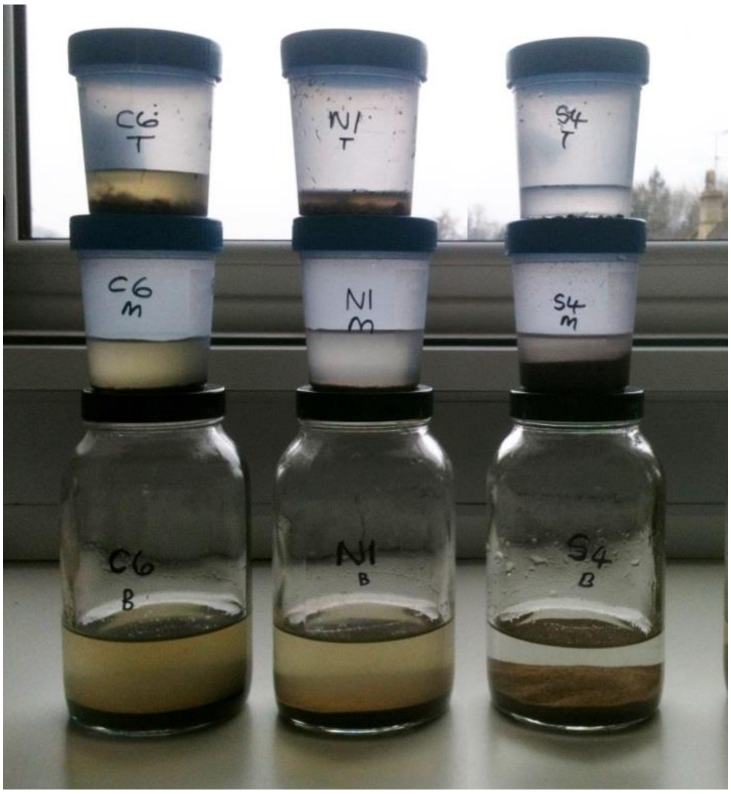


Figure 19. Particle analysis of the three chosen treatment soils.
Key: C - calcareous loam, N - neutral loam, S - sand; T - (Gravel >500 µm), M - (>250µm), B - (>63µm); the numbers refer to which replicate number they are.

9.3.5 Data Analysis

All parameters were first analysed with box charts, histograms and scatter graphs in Excel (Microsoft, 2013 version 15.0.4551.1005) to highlight particular correlations and differences to further compare and establish whether data were normally distributed.

Differences in soil results were non-parametric so were examined for significance using a critical U value table (Hole, 2011) with figures generated using the Mann-Whitney U formula in Excel (Microsoft, 2013 version 15.0.4551.1005).

Data were entered into 'Multi-Variate Statistical Package' (Kovach, 2006), ordinated (Palmer, undated) in a Principle Components Analysis (PCA) and a scatter graph generated from these results.

9.4 **Treatment Soil Results**

9.4.1 Results of All Potential Treatment Soils

Results of the four initial treatment soils are displayed in Table 10, with raw data in Appendix VI.

Treatment soils (Table 10) showed similar patterns to ecotype soils (Table 3), with higher pH of the calcareous loam soil (7.17) and the sand soil (7.79), similarly reflected in higher calcium levels of these. Organic matter was again lowest in the sand soil (0.72%) and the two loam soils contained highest macro-nutrients nitrate and phosphate. There were many differences between treatment soils and chosen ecotype soils; Most treatment soil elements were significantly lower than the ecotypes, with exception of significantly higher results for nitrate (38.78ppm, compared to highest at 19.96ppm) and organic matter (29.94% compared to highest at 22.55%) for calcareous soils, and phosphate which was significantly higher for neutral soil (14.37ppm compared to highest at 11.81ppm). The remaining phosphate and potassium for calcareous soils, pH and calcium for sand soils, and sodium for all soils showed no significant differences. These differences were expected as treatment soils were picked to represent typical receptor sites. It should also be noted that the comparison was between 6 samples (treatment sites) and 18 samples (ecotype sites), the latter therefore having larger standard errors (Table 3).

Table 10. Summary soil results from treatment soils (n=6.) The letter in brackets is the code used in later analysis for that treatment. Mann-Whitney P values are from comparison of these results with chosen ecotype soils Table 3.

		Calcareous loam (C) Win Green	Neutral loam (N) Avis	Sand (S) Woolacombe Warren	Rejected Calcareous loam (CB) Silk Hill
pH	**Median**	**7.17**	**5.62**	**7.79**	**8.23**
	Min	7.08	5.47	7.59	8.02
	Max	7.33	6.32	7.87	8.28
	Mann-Whitney P	*0.01*	*0.01*	*>0.05*	*0.01*
Cond. mS m^{-1}	**Mean**	**0.18**	**0.07**	**0.10**	**0.20**
	Standard Error	0.01	0.01	0.01	0.02
	Mann-Whitney P	*0.01*	*0.01*	*0.01*	*0.01*
N (ppm)	**Mean**	**38.78**	**5.98**	**2.76**	**26.64**
	Standard Error	2.38	1.05	0.71	3.55
	Mann-Whitney P	*0.01*	*0.05*	*0.01*	*0.05*
P (ppm)	**Mean**	**19.74**	**14.37**	**1.58**	**30.44**
	Standard Error	1.67	0.69	0.26	2.11
	Mann-Whitney P	*>0.05*	*0.01*	*0.05*	*>0.05*
OM %	**Mean**	**29.94**	**13.72**	**0.72**	**12.73**
	Standard Error	0.75	0.53	0.05	0.21
	Mann-Whitney P	*0.01*	*0.05*	*0.01*	*0.01*
K (ppm)	**Mean**	**66.33**	**72.12**	**17.92**	**77.56**
	Standard Error	7.89	3.92	0.84	2.09
	Mann-Whitney P	*>0.05*	*0.01*	*0.01*	*>0.05*
Na (ppm)	**Mean**	**21.99**	**24.06**	**21.44**	**13.03**
	Standard Error	1.47	0.67	0.50	0.23
	Mann-Whitney P	*>0.05*	*>0.05*	*>0.05*	*0.05*
Ca (ppm)	**Mean**	**14872.16**	**485.20**	**74215.85**	**368077.94**
	Standard Error	2037.50	7.29	11626.00	30708.85
	Mann-Whitney P	*0.01*	*0.01*	*>0.05*	*0.01*

Particle analysis was conducted on the three chosen treatment soils only (reasoning behind treatment soil final choice in later results), the percentages are displayed by stacked bar chart (Figure 20) with data in Appendix VI. As with the ecotype soils, the proportion of organic matter from hydrochloric acid removal was replaced by the original organic matter results (Table 10) due to inconsistencies noticed with the results from the removal method, and the need to keep methods used in treatment soil analysis consistent with those of ecotype soil analysis.

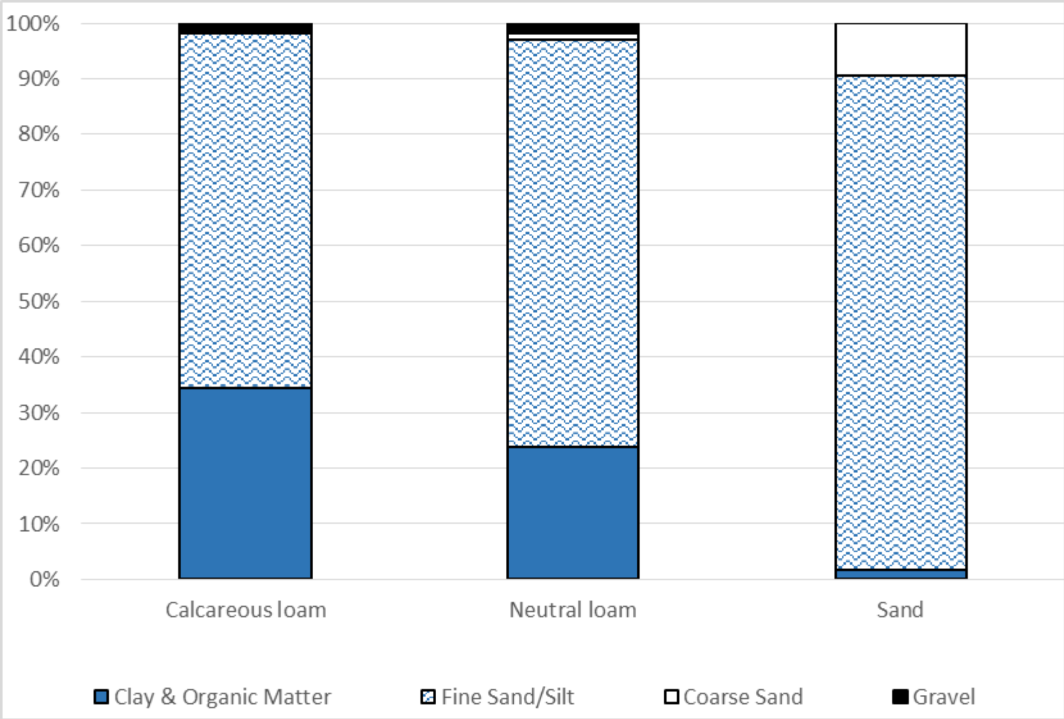


Figure 20. Particle analysis for treatment soils (means, %). Means from *n*=6. Organic matter figures are taken from previous muffle furnace analysis and deducted from smallest particle size portion (>63µm).

Figure 20 shows sand (Woolacombe Warren) had the majority of its soil make-up from sand and silt (>63 µm), where as the other two treatment soils also had approximately one quarter of their analysis from clay and organic matter (<63 µm).

The chemical analysis results of all four potential treatment soils and all ecotype soils were ordinated by Principle Components Analysis (PCA) and a scatter graph generated as shown in Figure 21.

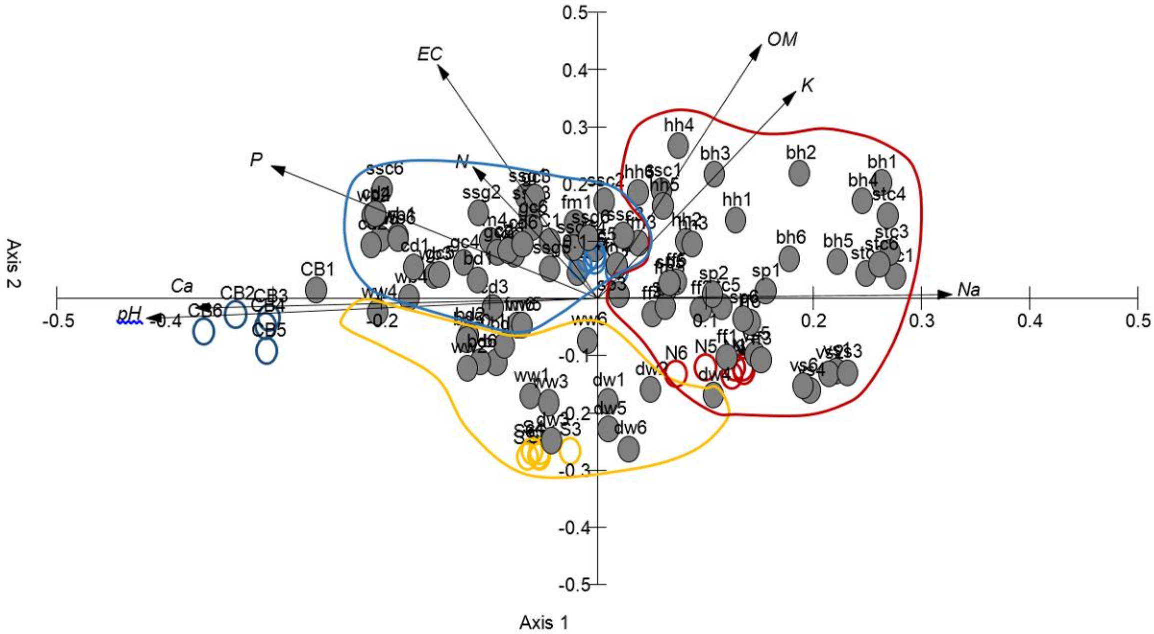


Figure 21. PCA scatter graph of all ecotype soils and all treatment soils before selection. Red outline around neutral loam ecotypes, blue outline around calcareous loam ecotypes and yellow outline around sand ecotypes. The unfilled hoop markers show location of treatment soils: yellow 'S' – Sand (Woolacombe Warren), red 'N' - neutral loam (Avis Meadow), bright blue 'C' - calcareous loam (Win Green), navy blue 'CB' - Calcareous loam (Silk Hill). Ecotype Key: bd - Burrow Dunes, bh - Burledge Hill, cd - Cockey Down, ff - Folly Farm, fm – Fontmell, gc - Goblin Combe, hh - Hellenge Hill, sp - Salisbury Plain, ssg/c - Southstoke grazed/cut, stc - St.Catherine's, vs - Valley of Stones, wb - Woodborough Hill, ww - Woolacombe Warren.

Figure 21 groups the three main ecotype soil types in this study, the three treatment soils; Avis Meadow, Win Green and Woolacombe Warren were also placed within these groupings in the expected soil types. The neutral loam treatment soil (Avis Meadow) was close to the sand ecotypes and the calcareous loam treatment soil (Win Green) was situated close to the neutral loam sites. The second calcareous loam site (Silk Hill) wasn't located within one of the ecotype soil type boundaries. The longest soil vector was pH and was shown to be the most influential vector for the whole data set as well as for Silk Hill which was located at the most positive point of the vector. As Table 10 shows this ecotype as having a median pH of 8.23, which was more alkaline than all ecotype median pH results it is thought this was the primary reason for Silk Hill's remote location on the scatter graph.

From comparison of results in Table 10 with ecotype soils results in Table 3 as well as the PCA results in Figure 21, it was decided that Silk Hill calcareous loam treatment soil would be rejected in favour of Win Green. Therefore all further analysis of treatment soils only includes the three soils used in the experiment.

9.4.2 Further Results of Chosen Treatment Soils

It was desired that differences between the three treatment soils were statistically significant, similarly to the differences between ecotype soils (Table 4). Therefore non-parametric Mann-Whitney U tests were used to study where differences were significant between the three soil types (Table 7).

Table 11. Mann-Whitney U-Test of treatment soil results. These results were generated from results of six replicates of each treatment soil used in the glasshouse studies, each soil type was compared against each other. As multiple tests were carried out, the significance level was decreased to <0.01. A sample size of *n*=6 and *n*=6 was used, therefore the critical U value that significant results (<0.01) will equal or be lower than is 2 (Hole, 2011), such values have been shown in bold.

	Neutral loam Avis	**Calcareous loam** Win Green	**Neutral loam** Avis	**Sand** Woolacombe Warren	**Calcareous loam** Win Green	**Sand** Woolacombe Warren
	Compared		**Compared**		**Compared**	
pH	**36**	**0**	**36**	**0**	**36**	**0**
Conductivity	**36**	**0**	26	10	**0**	**36**
Nitrate	**36**	**0**	7	29	**0**	**36**
Phosphate	30	6	**0**	**36**	**0**	**36**
OM	**36**	**0**	**0**	**36**	**0**	**36**
Potassium	11	25	**0**	**36**	**0**	**36**
Sodium	15	21	24	12	27	9
Calcium	**36**	**0**	**36**	**0**	**36**	**0**

Table 11 shows that for the three treatment soils significant differences were found between all soil types for pH, organic matter and calcium. There were other significant differences between these treatment soils; out of the eight soil parameters tested there were five between neutral and calcareous, five between neutral and sand, and seven between sand and calcareous loam.

There was found to be no significant difference between sodium levels throughout.

Chemical analysis results of the three chosen treatment soils were ordinated with all ecotype soils in MVSP with Principle Components Analysis (PCA) and a scatter graph generated as shown in Figure 22.

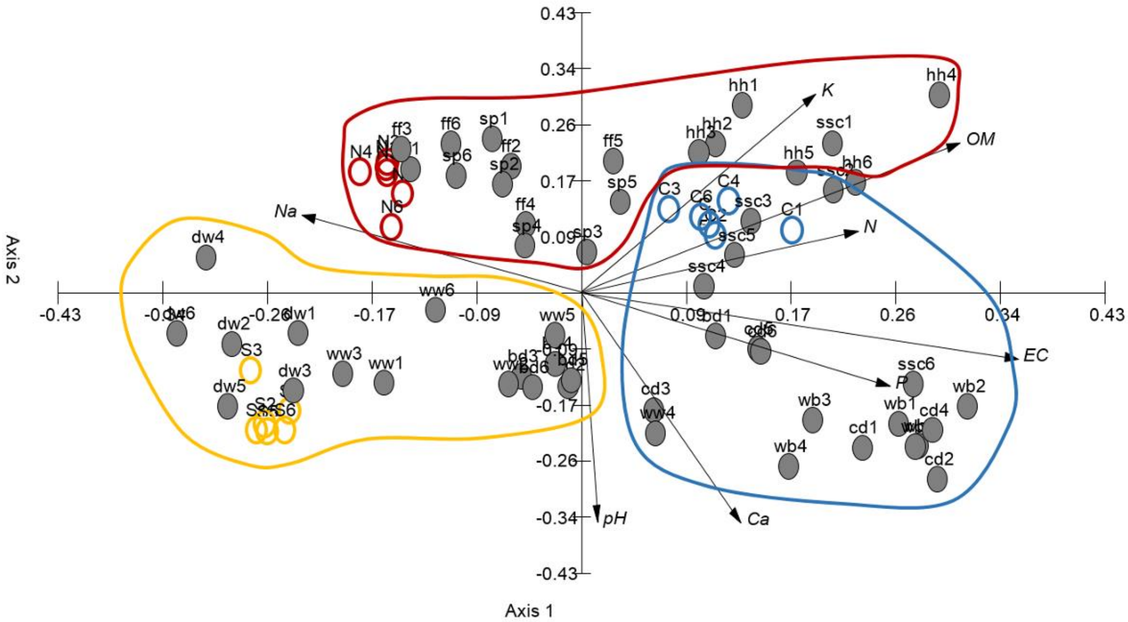


Figure 22. PCA scatter graph of chosen ecotype soils and chosen treatment soils. Red outline around neutral loam ecotypes, blue outline around calcareous loam ecotypes and yellow outline around sand ecotypes. The unfilled hoop markers show location of treatment soils: yellow 'S' - sand (Woolacombe Warren), red 'N' - neutral loam (Avis Meadow), blue 'C' - calcareous loam (Win Green). Ecotype Key: bd - Burrow Dunes, cd - Cockey Down, ff - Folly Farm, hh - Hellenge Hill, sp - Salisbury Plain, ss - Southstoke, wb - Woodborough Hill, ww - Woolacombe Warren.

The PCA illustrates the chosen ecotype soils grouped into the main soil types. When all other unchosen ecotypes and treatment soil were removed the treatment soils were further outside of the groupings than initially intended. The overlap already noticed (Figure 11) with Hellenge Hill is then near to the calcareous loam treatment soil. As with the ecotype soils PCA scatter graphs (Figure 11), conductivity was the longest and most influential vector, with organic matter closely following this, there were no particularly short vectors.

9.5 Treatment Soil Discussion

The treatment soils were chosen as more representative of typical receptor sites therefore differences seen between these and ecotype soils were anticipated. Woolacombe Warren sand (sand treatment soil) taken from foredunes compared to the fixed dune (Woolacombe Warren ecotype soil) showed noticeably lower metal levels which is thought to be due to the very low organic matter here, with no vegetation, allowing nutrients to leach easily (Rosen *et al.,* 2008). Differences were also shown in the Win Green calcareous loam, with lower pH, greater organic matter and nitrate ppm compared to the calcareous loam ecotypes, and significantly higher ($P<0.050$) nitrate compared to the other treatment soils which may reflect the historic agricultural improvement and suggest lack of grazing is still influencing the soil (O'Leary *et al*., 2002; Grogan, 2010). This higher nitrate level may also partly be due to the soil collection method of using molehills which have been found to contain elevated levels of inorganic nitrogen compared to surrounding soils (Canals & Sebastia, 2009). Avis Meadow had significantly greater phosphate (14.37ppm) than the neutral ecotypes, an element which takes a long time to decrease from previously agriculturally improved or fertilized soils (Gough & Marrs, 1990; Walker *et al*., 2004[c]).

The Silk Hill calcareous loam treatment soil was removed from the final choice, mainly due to its extremely high pH and calcium and very low organic matter. Not only did it compare poorly with the calcareous loam ecotype soil but also didn't represent a typical receptor site. It was thought the mean results of Win Green were better suited than Silk Hill when compared to the means of all ecotype calcareous loam soils, and also better represented a typical site in need of restoration or enhancement.

9.6 Treatment Soil Final Choice

The choice of treatment soil had to be made before ecotype seed was sown and therefore comparisons had to be made with the whole ecotype dataset rather than those finally chosen, the amount of soil needed also limited the availability of sites where permission for collections could be made. However, it was thought that similarities to typical receptor sites of these soils was suitable.

9.6.1 Win Green – Calcareous loam

Win Green, the highest point in the Cranbourne Chase AONB, is a calcareous clay loam hill with underlying chalk geology (Geddes, 2003; Magic, 2013). The National Trust bought this downland in 1937 (Wiltshire Council, 2011), it was ploughed during the Second World War, after which it was reverted back to grassland and cut for hay. Once part of the Win Green Down SSSI, the SSSI status was withdrawn from this section in the late 1970s due to loss of diversity. Since then the area was only cut for hay until approximately eight years ago when sheep were introduced for aftermath grazing. Cattle have also been used in recent years (Whitbourn, 2011).

9.6.2 Avis Meadow – Neutral loam

Avis meadow soil was neutral Minety Rolling Clay, derived from underlying Oxford Clay (Geddes, 2003; Landuse Consultants, 2005). Historically, this site was managed as a hay meadow until 1980 when it became intensive horse grazing (Wiltshire Wildlife Trust, 2003[a]) The Wiltshire Wildlife Trust acquired the field in 1997 since which they have been managing it by cutting with aftermath grazing to restore the grassland back to a species-rich sward (Wiltshire Wildlife Trust, 2003[a]). Regeneration has been slow with a classification of improved/semi-improved still current (Mortimer, 2010).

9.6.3 Woolacombe Warren – Sand

Woolacombe Warren, a National Trust-owned dune system, is the same site used for the ecotype sites. However, ecotype seed and soil samples previously collected were taken from the grey dunes which are semi-stabilised with succession of vegetation cover, whereas treatment soil was taken from the mobile dunes further west with very little or absent vegetation cover. This was thought to represent a typical dune receptor site in need of reseeding for vegetating and stabilisation purposes.

10 MANAGEMENT REGIME TREATMENT

10.1 **Management of Grasslands**

A key influence on grassland establishment and longevity is its subsequent management after reseeding. Management is a large influence in the composition of herb and grass species with a fine balance needing to be found (Hurben & Huber-Sannwald, 2002; Flower, 2008). When this balance is lost there is shown to be a succession towards a less species-rich sward.

10.1.1 Intensive Management

Intensive management can cause problems by unbalancing factors influencing grassland composition. As already discussed (Chapter 1), much of the intensive agricultural management came about since the Second World War with a key management change being the regular large-scale re-seeding of grasslands (Shrubb, 2003; Goulson *et al*., 2005). As well as such direct intensive management changes, sward composition can also result from over-grazing. Dorrough *et al.* (2004) showed frequent grazing reduces persistence in perennials and Pavlů *et al.* (2007) found a pattern emerged for prostrate herb cover which increased with intensive management becoming widespread. It is thought that continuous, heavy grazing allows little opportunity for flowers to set seed (Flower, 2008). Another problem with intensive grazing is when high stocking rates cause localised poaching and erosion of the soil (Wells, 1969) by increasing soil bulk density and therefore impeding drainage (Mulholland & Fullen, 2007). The impacts of soil compaction by grazing animals was reviewed by Greenwood and McKenzie (2001) who suggested the pressure by cattle at high stocking rates was comparable to agricultural machinery.

10.1.2 No Management

The other end of the spectrum with inappropriate grassland management can be from cessation of any grazing or cutting regime, which has been found to decrease species diversity rapidly (Jacquemyn *et al*., 2003). Suggestions why this happens include the loss of gap formation, decreased light availability, and proliferation of strong competitors (Smith *et al*., 2000; Jacquemyn *et al*., 2003). In support of the latter of these suggestions are studies where taller, more aggressive species have been shown to increase in unmanaged swards and will dominate and shade out herb species; a study by Kydd (1964) showed an increase in total grass cover (particularly *Festuca rubra* L., *Dactylis glomerata* L., *Holcus lanatus* L. and *Arrhenatherum elatius* L.) within the undergrazed control in a four-year grazing intensity trial. Also, more recently, Pavlů *et al.* (2007) found tall forbs such as *Cirsium arvense* L. and *Galium album* Mill. and tall grasses such as *Alopecurus pratensis* L. and *Elytrigia repens* L. thrived in the unmanaged control of the trial, with higher species richness emerging in all cut and/or grazed treatments compared to the unmanaged.

Problems with undergrazing have become more of an issue in recent years due to an increase in abandonment of farmland, particularly pasture. A report by the European Commission (2013) into farmland abandonment highlighted the main pressures to include weak land market and low farm income. These findings are unsurprising as the modern financial hardships of the industry have been well documented in the media (BBC News, 2012; Gray, 2012; Rayner, 2013). Lamb and milk prices decreasing as well as increased disease risk such as Schmallenberg and Bovine TB are a few factors causing difficulties in gaining income from farm products for more than they cost to produce (Rayner, 2013). These economic problems therefore increase the amount of abandoned grassland and loss of diversity (English Nature, 2005).

10.1.3 Cut with Aftermath Grazing

This was a traditional management of hay meadows where only a light spreading of fertilizer would be applied on a rotational basis; this was in the form of manure from winter sheltered livestock. A hay cut would be made on or soon after the traditional date of 25th July when a peak grasses and wildflower yield could be achieved. The fields would then be left to recover for six to eight weeks after which the livestock would be added to graze the re-growth in September / early October (Flower, 2008). The aftermath grazing was important for various reasons both agriculturally and botanically; it not only delayed the need to feed expensive winter hay, but the action of grazing animals at this time would also open up the sward to create gaps where seed could be trodden in which aided germination (Smith *et al*., 2000; English Nature, 2005; Flower, 2008).

This form of management has decreased with the modern changes in land use (English Nature, 2005). The reduction in dairy farming due to economic and political pressures has resulted in lower demand for a hay crop. Also, the popularity of silage-making which increased from the 1950's (Dunmore and District Vintage Club, undated) has reduced the requirement for hay further and has changed the timings of many cut grasslands. Silage production involves the application of chemical fertiliser in April creating a grasses peak in May when the first cut is made, this is repeated one or two more times the same year (Living Countryside, 2013), and therefore alters which species are able to set seed.

10.1.4 Extensive Grazing

Not all grassland was traditionally cut. Those areas which did not need a hay cut, particularly larger areas such as Salisbury Plain and land too awkward for machinery such as steep chalk escarpments and coastal sand dunes, are often extensively grazed. Grazing controls scrub encroachment and suppresses aggressive plant species. It also removes the grass growth more gradually than other techniques such as cutting or burning (English Nature, 2005), so can be beneficial to slower growing species. The action of the seed being digested by ruminants has also been shown to aid germination of some hard coated species (Lowry, 1996; Fredrickson *et al*., 1997) and can help to disperse species that withstand the environment of the ruminant gut. Vegetation trampling and return of nutrients from dung are also positives in the management (Wells, 1969). The grazing technique between large herbivores differs and is also a consideration when deciding how to graze a habitat; cows are generally not selective grazers and use their large tongues in a tearing action on the vegetation therefore leaving long tussocky areas (English Nature, 2005). Sheep are more selective than cattle (Wells, 1969; English Nature, 2005), often picking flower heads, they graze to a short length and can have difficulty grazing long vegetation. The angle of horses' teeth mean they can graze down to as short a length as rabbits, although they selectively leave longer areas, such as those used as latrines (English Nature, 2005).

10.2 Species Adaptation to Management

As already discussed, it has been found that some species of plant are better adapted to certain managements than others. *Festuca rubra* and *Dactylis glomerata* have been found to be associated with both extensively and intensively grazed pastures, whereas *Elytrigia repens* L. and *Alopecurus pratensis* L. were more abundant in unmanaged grassland (Pavlů *et al*., 2007). Poschlod *et al.* (2011) studies showed management affected population density and age structure of *L. corniculatus* with grazing producing the highest numbers, and lowest numbers found in the unmanaged (succession) treatment. There is also known to be some genetic variation to this within species, as shown in a study where populations of *Poa annua* L. responded differently to management regime (McNeilly, 1980).

10.3 Grassland Restoration

Often the first decision made in restoring habitats is whether it can be done by creating the correct conditions to allow it to develop naturally. In many cases the past management is an important determinant of such a decision (Walker, 2003[c]). Wells (1969) found that grassland unmanaged for a minimum of ten years will have such a depleted diversity and seed bank that many species will be unable to re-establish. On ex-arable sites, soil nutrients can be high and seed banks depleted (Walker, 2003[c]; Flower, 2008). In such cases, reseeding or enhancing by seed or plug planting is necessary. Often, first decreasing the soil nutrients, particularly phosphate will be needed. Walker (2003[c]) states that on brown earths, nutrient stripping by deep cultivation or removing turf may be required when soil phosphorus levels are too high. A P index of 0-1 is the level of available phosphorus recommended by the Natural England Technical Advice Note TIN066 (Natural England, 2010), this index amount is equivalent to 0-15mg/L (Olsen's method) and 0.5-4.4mg/L (Morgan's Method) of soil phosphorus (Natural England, 2008[b]).

There are various techniques for re-seeding grassland, often with site-specific tailoring. Generally, if the land is ex-arable, cultivation after the crop and removal of stubble, followed by cultivation again the following year can prepare the ground sufficiently. An improved grassland conversion will likely need to be sprayed off during the spring and cultivated in summer. The seed bed is prepared by harrowing during late summer and the seed-sown and rolled on the soil surface in September (Flower, 2008). If only enhancing the sward, the technique usually involves grazing hard in late summer and harrowing hard to bring up approximately 30 to 50% bare earth, the seed is broadcast in late summer/early autumn and either rolled or livestock are replaced to tread in the seed (Flora Locale, 2005[a]; Flower, 2008). Broadcasting of the seed is best done by hand, otherwise it's suggested that using a fertilizer spinner would be successful, methods using green hay require different methods and timing (Flora Locale, 2005[b]).

The initial years of establishment are very important in restoration. Flora Locale (2005[c]) guidelines indicate that in the first year the sward should be lightly mown or grazed in early July with any cuttings removed. Conversely, Natural England (2010) guidelines advise that after an autumn sowing, the vegetation is cut or grazed in early spring when the sward has reached 10-15cm to a height of 5-7cm and livestock only returned once a height of over 10cm is again achieved, which process should continue regularly until autumn.

10.4 Management Treatment in Experiment

For this experiment, the Natural England recommendations were used as a guide for sward height in the initial year of establishment. Once the majority of plants (for this study it was over 50% of the plants) had stems that were longer than 10cm, they were cut with scissors to 7cm to simulate grazing (Wilson & Jones, 1981), this was carried out with a pre-measured plant marker (Figure 23) and is referred to as 'grazed treatment' throughout the proceeding chapters.

Figure 23. Grazed treatment application: Cutting plants to plant marker height of 7cm, to assimilate grazing.

In March 2012 the management treatment commenced, with half (216) of the plants receiving a grazed treatment cut which was repeated regularly, every four weeks. Clippings were removed, oven-dried and weighed (Wilson & Jones, 1981; Wardle *et al.*, 1998). The other 216 plants were classed as the 'unmanaged' standard. The grazed treatment was applied six times in the first (main) year before the July/August 2012 harvest.

The study was extended for another 12 months after the harvest, therefore the management treatment was recommenced in the April of the second year. The Natural England guidelines suggest that after the first full season it may be beneficial to allow flowers to set seed before grazing. However, it was decided that the management needed to be in place to get another whole season of grazed treatments for comparison reasons between the managements. A further five grazed treatments were applied in the second year, the first in May 2013, then every four weeks until August 2013. A further grazed treatment was not applied after August due to lack of growth.

11 EXPERIMENTAL DESIGN AND MEASUREMENT METHODS (STUDIES 1 & 2)

Please note that within this chapter, calcareous sand is referred to as ‘sand’ and cut with aftermath grazing is referred to as ‘cut’. ‘Grazing treatment’ refers to simulated grazing from cutting by hand.

11.1 Introduction - Experimental Design and Measurements Methods

11.1.1 Glasshouse Studies

There is no standardised methodology for investigating plant nutrition and fitness (Rorison & Robinson, 1986). Depending on the study, methods will vary according to the variables and controls. For plant nutrition the elements in the soil can be measured although results will be limited in terms of potential availability for the plants (Taiz & Zeiger, 1991), therefore plant material should also be tested for essential elements. For nutritional importance of plants to herbivores and pollinators the plant material will again only show availability, this could prove less substantial if other secondary plant chemicals are present to impede the assimilation by the animal, as outlined in the example of the *Colias croceus* assimilation of Carbon (Chapter 1).

When studying the effects of one variable (e.g. soil) it is important that all other possible contributing factors are disregarded such as climatic or topographical differences. Often the most appropriate way to do this is by ‘greenhouse’ or ‘common garden’ experimentation where all environmental factors can be standardised, resulting in a more reliable means of testing a hypothesis than a field-based investigation (Moore & Chapman, 1986). However, this [form of] methodology does have disadvantages, certain biological interactions in the field cannot be portrayed adequately; interspecific competition is one such factor (Gibson *et al*., 1999). In the investigation into the effect of a different soil type, factors of that soil type such as natural drainage, soil aeration and temperature are also excluded and must be artificially replaced by media such as perlite. Although perlite has a neutral pH (United States Department of Agriculture, 1999) there may still be slight changes to soil in terms of chemical properties. Conductivity and water release have been found to be higher in perlite than sandy loam (Jackson, 1973).

11.1.2 Factorial Designs

When there is more than one independant variable in an experimental design measured simultaneously then the experiment becomes a factorial design. There are different forms of factorial designs, when there are several predictors or independent variables and some of these have been measured with the same units and others have been measured with different units this becomes a 'Mixed Design'. At least two independent variables are needed for a mixed design (Underwood, 1997; Field *et al*., 2012). In this study the treatment soil and management regime are the fixed factors, these are independent variables measured with different units to each other, and within these the three soil types and two management regimes are measured with the same units.

11.1.3 Individual Plant Measures of Fitness

To determine the success of plant translocation the key parameter is plant fitness, an essential component of which is survival. Biomass production is thought to be a key factor determining fitness (Schroder & Prasse, 2013) as it can help evaluate traits such as competitiveness and photosynthetic capacity. Other important factors include vigour (strength and health) and fecundity (Himanen *et al.,* 2012) which determines longevity of many species (Crawley, 1983). As well as indicating the fitness of a plant/ecotype the measurement of certain parameters can also provide useful indicators of which factors may be influential in successful habitat restoration. Such parameters include main stem length (and 'stretched length'), growth form and leaf-nitrogen which can all vary in response to climate, soil, competition, disturbance, herbivory and plant defence (Cornelissen *et al*., 2003).

11.1.4 Herbivore Requirement Measures of Plants

Theories differ in terms of which plant material represents the best nutritional value to herbivores (Crawley, 1983). One view is that plants with lower chemical defences are a higher quality food and that this factor is of greater importance to selective herbivores than is gross energy or nutrient content (Bryant & Kuropat, 1980). This assertion seems logical if the herbivore in question does not have the capacity to digest certain toxins or browses plants with digestibility inhibitors thus decreasing its ability to utilise any nutrients available (Howe & Westley, 1988). Difficulty in determining what represents a nutritious plant is a consequence of the extensive variation between herbivores in primary food materials and complexity in diets (Crawley, 1983). Nitrogen is one element commonly known to be vital for all herbivore nutrition (Mattson, 1980; Crawley, 1983). As well as playing a crucial role in cell structure and metabolic processes (Mattson, 1980), reduced herbivore fecundity has also been correlated with nitrogen deficiency in plants (Crawley, 1983). The most efficient approach to examining herbivore requirements therefore is to test for elements needed in growth and fitness such as nitrogen, as well as for less desirable secondary compounds. When Schroeder (1986) tested nutrient quality of leaves, he measured the attractive qualities such as nitrogen, sugar and water content as well as the less desirable hydrogen cyanide and the difficult-to-digest polymers'; cellulose and lignin.

11.2 **Potting On and Experimental Design**

Although three interaction studies were carried out, the experimental model was conducted in a Full Factorial Design to allow study of each of the variables and also their interactions with each other on the response variable. Therefore methodology was identical for all studies unless otherwise stated.

Originally nine replicates were to be used but due to plant failures this was reduced to eight (see Chapter 16). Therefore each ecotype was planted within six soil-management treatment combinations and replicated eight times, this design is illustrated in Figure 24, and bench layout is shown in Appendix VII. Treatments were; Calcareous loam soil+unmanaged, Calcareous loam soil+grazed, Neutral loam soil+unmanaged, Neutral loam soil+grazed, [Calcareous] sand+unmanaged, [Calcareous] sand+grazed.

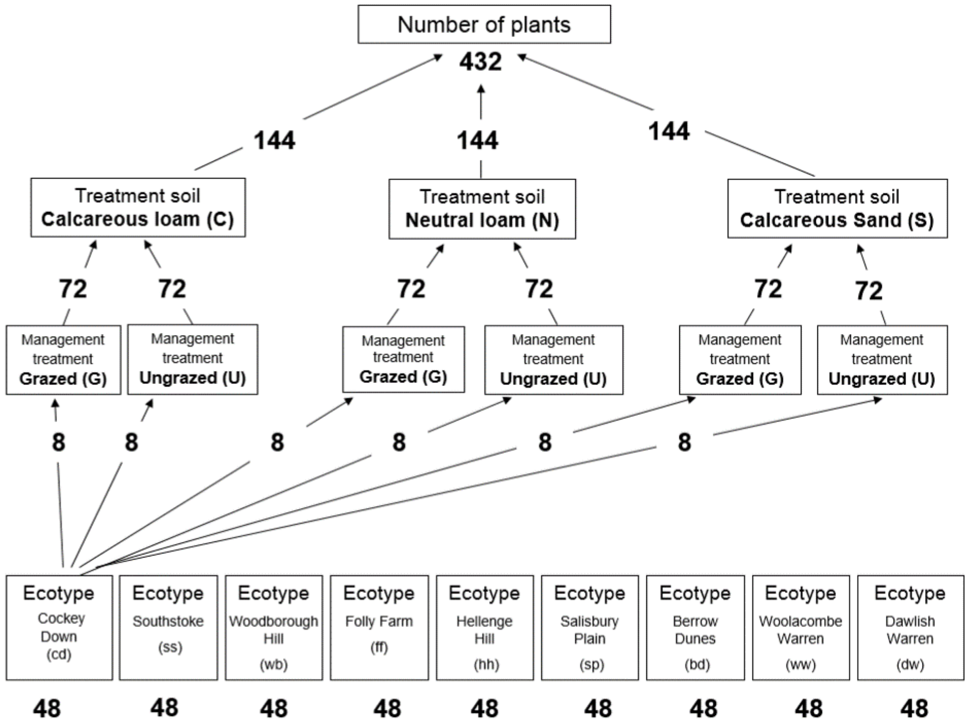


Figure 24. Experimental design.

A mixture of 3:1 sieved treatment soil:perlite was used to fill 432 1L pots. As a particular interest in this study is on the soil effects, a low ratio of perlite was used to reduce dilution of the soils and yet eliminate container issues mentioned previously. These proportions were also used to avoid reducing easily available water (EAW) which Bunt (1983) found to be an issue if using more than 25% in the mix.

Randomisation of seedlings was generated using Agricolae package version 1.0-9 (De Mendiburu, 2010) in R statistical software version 3.1.0 (R Core Team, 2013) (Appendix VIII).

Seedlings were potted up on 10th October with a second batch from the re-sown seedlings planted on 25th October 2011 (Figure 25).

Figure 25. Experimental design layout with newly potted up seedlings October 2011.

Not all ecotypes had nine samples with a full set of eight replicate seedling survivals each, therefore some had to be supplemented with reserve seedling sample replicates from the same ecotype. Samples used are illustrated in Table 12.

Table 12. Final sample numbers used for Ecotype replicates, grouped by ecotype soil type

	Ecotype sample numbers used to make eight planting replications (r.)								
	r.1	**r.2**	**r.3**	**r.4**	**r.5**	**r.6**	**r.7**	**r.8**	**spare**
Cockey Down	2	4	6	8	12	14	16	18	20
Southstoke	2	4	8	10	12	14	16	18	20
Woodborough Hill	2	4	6	8	10	12	14	16	20
Folly Farm	1	2+4	3	5+7	6	8	9	10	12
Hellenge Hill	2	4	6	9+10	12	14	16	18	20
Salisbury Plain	2	4	6	8	10	12	14	16	18
Berrow Dunes	2	4	6	7	8	10	11	12	13
Woolacombe Warren	2	4	8	10	12	14	16	18	20
Dawlish Warren	1	2+8	3	6	9	10	12	13	17

All plants received a minimum of 16 hours of light per day (Stephenson & Windsor, 1986), which was supplemented during winter with Maxibright T5, a blue-white tri-phosphor 6500K lighting system. A system of capillary matting with water reservoirs (manually topped up) was used to maintain water supply from the base, this was increased with manual spray watering of the above soil plant material in hot weather and when plants were too young for roots systems to benefit from the capillary matting system. The plants were also weeded fortnightly, and the glasshouse boiler (ITT Reznor XD225 OF Warm Air Heater) set to a minimum temperature of 15ºC (Fern, 2007) throughout the period of the experiment.

A Tinytag data logger (Gemini Data Loggers) was used throughout the experiment to record 30 minute readings.

The potted on plants were grown in the experiment for 24 months, with the main harvest being carried out nine months after potting on.

11.2.1 Recording Glasshouse Temperature and Humidity

Results from the Tinytag thermometer were collated with means calculated as shown in Appendix IX. The Tinytag was removed from the glasshouse at a time unknown to the author during 2013, therefore Met Office (2013) readings were obtained from the closest weather station in Yeovilton for that year, no standard error could be calculated as means were only given of Met Office results.

The temperature and humidity means Appendix IX were plotted into a combination line/bar graph Figure 26 for comparison with subsequent plant results, to identify if patterns relate to these particular weather details. Although there will be small discrepencies between the two sources of information used for temperatures, the more southerly aspect of Yeovilton compared to Bath should compensate somewhat for the warmer temperatures of the glasshouse as shown in the similarity between the two sources for 2012 temperature. These results show the cold winter temperatures of 2013 were lower for a longer period than 2012, there was also a more steady incline to the peak high temperatures of July 2013, whereas temperatures in 2012 were more erratic with lower summer mean temperatures and two warmer peaks in May and August. Highest humidity in 2012 was during August to October.

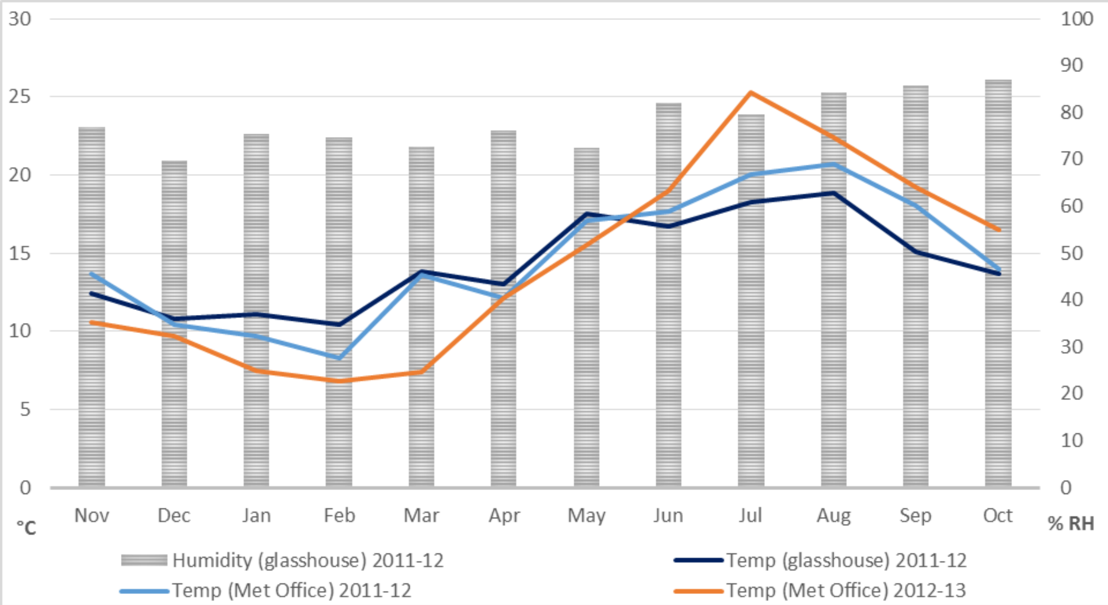


Figure 26. Mean air temperatures and humidity readings taken from the glasshouse Tinytag during 2011-2012 (based on 30 minute readings) and Met Office (2013) mean air temperatures from closest weather station (Yeovilton) during 2012 - 2013

11.3 **Plant Fitness - Measurements Methods**

11.3.1 Regular Monitoring Parameters

Plants were sown, planted on and given treatments as detailed in chapters 7-10.

Plant growth and development parameters were measured on the day potting-up of plants was completed (13th October 2011) and thereafter at four-weekly intervals. Seeds which failed to germinate were resown 12 days after the main sowing (Chapter 7), these plants were potted up on 25th October 2011, and were always measured approximately 12 days later than the main sowing batch, these were termed ‘second batch’.

For the first six months of the experiment, ‘plant fitness’ parameters quantified were; main stem length; number of stems per plant; number of leaflets per main stem; and number of branches per main stem (Ferris & Taylor, 1993; Carter *et al*., 1997; Vuckovic *et al.,* 2007; Smith *et al.,* 2009). To clarify, only fully extended leaflets over 2mm in length were counted and branches on the main stem were only recorded if they were situated over 2mm up the stem.

Figure 27. Plant development by May 2012

Figure 28. Plant development by July 2012

By March 2012, time taken to obtain all parameter results from both plant batches was five days per monitoring event and as the plants had not reached full maturity at that point, this length of time would have increased. Such detailed and lengthy recording proved impractical and therefore subsequent monthly monitoring was reduced to cover main stem length and presence of seed pods. Monitoring dates as well as parameters measured are shown in Table 13.

In addition to quantitative parameters (Table 13), other observations made throughout the recording period included: mortality; wilting; leaf colour; and, herbivory.

After management treatment commenced in March 2012, the regular growth and development parameters were always measured immediately prior to ‘grazing’ treatment so growth between management treatments could be accurately recorded.

Table 13. Date and type of 'Plant Fitness' growth measurements recorded with period reference used in results.

First (main) batch	Second batch	Reference used in Chapter 12 Results	Parameters measured of each plant
13th October 2011 8th November 2011 6th December 2011	25th October 2011 22nd November 2011 20th December 2011	October 2011 November 2011 December 2011	Main stem length Stems per plant Leaflets per main stem Branches per main stem Mortality
3rd January 2012 31st January 2012	17th January 2012 14th February 2012	January 2012 February 2012	Main stem length Stems per plant Leaflets per main stem Branches per main stem Mortality Seed pod presence
29th Feb, 2nd & 5th March 2012	13th & 15th March 2012	March 2012	Main stem length Stems per plant Leaflets per main stem Branches per main stem Mortality Seed pod presence
27th March 2012 24th April 2012 22nd May 2012 19th June 2012	10th April 2012 8th May 2012 5th June 2012 3rd July 2012	April 2012 May2012 June 2012 July 2012	Main stem length Mortality Seed pod number
15th July 2012	31st July 2012	August 2012	All Harvest measurements
23rd August 2012	6th September 2012	September 2012	Main Stem Length Mortality Seed pod presence
25th April 2013	25th April 2013	April 2013	Main Stem Length Mortality Seed pod presence
25th May 2013 27th June 2013 25th July 2014 27th August 2013	25th May 2013 27th June 2013 25th July 2014 27th August 2013	May 2013 June 2013 July 2013 August 2013	Mortality Seed pod presence
28th October 2013	28th October 2013	October 2013	Main Stem Length Mortality Seed pod number Seed number per pod

11.3.2 Harvest Measurements

After a growth period (within treatment soils) of approximately nine months, all growth and development parameters were recorded for harvest plants (main stem length, stems per plant, leaflets per main stem, branches per main stem, longest branch per main stem).

11.3.3 Fecundity

In addition to regular presence/absence of seed pods noted during the fortnightly monitoring, seed pods were counted at harvest for all plants, after which they were fresh weighed, then oven dried and reweighed for dry biomass results.

Seed pods were again counted for all plants at the end of the experiment in the second year. At this point an estimate was also calculated for seed per pod by selecting up to six seed pods per plant (where available), counting seeds for those seed pods and calculating the mean for each seed pod. Seeds per plant were then estimated using the seeds per plant mean and the seed pods per plant totals.

11.3.4 Mortality

During every measurement visit to the glasshouse, plants were checked for survival. Any mortalities were logged and replaced with a new (matching ecotype) replicate from the excess seedlings, and new measurements recorded. The last replacement was made in April 2012. It was thought replacements up to this time would still receive a satisfactory time within both treatment soil and management.

11.4 **Herbivore Requirements - Measurements Methods**

11.4.1 Regular Monitoring Flowering Phenology and Grazed Treatment Clippings Biomass

During the regular monitoring parameters taken for 'plant fitness' analysis, flowering phenology was also recorded from January when the first flower was produced. Parameters were; number of flowers per plant; and flowering period (Carter *et al*., 1997; Vuckovic *et al.,* 2007).

In March 2012 the grazed treatment commenced, therefore a further parameter, (dry biomass of clippings taken) was also included. Monitoring dates as well as parameters measured are shown in Table 14.

Table 14. Regular Herbivore Requirement Parameters Taken

First (main) batch	**Second batch**	**Reference used in results section**	**Parameters measured of each plant**
3rd January 2012 31st January 2012	17th January 2012 14th February 2012	January 2012 February 2012	Flower number
29th Feb, 2nd & 5th March 2012	13th & 15th March 2012	March 2012	Flower number Grazed treatment weights
27th March 2012 24th April 2012 22nd May 2012 19th June 2012	10th April 2012 8th May 2012 5th June 2012 3rd July 2012	April 2012 May2012 June 2012 July 2012	Flower number Grazed treatment weights
15th July 2012	31st July 2012	August 2012	All Harvest measurements
23rd August 2012	6th September 2012	September 2012	Flower presence
25th April 2013	25th April 2013	April 2013	Flower number Grazed treatment weights
25th May 2013 27th June 2013 25th July 2014 27th August 2013	25th May 2013 27th June 2013 25th July 2014 27th August 2013	May 2013 June 2013 July 2013 August 2013	Flower number Grazed treatment but clippings not weighed
28th October 2013	28th October 2013	October 2013	Flower number

11.4.2 Harvest Flowers

At harvest time, flower number was again taken, flower aroma was also graded both during pre-harvest measurements and after harvest following methods described by Murrell *et al.* (1982) whereby “single umbels are sniffed and assigned a rank” of 0 (no odour) to 4 (strong odour) (Murrell *et al.,*1982). During harvest, flowers were placed in sealed plastic bags to allow compounds to concentrate inside. The air sample inside the bag was then sampled near the end of the day and again ranked from 0 (no odour) to 5 (very strong odour).

After the flower scent test was complete all flowers were fresh weighed, these were then dried and reweighed for the dry biomass measurement (Figure 29).

Figure 29. Separating dry plant parts and weighing dry flower biomass.

11.4.3 Growth Habit and Hirsuteness

Additional observations were made immediately prior to harvest, of growth habit (prostrate, decumbent or erect) and hirsuteness (glabrous, very sparsely hairy, sparsely hairy or hirsute) (Smith *et al.,* 2009).

11.4.4 Harvest Biomass

Plants were harvested by cutting to 5cm above soil level. Although a shorter cut than the grazed treatment, it was thought that this would still be comparable to cattle grazing due to Natural England guidelines (2010) advising grazing to a height of 5-7cm. With this height plants could still survive for further analysis yet a suitable amount of plant matter would be obtained. Fresh and dry shoot weights were calculated.

11.4.5 Plant Chemical Analysis

To establish the nutritional value and levels of defence against herbivores, plants were analysed to determine nitrogen and hydrogen cyanide (HCN) content of the leaves (Table 15). Originally tannin levels were to be tested, but due to limited amounts of suitable plant material, leaf-nitrogen and leaf-HCN analyses were prioritised.

Figure 30. Digestion of dried plant material with sulphuric acid and a Calgon tablet to transform organic nitrogen into ammonium for leaf-nitrogen analysis.

Table 15. Methods of plant chemical analysis

Chemical	Solution/Method	Reference
Leaf Nitrogen (N)	Determined by Kjeldahl For each sample, approximately 0.5g of dry leaves were accurately weighed into a Kjeldahl digestion tube. Organic nitrogen was first transformed into ammonium by digesting the dried leaves with addition of 5mL sulphuric acid and a Calgon tablet as a catalyst (a blank is digested first) (Figure 30). The amount of ammonium produced was allowed to cool and 20mL of deionised water added. Sodium hydroxide was then added (25mL) to the solution to increase pH. The solution was steam distilled in the Kjeldahl apparatus, boric acid indicator was used in a 50mL collection flask, placed under the condenser tube. Sulphuric acid was then used to titrate the solution until it turned pink. The formula to determine percent leaf-nitrogen was then calculated: $\%N = \frac{(T - B) \times N \times 1.401}{\text{g sample}}$ Key: T = mL of titrated sample B = mL of titrated blank *N* = acid normality	Baker & Thompson1992
Hydrogen cyanide (HCN)	Determined using the Dawson's qualitative method. Sodium picrate papers were prepared beforehand by cutting chromatography paper into strips which were then soaked in a solution of sodium carbonate, picric acid and distilled water. A small leafy branch of approximately 0.1g (exact weights recorded) was randomly selected from the fresh plants. Plant material was placed in a boiling tube, mixed with five drops of toluene and crushed with a rod to aid cell breakdown. Picrate papers were then placed in the tube, suspended above the sample and stoppered (Figure 31). Tubes were left in a fume cupboard for two hours then picrate papers removed. In the presence of HCN, the yellow picrate paper would turn an orange/brown colour.	Gebre-hiwot & Beuselinck2001; Smith *et al.*, 2005

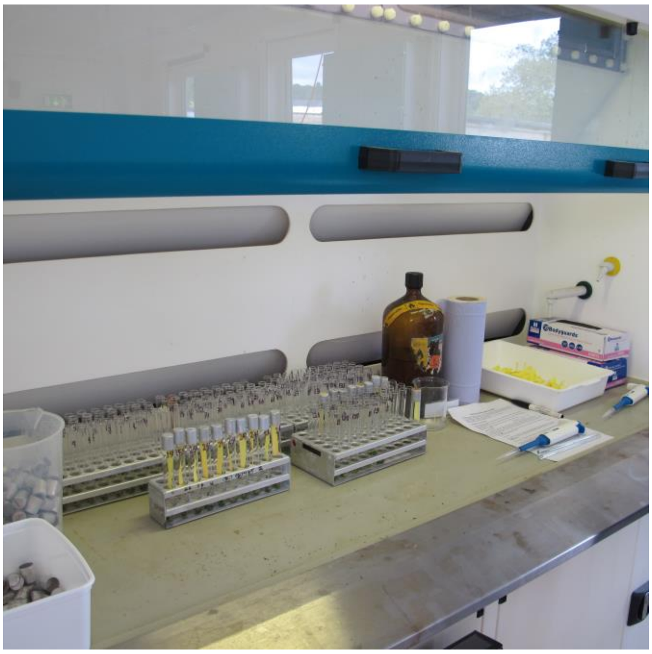


Figure 31. Picrate papers suspended over crushed plant samples with toluene for leaf-HCN analysis.

11.5 **Post-Harvest: Grading Leaf-HCN – The Eyedropper Technique**

For the qualitative leaf-HCN results obtained, picrate papers used in the leaf-HCN test were graded by eye according to the darkness of colouration from 0 (no colour change) to 4 (dark orange/brown) a method adapted from that of Egan *et al.* (1998). These eye-graded results were also used for 'presence and absence' tabulation.

To quantify these results more accurately for both statistical analysis and to eliminate human error or bias, a novel method was developed. All picrate papers were photographed on A4 paper using a Canon Powershot G12 camera (2736 pixels, 180dpi), at no flash, auto mode, held approximately 30cm above the papers in natural daylight conditions on the day of analysis. Two batches of picrate papers were made for the experiment which differed slightly in colour. To counteract this inconsistency each page of used picrate papers was photographed together with an unused standard picrate paper from that batch as a control (Figure 32).

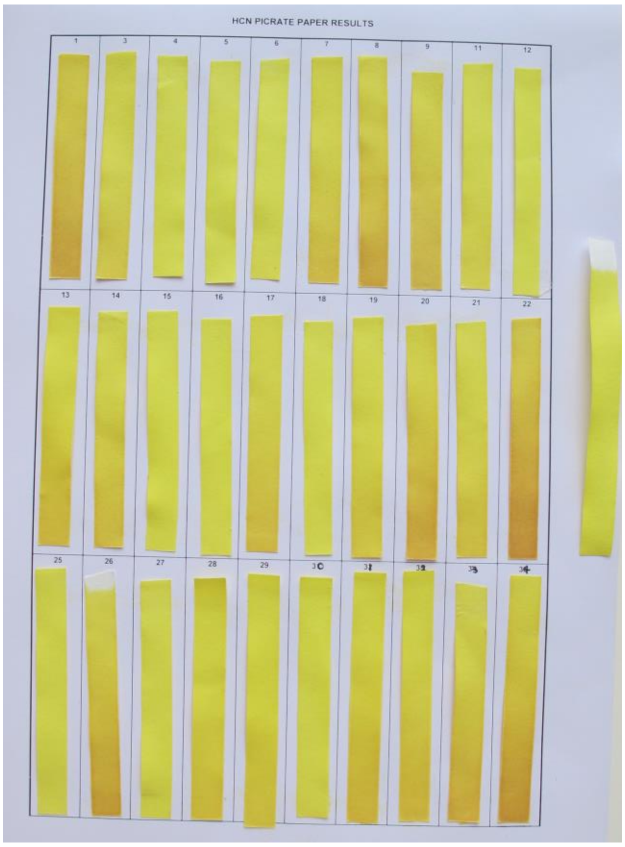


Figure 32. Picrate papers image for The Eye-dropper Technique. The single paper on the right of the page is the standard.

Images were opened up in Adobe Illustrator CS version 11.0.0 (Adobe Systems Incorporated, 2003) and the eyedropper tool used to first select six random areas from top to bottom of the standard picrate paper. From the colour identified by the eyedropper tool the green hue number (degrees of colour) was recorded each time. It was found that the darker orange the picrate paper was, the lower the green hue would be and was the most reliable of the three colours to use (based on trials using both visual ranking and the eyedropper technique). Out of these six figures the minimum value was picked as the standard zero HCN for that batch. The eyedropper was then placed over the darkest area on the bottom of each picrate paper with the green hue figure recorded. Each green hue figure was subtracted from the standard and the difference used as the leaf-HCN amount. To ensure those with no leaf-HCN were recorded as such, the confidence interval from the six standard results was used, i.e. a confidence interval of seven would mean that a difference between the picrate paper and the standard, up to and including seven, would be replaced with a zero.

11.6 **Data Analysis**

11.6.1 Initial Data Exploration

All parameters were first tabulated with means, standard deviations and standard errors calculated. Bar charts and line graphs were produced in Excel (Microsoft, 2013) (version 15.0.4551.1005) to identify initial differences between ecotypes and treatments.

Data were then saved as CSV files for use in R statistical software version 3.1.0 (R Core Team, 2013). Histograms were created using Rcmdr version 2.0-4 (Fox & Bouchet-Valat, 2014) to help determine the distribution of each data set; ANOVA (for parametric data) and Kruskall-Wallis (for non-parametric data) was also used in this program to determine initial significant differences.

Scattergraph matrices and boxplots were created using RStudio (RStudio, 2013) (version 0.98.994) to identify similarities and correlation between datasets.

11.6.2 Generalized Linear Mixed Model (GLMM)

Generalized linear mixed models (GLMM) were used to statistically predict ecotypic distributions with specific sub-sets of response variables, as this statistical technique is good for species-specific models (Guisan *et al.,* 1999). GLMM tuition and written aids were used in the application and interpretation of the models in R Studio (RStudio, 2013) (version 0.98.994) (Field *et al.,* 2009; Zuur *et al.,* 2009; Winter, 2013; Smith, 2014). It was originally hoped that one model could be used (with the response variable changed for each dataset). However, due to the high number of variables and interactions, the sample size prevented a full model analysis (Smith, 2014). Partial models were therefore processed and resulting effects plots along with the Akaike Information Criterion (AIC) and P values from ANOVA comparisons values used to determine the most important factors for the final model (Winter, 2013, Smith, 2014). The standard used in all models was Cockey Down (a calcareous loam/grazed ecotype), in calcareous loam and grazed treatment. The standard was picked to represent the [presumed] best combination for *Lotus corniculatus,* a calcareous loam ecotype was chosen as calcareous pastures represent the most common habitat for the plant (Grime *et al.,* 1992), and grazed treatment selected as this management [or cutting with clippings removed] is recommended in Natural England guidance after species-rich grassland creation (Natural England, 2010).

For the first model (Model A) it was hoped to include each ecotype, the treatment soil and management treatment. The following are the partial models used with each dataset (the bold text is the response variable which was changed to that being investigated at the time of each analysis). The most suitable combination for model A was selected for each response variable (Table 16).

Model A.1:

<-lmer(**response_variable**~EcotypeSeed+soil+management+(1|f.replic), data=**response_variable**)

This model uses the factors of ecotype seed (EcotypeSeed), soil treatment (soil) and management treatment (management) as fixed effects, it blocks them by replicate to eliminate any glasshouse effect.

Model A.2:

<-lmer(**response_variable**~EcotypeSeed+soil+(1|management/f.replic)data= **response_variable**)

This model variation differs in that it uses management treatment and replication as a nested random effect.

Model A.3:

<-lmer (**grazed_biomass**~seed+soil+(1|f.replic),data=**grazed_biomass**)

Model A.3 was only used on grazed treatment clippings biomass due to this part of the experiment only involving the grazed treatment plants.

To determine significance P values of factors the Likelihood Ratio Test was conducted (Winter, 2013). The chosen (A, B and C) model was repeated, each time with one of the factors removed (the null model). ANOVA was calculated for the comparison between the full model and null model each time. AIC and P values for each comparison indicated which factors were significant and accounted for most effect in the model (Winter, 2013).

As the largest variation in the data was seen to be the management treatment it made sense to repeat those models separately between the two managements (therefore the datasets were split between managements ‘Model B.1 and B.2’).

Model B.1:

<-lmer (**response_variable**~seed+soil+(1|f.replic), data=**response_variable.grazed**)

Model B.2:

<-lmer (**response_variable**~seed+soil+(1|f.replic), data=**response_variable.unmanaged**)

The last model was intended to look at any interaction between ecotype with treatment and also look at donor site soil and management effects. When the dataset had already been split by management, the split was carried out again for this model (Model C.1 and C.2), when there was no management split the dataset was left intact (Model C.3).

Model C.1:

<-lmer(**response_variable**~EcotypeSoil+soil+EcotypeMgmt+EcotypeSoil *Soil+ (1/f.replic),data=**reponse_variable.grazed**)

Model C.2:

<-lmer(**response_variable**~EcotypeSoil+soil+EcotypeMgmt+EcotypeSoil *Soil+ (1/f.replic),data=**reponse_variable.unmanaged**)

Model C.3:

<-lmer(**response_variable**~EcotypeSoil+soil+EcotypeMgmt+management+ +EcotypeSoil*Soil+EcotypeMgmt*management+(1/f.replic),data=**reponse _variable**)

As before, these models were repeated with ANOVA comparison of null (partial) models to establish P value significance of factors.

All of the above models are calculated using a Gaussian distribution. For those which needed Poisson distribution due to the nature of the data (count data or non-parametric data), 'family = poisson' was entered into the model equation before 'data='.

The following table (Table 16) outlines which models were used for each response variable.

Table 16. Models used with each response variable. Refers to model descriptions in 'Generalized Linear Mixed Models' and 'Spatial Autocorrelation' in Chapter 11.

Response Variable	GLMM (lme4) models used	Spatial Autocorrelation GLMM (nlme) models used
Main Stem Lengths (Harvest)	A.1, B.2, B.3, C.1, C.2	D.1, D.2
Main Stem Lengths (Soil+Mgmt)	A.1, B.2, B.3, C.1, C.2	N/A
Leaflets per main stem (Harvest)	A.2, B.2, B.3, C.1, C.2	D.1, D.2
Stems per plant (Harvest)	A.2, B.2, B.3, C.1, C.2	D.1, D.2
Hirsuteness	A.1, C.3	D.3
Time taken for seed pod formation	A.1, B.1, B.2, C.1, C.2	D.1, D.2
Seed pod number	A.1, B.1, B.2, C.1, C.2	D.1, D.2
Seed pods sampled	A.1, B.1, B.2, C.1, C.2	N/A
Mean seeds per pod	A.1, B.1, B.2, C.1, C.2	D.1, D.2
Grazed treatment clippings dry biomass	A.3, C.3	D.3
Harvest dry biomass (vegetation)	A.2, C.3	D.3
Harvest relative moisture content (%) of biomass (vegetation)	A.1, B.2, B.3, C.1, C.2	D.1, D.2
Hydrogen Cyanide (HCN)	A.1, C.3	D.3
Nitrogen	A.1, B.2, B.3, C.1, C.2	D.1, D.2
Total flower number	A.2, C.3	D.3
Pre harvest flower scent	A.1, C.3	D.3

11.6.3 Spatial Autocorrelation

The mixed modelling package NLME (Non-linear Mixed Effects) in RStudio (2013) (version 0.98.994) was used to look at the spatial autocorrelation between ecotype sites. Additional columns of OS Grid Northing and Easting data were entered into each dataset. The model was split between management treatments again when relevant.

Model D.1:

<-lme(**response_variable**~EcotypeSeed+Soil+Management,data= **response_ variable**.unmanaged, random = ~1|f.replic)

Model D.2:

<-lme(**response_variable**~EcotypeSeed+Soil+Management,data=na.omit (**response_variable**. grazed),random= ~1|f.replic)

These two models split the data between management treatment, Model D.2 also has the addition of 'na.omit' to allow data with NA (zero) values to be used by ignoring them.

Model D.3:

<-lme(**response_variable**~EcotypeSeed+Soil+Management,data= **response_ variable**, random = ~1|f.replic)

This model was used when the management split was not needed.

A semi-variogram was then created for each, to show the effect which spatial proximity between ecotype sites had on the response variable, these model graphs were built and interpreted with tuition and written aids (Neilson and Wendroth, 2003; Winter, 2013; Smith, 2014). To establish whether spatial distance between sites was a significant factor in the models, an ANOVA test was completed each time, to compare the model containing the spatial factor to the model without. The AIC and P values were then used to see which model showed the best fit and if there was significant difference with the spatial factor. Table 16 indicates which models were used for each response variable.

Spatial Results

The variograms generated can be found in Appendix XI, along with AIC numbers and ANOVA P values between the null model and the spatial model. As the ANOVA AIC numbers and P values ruled out any significant effect on the models from spatial distance between ecotype sites they have been removed from the results write-up. The pattern shown in each variogram also did not indicate any spatial autocorrelation was influencing these parameters apart from for main stem length. Therefore a small paragraph has been kept in this sub-chapter.

12 RESULTS (STUDIES 1 & 2)

Please note that within this chapter, calcareous sand is referred to as ‘sand’ and cut with aftermath grazing is referred to as ‘cut’. ‘Grazing treatment’ refers to simulated grazing from cutting by hand.

Once the seedlings had been potted on, the experimental design detailed in chapter 11 was implemented and parameters monitored for use in identifying plant fitness and/or changes in relation to herbivore nutrition in relation to treatments. This chapter displays the growing and harvest results of the main experiment of this thesis.

Table 17 lists abbreviations used in results for ecotypes and treatments and should be used in conjuction with subsequent tables and figures.

Table 17. Key to ecotype references used in results

Ref.	Ecotype Name	Ecotype Management	Ecotype Soil
cd	Cockey Down	Grazed	Calcareous loam
ss	Southstoke	Cut [with aftermath grazing]	Calcareous loam
wb	Woodborough	Cut [with aftermath grazing]	Calcareous loam
ff	Folly Farm	Cut [with aftermath grazing]	Neutral loam
hh	Hellenge Hill	Grazed	Neutral loam
sp	Salisbury Plain	Unmanaged	Neutral loam
bd	Berrow Dunes	Unmanaged	[Calcareous] sand
ww	Woolacombe Warren	Unmanaged	[Calcareous] sand
dw	Dawlish Warren	Grazed	[Calcareous] sand

Ref	Treatments
C	Calcareous loam treatment
N	Neutral loam treatment
S	[Calcareous] sand treatment
G	Grazed treatment
U	Unmanaged treatment

12.1 **Plant Fitness Results: Regular Monitoring (Vegetation)**

12.1.1 Main Stem Lengths

Means were calculated for monthly main stem length, (Appendix XII) and plotted into line graphs (Figure 33 and Figure 34). Plants grown in calcareous loam soil treatment had main stems that were consistently the longest, significantly so between April and August 2012 (Figure 33). Neutral loam soil treated plants grew least until July where it overtook the sand plant growth which therefore became shortest. Sand treatment remained the shortest during the second year, with less variation between neutral loam and sand treatment. Unmanaged treatment was significantly higher than grazed treatment between April and August 2012.

Little variation was evident between ecotype main stem length during the first few months (Figure 33), with Woodborough and Folly Farm generally the shortest. Salisbury Plain ecotype had the least after-growth a month after harvest and was again the shortest the following year.

When ecotypes were grouped by their home-site soil types (Figure 34), there was seen to be little variation and no significant differences in any given month.

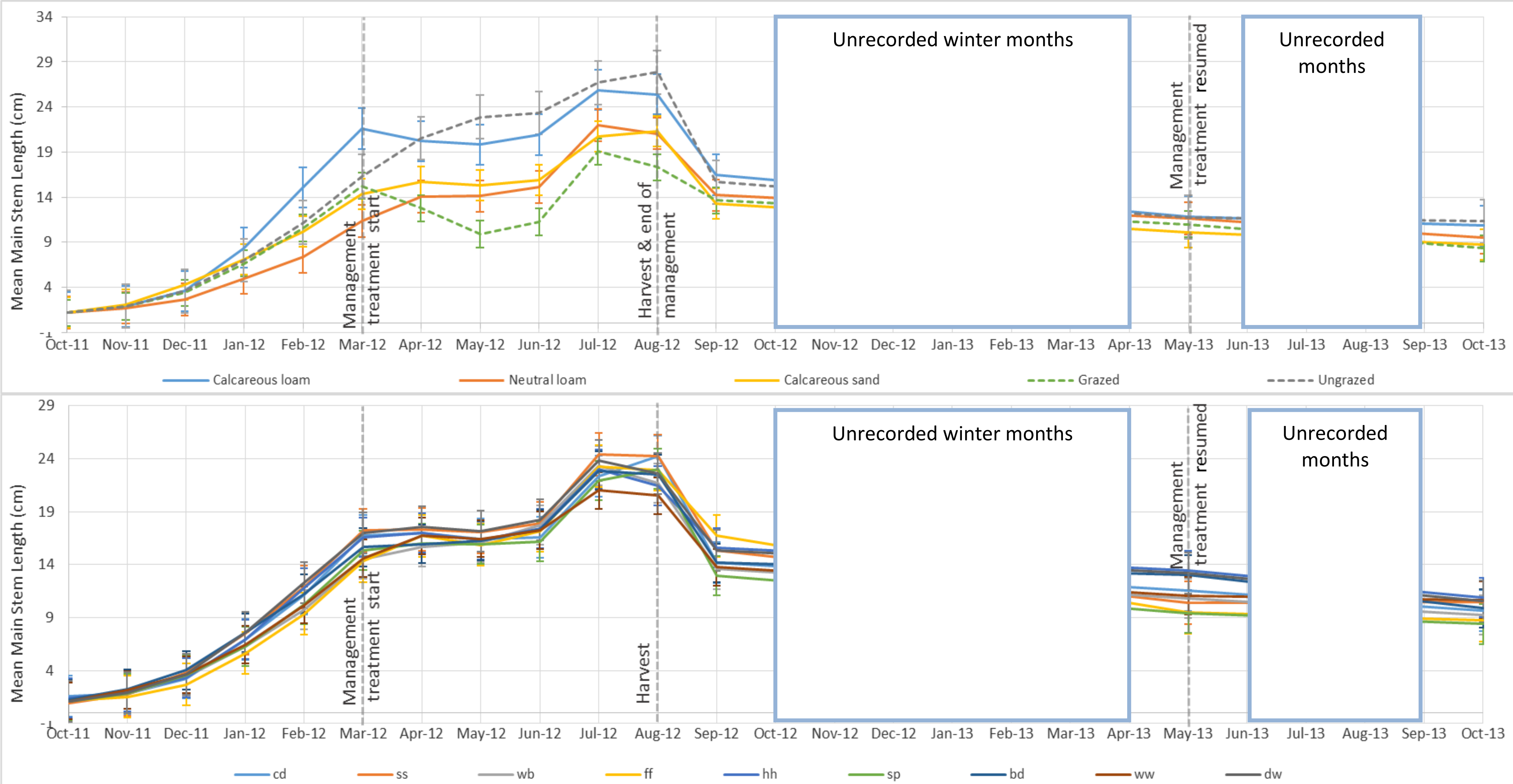


Figure 33. Mean main stem lengths (+ 1 SE) recorded monthly, upper graph is grouped by treatment (soil treatment n=144, management treatment n=216), lower graph is grouped by ecotype (n=48). See Table 21 for ecotype key. P Values are shown in Table 18.

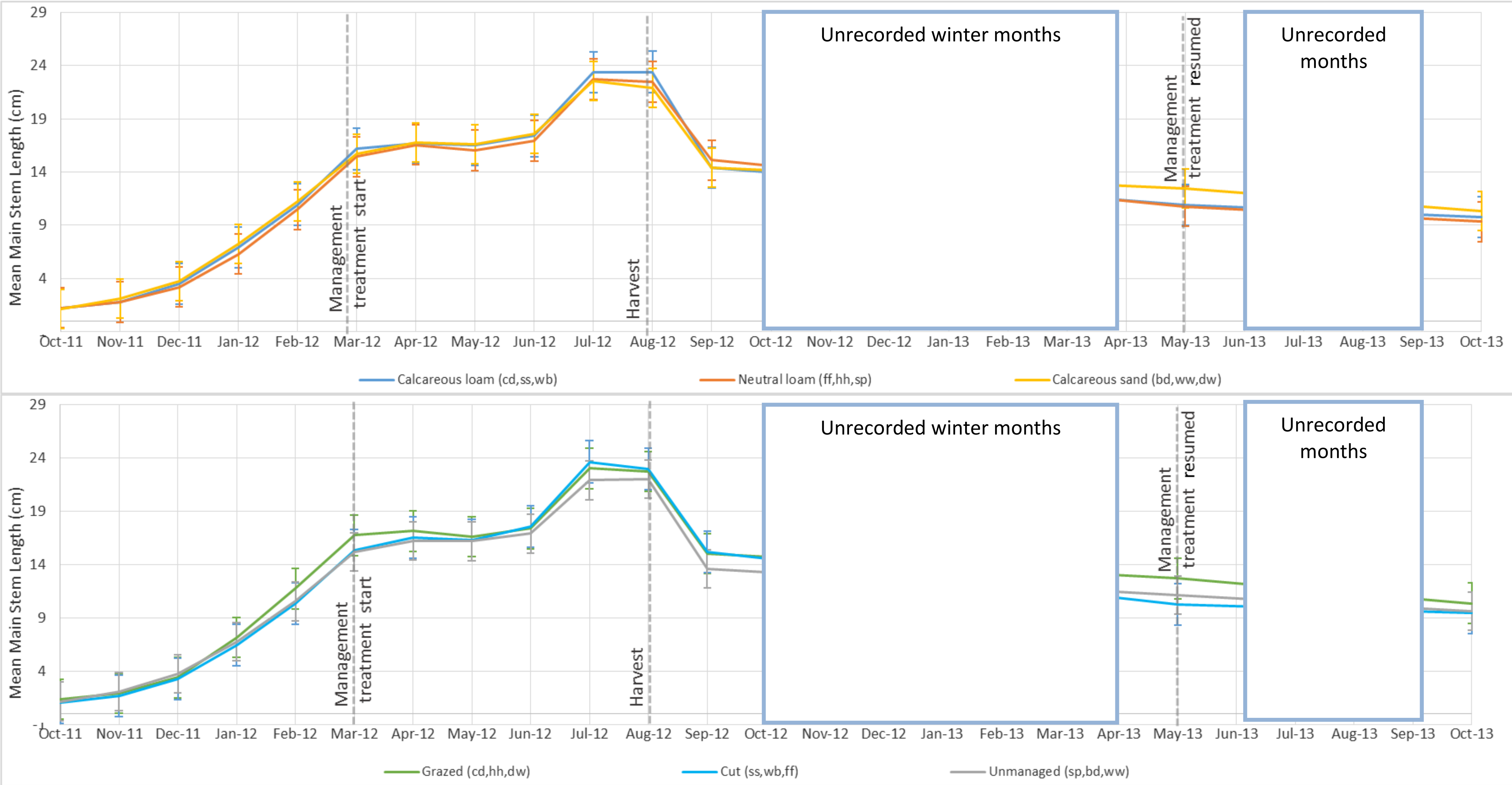


Figure 34. Monthly mean (+ 1 SE) main stem lengths. Top graph grouped by Ecotype site soils (n=144), bottom graph grouped by Ecotype site managements (n=144). Greyed out blocks are months when main stem length was not recorded. P Values are shown in Table 18.

12.1.2 Stems per Plant

Results of stem number counts per plant are from monthly recordings between October 2011 and March 2012 with a final recording at harvest (tabulated data in Appendix XII). There was slow formation of additional stems between October 2011 and February 2012 (Figure 35), with a faster increase in mean number of stems per plant between February and March 2012.

All ecotypes (Figure 35 and Figure 36) had similar stem number until February 2012. Sand ecotypes (Woolacombe Warren, Berrow Dunes and Dawlish Warren) had the highest number of stems throughout (P=0.001), this difference became more pronounced by harvest in August 2012. Ecotypes from cut management sites had the least number of stems from March to harvest (P=0.050). The ecotype with smallest number of stems at harvest was Woodborough (P=0.020).

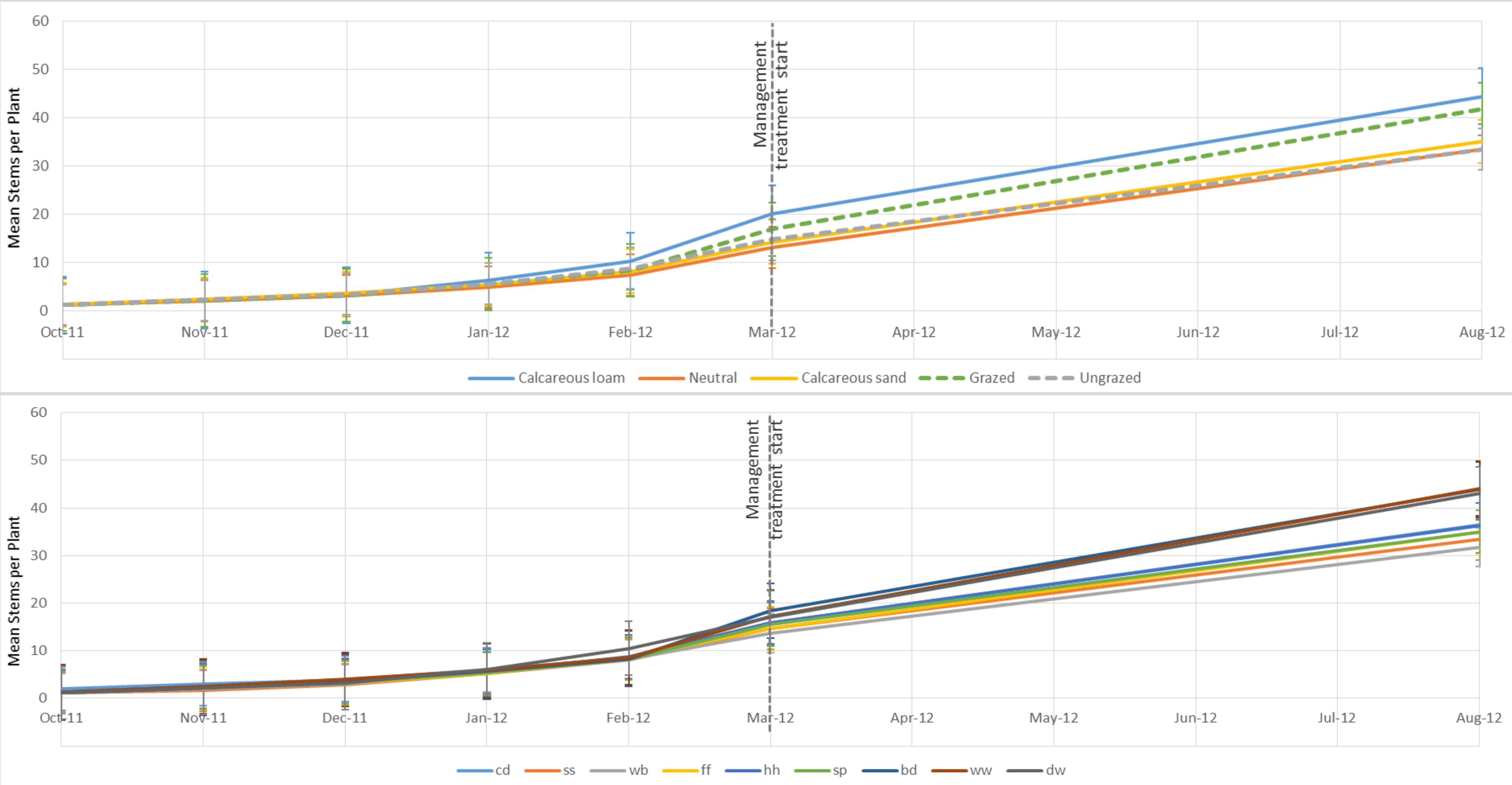


Figure 35. Monthly mean (+ 1 SE) stem number per plant. Top graph is grouped by treatment (soil treatment N=144, management treatment N=216), bottom graph is grouped by ecotype (N=48). See Table 21 for ecotype key. P Values are shown in Table 18.

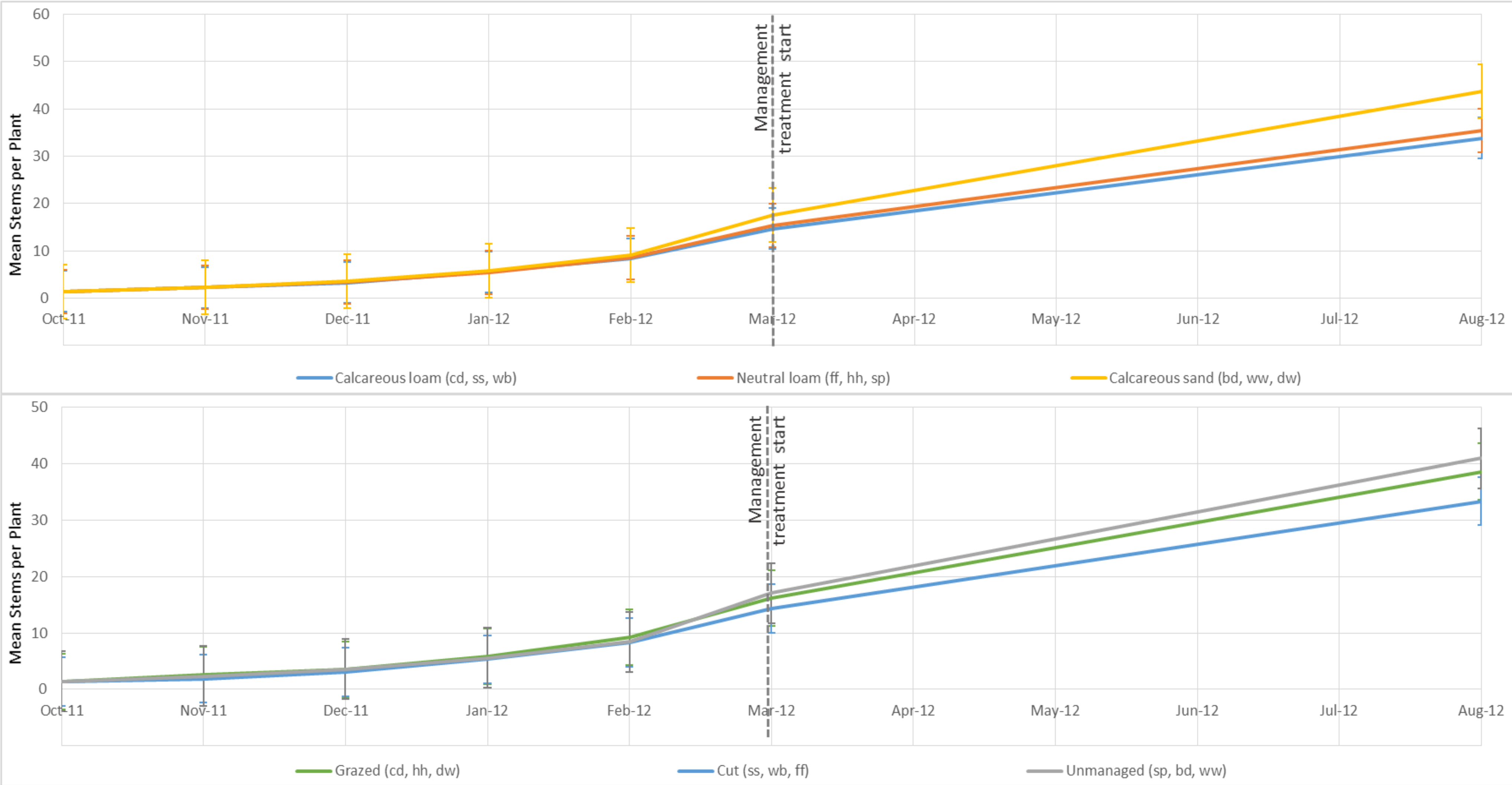


Figure 36. Monthly mean (+ 1 SE) stem number. Top graph is grouped by ecotype site soils (N=144). Bottom graph is grouped by ecotype site managements (N=144). See Table 21 for ecotype key. P Values are shown in Table 18.

12.1.3 Leaflets per Main Stem

Plots by ecotype site soils and management regimes (Figure 37) (tabulated data in Appendix XII) showed there was a greater number of leaflets per main stem on plants grown in calcareous loam soil treatment compared to other soils ($P=0.002$), and also more in the unmanaged treatment ($P<0.001$). By harvest time in August 2012 there is least number of leaflets (per main stem) on plants grown in sand soil treatment ($P=0.001$).

A similar pattern was found througout the ecotypes for leaflet number (per main stem) (Figure 37) as for stem number (Figure 35) except for Woolacombe Warren which went from having the least leaflets per main stem in March to having the most by August 2012.

When ecotypes were grouped by Ecotype soil and Ecotype management (Figure 38), sand ecotypes (Berrow Dunes, Woolacombe Warren, Dawlish Warren) had a noticably high number of leaflets (per main stem) compared to the other ecotypes ($P<0.001$) from November 2011, this difference is enlarged from May to August 2012. Ecotypes from cut management sites have a lower number of leaflets (per main stem) from November 2011 to August 2012 ($P=0.004$).

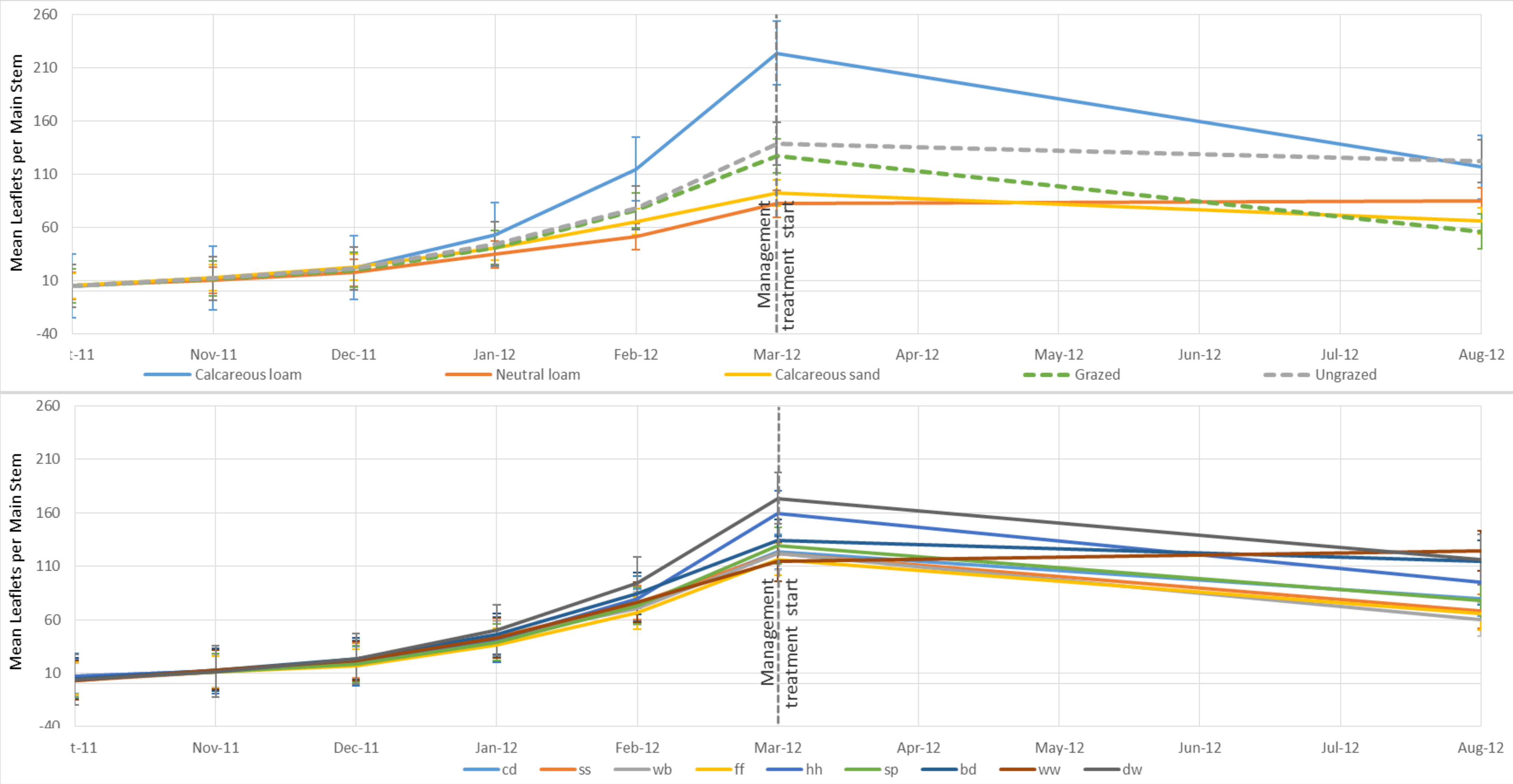


Figure 37. Mean (+ 1 SE) leaflets per main stem. Top graph is grouped by treatment (soil treatment n=144, management treatment n=216, and the bottom graph is grouped by ecotype (n=48). See Table 21 for ecotype key. P Values are shown in Table 18.

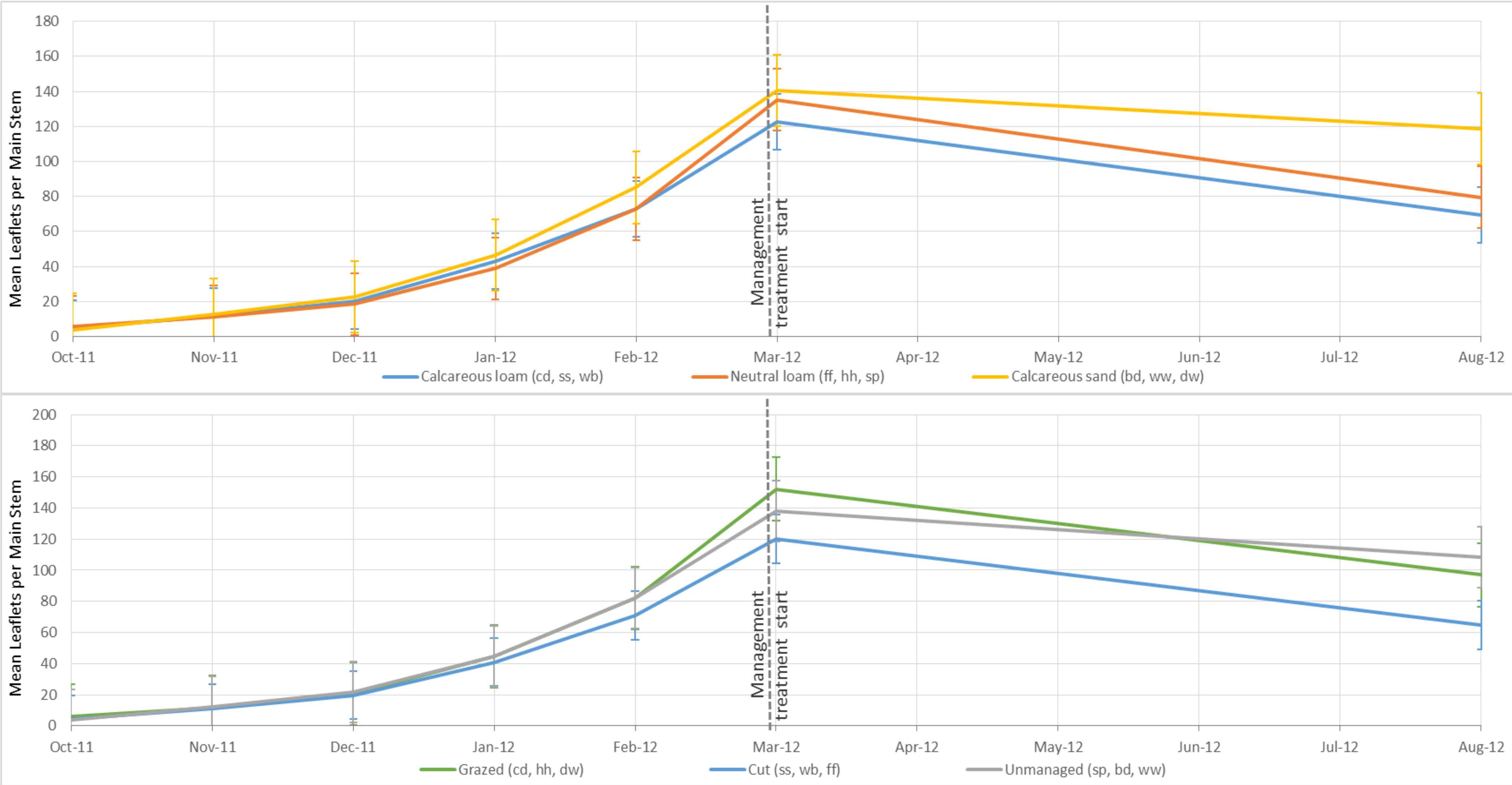


Figure 38. Monthly (+ 1 SE) mean leaflets per main stem. Top graph is grouped by ecotype site soils (n=144). Bottom graph is grouped by ecotype site managements (n=144). See Table 21 for ecotype key. P Values are shown in Table 18.

12.1.4 Branches per Main Stem

There was little variation between branch number per main stem (Figure 39) (tabulated data in Appendix XII). Most of the variation is seen in the treatments line graph (Figure 39), with unmanaged treatment plants having more branching per main stem than grazed treatment (significant at harvest P=0.018). Calcareous loam soils produce greater number of branching than the other treatment soils (significant in February and March).

Ecotypes from sand home-sites (Figure 40) had early formation of branching during December to February but were lowest at March, they were again highest by the August harvest.

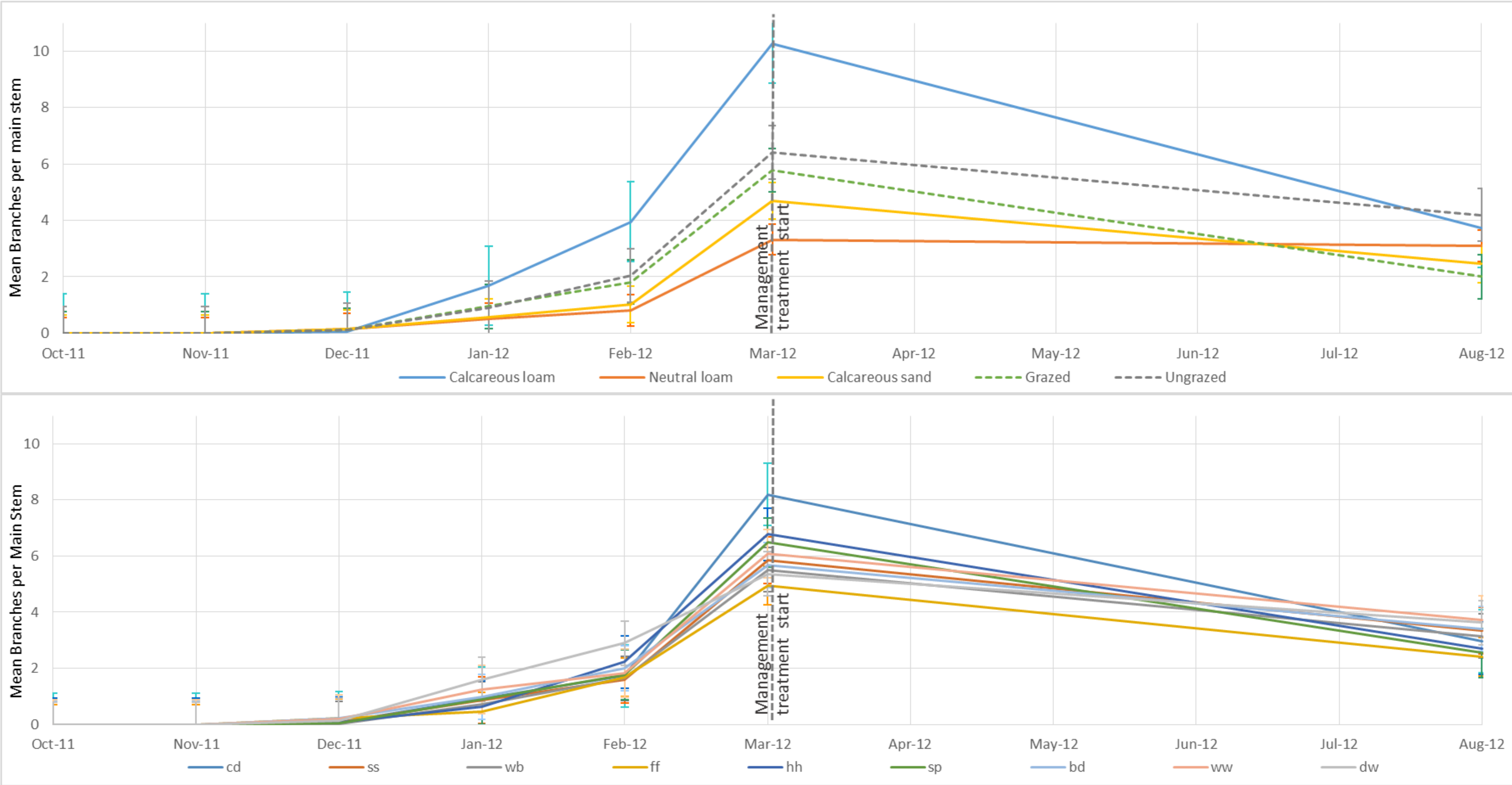


Figure 39. Mean (+ 1 SE) monthly branches per main stem. Top graph is grouped by treatment (soil treatment n=144, management treatment n=216), bottom graph is grouped by ecotype (n=48). See Table 21 for ecotype key. P Values are shown in Table 18.

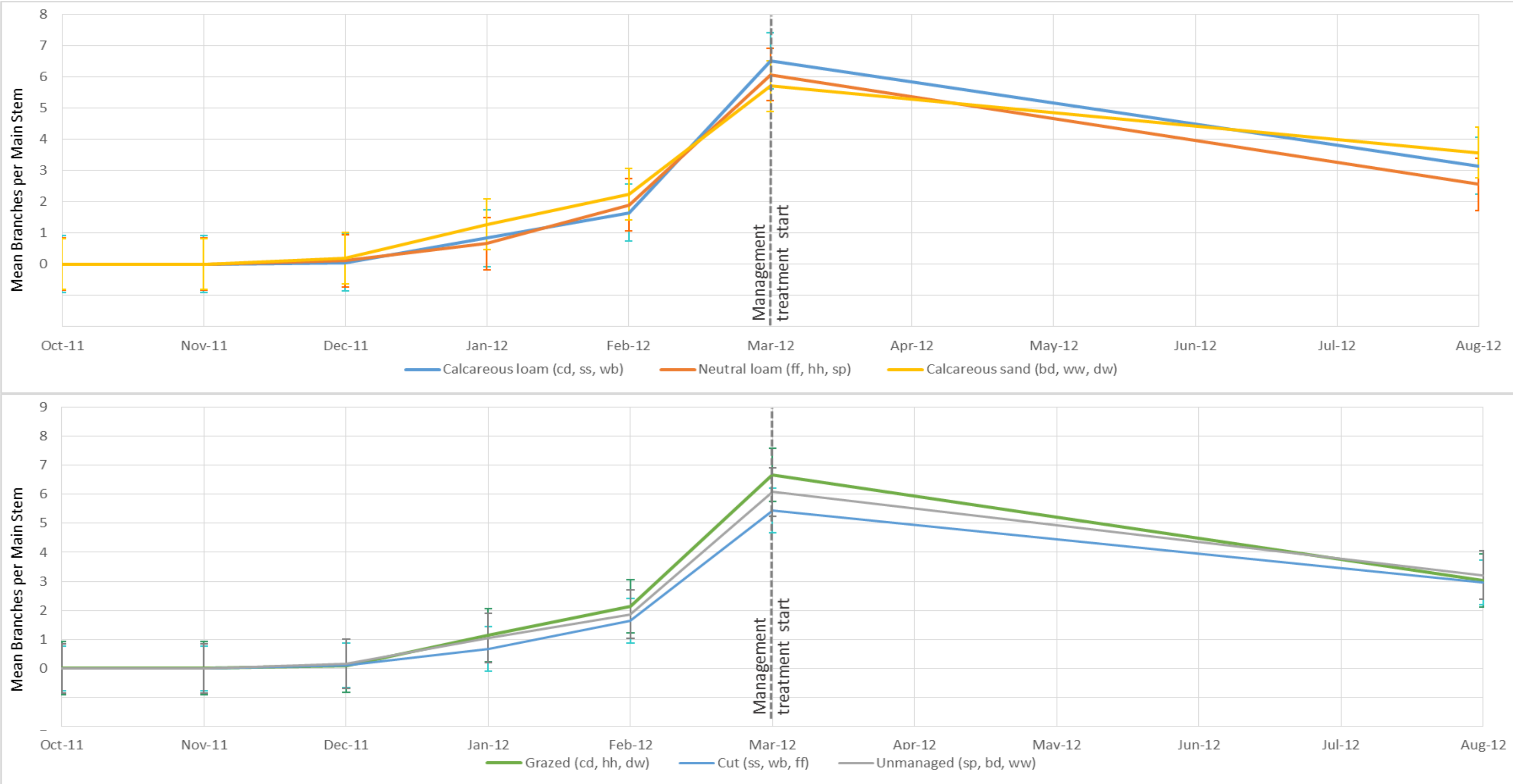


Figure 40. Mean (+ 1 SE) monthly branches per main stem. Top graph is grouped by ecotype site soils (n=144). Bottom graph is grouped by ecotype site managements (n=144). See Table 21 for ecotype key. P Values are shown in Table 18.

12.1.5 Summary of Results – Regular Monitoring Parameters (Vegetation)

Main stem length: Calcareous loam soil produced significantly longer main stem length. Plants grown in sand treatment soil were significantly shorter than the rest by the end of the first year and throughout the second year. Salisbury Plain ecotype took longest to recover main stem growth after harvest.

Stems per plant: Plants grown in calcareous loam soil and those receiving grazed treatment had significantly more stems than other treatments. Woodborough had lowest (significant) stem number. When grouped together, sand ecotypes had significantly higher stem number, becoming more pronounced by harvest. Ecotypes from cut sites had least (significant) stems from management commencement in March to harvest in August.

Leaflets per main stem: Plants grown in calcareous loam soil and those in unmanaged treatment had significantly higher leaflet number. By harvest there were least leaflets on plants grown in sand. Woolacombe Warren had fastest growth of leaflets, going from least of all ecotypes in March to most by August. When grouped by ecotype, sand ecotypes had significantly more leaflets from November onwards, and ecotypes from cut sites had lower leaflet number from November onwards (significant).

Branches per main stem: Unmanaged treatment plants had more branching, which became significant at harvest.

12.2 **Plant Fitness Results: Harvest Measurements (Vegetation)**

12.2.1 All Vegetative Growth Parameters

Mean harvest results of main stem lengths; stems per plant; leaflets per main stem; branches per main stem; and longest branch per main stem are tabulated in Table 18. Vegetative growth is shown at the time of harvest for each parameter, titled ‘Harvest’. To examine the growth which happened only after both management treatment and soil treatment were in place (April-August) a separate data set was collated, denoted ‘Soil+Mgmt’ and is the harvest measurement minus the growth measurement immediately prior to first management in March. The main stem lengths ‘Soil+Mgmt’ were calculated differently to the other parameters: For grazed treatment plants, the main stem length prior to each grazed treatment minus 7cm (the length ‘grazed’ to) was added to the final harvest measurement to show actual growth. Main stem length throughout the whole experiment was also calculated in this way but added all growth after the potting up stage (October-August), this is denoted ‘All’ in the figures and tables (and was only calculated for main stem length). When interpreting the tabulated results, ANOVA (for main stem lengths) and Kruskal-Wallis (for leaflets per main stem, stems per plant, branches per main stem and longest branch per main stem) were used to establish which differences were significant.

Table 18. Mean growth measurements at harvest. KEY: 'Harvest' is the final parameter measurement at harvest. 'Soil+Mgmt' is harvest measurement minus growth prior to management treatment. 'All' is only calculated for Main Stem Length and is monthly growth prior to potting-up stage (Oct 11-Aug 12), 'Soil+Mgmt' for Main Stem Length is calculated the same, but prior to management treatment (April-Aug 12), this is to include monthly regrowth of grazed treatment plants. Longest branch per main stem was only measured at harvest (Ecotype *n*=48, Soil *n*=144, Management *n*=216). *P* numbers are from ANOVA for main stem lengths and Kruskall-Wallis for other parameters. See Table 17 for ecotype and treatment key.

			Ecotype										**Soil**		**Management**	
			cd	**ss**	**wb**	**ff**	**hh**	**sp**	**bd**	**ww**	**dw**	**C**	**N**	**S**	**G**	**U**
All (Oct 11-Aug 12)	**Main Stem Length (cumulative growth) cm**		**33.5**	**35.9**	**30.8**	**31.4**	**33.5**	**31.1**	**34.1**	**30.4**	**34.4**	**41.0**	**27.9**	**29.5**	**39.0**	**26.6**
		SE	1.8	2.5	2.2	2.3	2.6	2.0	1.8	1.8	2.3	1.3	0.8	1.3	2.7	1.9
			P=0.663									**P<0.001*			**P<0.001*	
Soil+Mgmt (April - August 2012)	**Main Stem Length (cumulative growth) cm**		**16.8**	**18.1**	**15.4**	**15.8**	**16.0**	**14.7**	**17.4**	**15.0**	**16.1**	**18.6**	**15.5**	**14.3**	**24.9**	**7.3**
		SE	1.5	2.1	1.7	2.1	2.2	1.5	1.2	1.6	1.9	1.3	0.8	0.9	0.8	0.6
			P=0.977									**P=0.024*			**P<0.001*	
	Leaflets per main stem		**-42**	**-54**	**-61**	**-51**	**-65**	**-52**	**-20**	**10**	**-57**	**-107**	**3**	**-27**	**-71**	**-16**
		SE	20	16	15	16	19	18	17	22	21	14	8	7	8	9
			P=0.102									**P<0.001*			**P<0.001*	
	Stems per plant		**21**	**19**	**18**	**20**	**21**	**19**	**25**	**27**	**26**	**24**	**20**	**21**	**25**	**19**
		SE	2	2	2	2	2	2	3	3	3	2	1	1	1	1
			**P=0.001*									*P=0.585*			**P<0.001*	
	Branches per main stem		**-5**	**-2**	**-2**	**-3**	**-4**	**-4**	**-2**	**-2**	**-2**	**-7**	**0**	**-2**	**-2**	**-4**
		SE	2	1	1	1	1	1	1	1	1	1	0	1	1	1
			P=0.422									**P<0.001*			**P=0.018*	
Harvest	**Main Stem Length cm**		**24.2**	**24.3**	**21.7**	**23.0**	**21.4**	**23.0**	**22.5**	**20.5**	**22.6**	**25.4**	**21.1**	**21.3**	**5.6**	**9.0**
		SE	1.3	1.4	1.3	1.4	1.5	1.1	1.5	1.1	1.1	0.8	0.6	0.8	0.4	0.6
			P=0.543									**P<0.001*			**P<0.001*	
	Leaflets per main stem		**80**	**68**	**60**	**66**	**95**	**78**	**115**	**125**	**116**	**117**	**85**	**66**	**56**	**122**
		SE	15	7	6	6	15	10	18	22	23	12	8	4	2	9
			**P=0.001*									**P=0.001*			**P<0.001*	
	Stems per plant		**36**	**33**	**32**	**35**	**36**	**35**	**44**	**44**	**43**	**44**	**33**	**35**	**42**	**33**
		SE	3	2	2	3	3	2	3	3	3	2	1	2	1	1
			**P=0.001*									**P<0.001*			**P<0.001*	
	Branches per main stem		**3**	**3**	**3**	**2**	**3**	**3**	**3**	**4**	**4**	**4**	**3**	**2**	**2**	**4**
		SE	1	0	0	0	1	0	1	1	1	0	0	0	0	0
			P=3236									**P=0.007*			**P<0.001*	
	Longest branch per main stem cm		**5.6**	**6.6**	**5.5**	**5.4**	**5.4**	**4.9**	**6.1**	**5.4**	**6.7**	**7.3**	**5.2**	**4.7**	**3.8**	**7.7**
		SE	0.6	0.8	0.6	0.9	0.8	0.6	1.0	0.7	0.9	0.5	0.4	0.4	0.2	0.4
			P=0.283									*P=0.151*			*P=0.741*	

Similar traits were shown throughout main stem lengths for ‘All’ and ‘Soil+Mgmt’ (Table 18). Grazed treatment produced highest growth (‘All’ P<0.001, ‘Soil+Mgmt’ P<0.001), but shortest at ‘Harvest’ (P<0.001). Calcareous loam treatment soils produced the most Main stem length growth (‘All’ P<0.001, ‘Soil+Mgmt’ P=0.024, ‘Harvest’ P<0.001).

Plants with greatest stem number were those grown in calcareous loam soil (‘Harvest’ P<0.001) and those with grazed treatment applied (‘Harvest’ P<0.001, ‘Soil+Mgmt’ P<0.001). Of all ecotypes, the three from sand home-sites had the most stems per plant at ‘Harvest’ (P=0.001) and in ‘Soil+Mgmt’ (P=0.001).

For leaflets and branches per main stem most results were negative values in the ‘Soil+Mgmt’ category even when receiving unmanaged treatment, suggesting branches and leaflets had been shed after their peak biomass production. Therefore looking at the ‘Harvest’ category, highest numbers of leaflets and branches per main stem were from plants grown in calcareous loam soil (P=0.001 and P=0.007 respectively). Of the ecotypes, those from sand home-sites had highest leaflet number per main stem (P=0.001). No significant variation was seen for branch number (per main stem) between management treatments.

From the results in Table 18 it was decided that only main stem length, stems per plant and leaflets per main stem would be analysed further as little variation was found within the branch number and branch length response. Scattergraph results of these parameters (Appendix XVII) revealed strong correlation between ‘All’ and ‘Soil+Mgmt’ for main stem length, therefore, as ‘Soil+Mgmt’ had weakest correlation with ‘Harvest’ out of the two, ‘Soil+Mgmt’ and ‘Harvest’ datasets were chosen for further analysis. There was also moderate correlation between ‘Soil+Mgmt’ with ‘Harvest’ for stems per plant and for Leaflets per main stem, therefore only ‘Harvest’ datasets for leaflets and stems were analysed further. All other relationships appeared weak.

12.2.2 Further Analysis: Main Stem Length, Leaflets per Main Stem, Stems per Plant

The results for ‘Harvest’ and ‘‘Soil+Mgmt’ growth for Main Stem Length, and ‘Harvest’ results for leaflets per main stem and stems per plant were placed into a General Linear Model (GLM). The distribution of main stem length data sets were found to follow a normal distribution therefore the model used for these was Gaussian. The other data sets were found to be skewed and as these were count data, the model was created using Poisson. As all had significant differences between management treatments the model was also split between them. Due to the large number of models processed, the results are tabulated (Table 19) for ease of interpretation. Effects plots for each of the model outcomes can be found in Appendix X.

Table 19. Linear Mixed Effects Regression (GLMM) models of harvest growth parameters. KEY: ‘Harvest’ is the final parameter measurement at harvest. ‘Soil+Mgmt' is only calculated for main stem length and is monthly growth prior to potting up stage (Oct 11-Aug 12), including monthly regrowth of grazed treatment plants. Cockey Down in calcareous loam + grazed treatment is used as the standard, ‘significant higher’ and ‘significant lower’ columns refer to significant differences in relation to this standard. Underlined ecotypes are those with greatest influence on the model. See Appendix X for P values and Table 17 for ecotype and treatment key.

		Management Treatment Split	**Ecotype** Significant Lower	**Ecotype** Significant Higher	**Soil Treatment** Significant Lower	**Soil Treatment** Significant Higher	**Management Treatment** Significant Lower	**Management Treatment** Significant Higher
Main Stem Length	**Harvest**	**All**	*nsd*	*nsd*	N, S	C	G	U
		Grazed	ww, bd	*nsd*	N, S	*nsd*		
		Unmanaged	*nsd*	*nsd*	N, S	*nsd*		
	Soil+ mgmt	**All**	*nsd*	*nsd*	N, S	C	U	G
		Grazed	*nsd*	*nsd*	N, S	*nsd*		
		Unmanaged	*nsd*	*nsd*	*nsd*	N		
Stems per Plant	**Harvest**	**All**	wb	bd, ww, dw	N, S	C	U	G
		Grazed	wb	bd, ww, dw, sp	N,S	C		
		Unmanaged	sp, ss	bd	N, S	C		
Leaflets per main stem	**Harvest**	**All**	wb, ff, ss	bd, ww, dw, hh	N, S	C	G	U
		Grazed	*nsd*	bd, ww, dw, hh, wb, sp, ss	S	*nsd*		
		Unmanaged	wb, ff, ss, sp	bd, ww, dw, hh	N, S	*nsd*		

The GLMM model results (Table 19) and P values (Appendix X) identified that Calcareous loam treatment soil led to significantly higher growth of harvest main stem length ($P<0.001$), stems per plant ($P<0.001$) and leaflets per main stem ($P<0.001$). The grazed treatment stimulated faster main stem length growth ($P<0.001$) and stem formation ($P<0.001$), though at harvest, grazed treatment plants had least main stem length ($P=0.001$) and leaflets per main stem ($P<0.001$). The only ecotype differences seen in main stem length were significantly lower results for Woolacombe Warren and Berrow Dunes in grazed treatments ($P=0.014$). These two ecotypes from sand sites, along with the third sand ecotype (Dawlish Warren) had significantly higher results for stem number per plant ($P<0.001$) and leaflet number per main stem ($P<0.001$), a result that carried through both management treatments for leaflet number (per main stem) (grazed treatment $P<0.001$, unmanaged treatment $P<0.001$), but only grazed treatment for stems per plant ($P<0.001$) (apart from Berrow Dunes $P<0.001$). Hellenge Hill also had significantly higher leaflet number (per main stem) throughout both management treatments.

As the only significant differences for Main Stem Length 'Soil+Mgmt' were seen for treatments rather than ecotypes this was eliminated from further analysis of ecotypic variation, only 'Harvest' main stem length, leaflets per main stem and stems per plant were studied further.

Further models were created to see if there were significant differences caused by the ecotype soil or ecotype management and also interactions from different ecotype/treatment combinations. However, there was seen to be no significant differences between ANOVA models with the original model for main stem length.

The interaction model for Stem number (Figure 41) showed a better fit than the original, with all factors having significant effects on the model (P values in Appendix X). This confirms the pattern for stem number per plant already shown in Table 19 with significantly higher results of sand ecotypes in grazed treatment ($P<0.001$). The ecotypes from cut sites were found to have significantly lower results for stems per plant in this treatment as well (P<0.001). The GLMM also shows a number of interactions. Sand and neutral loam ecotypes grown in sand and neutral loam treatments are significantly higher for unmanaged treatment plants (P<0.001) but significantly lower (except sand ecotypes in sand treatment) for grazed treatment plants (P<0.001), showing the management treatment had an effect on these interactions.

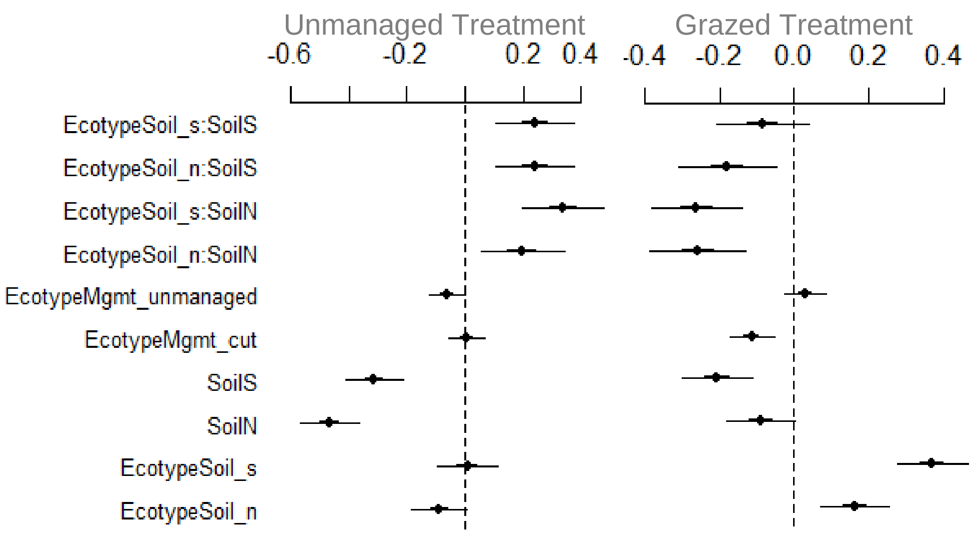


Figure 41. Effects plot of stem number GLMM with interactions between ecotype and treatment, split between management treatments. Factors with capital letter (S, N) are treatments, those with lower case letters (s, n, unmanaged, cut) are ecotype factors. (Unmanaged Treatment significance: Ecotype soil P<0.001, Ecotype mgmt. P=0.110, Treatment soil P<0.001, Interaction P<0.001. Grazed Treatment significance: Ecotype soil P<0.001, Ecotype mgmt. P<0.001, Treatment soil P<0.001, Interaction P<0.001).

The intraction model for leaflets per main stem (Figure 42) was shown to be a better fit than without interactions as a factor. The pattern seen with ecotypes in Table 19 is confirmed here, with significantly higher results for sand ecotypes in grazed treatment (P<0.001). Ecotypes from cut sites had significantly lower leaflet number per main stem in unmanaged treatment (P<0.001). The opposite pattern for interactions is seen compared to stems per plant (Figure 41), here sand and neutral loam ecotypes grown in sand and neutral loam soil treatment had significantly lower leaflet number per main stem (than the standard) in unmanaged treatment plants (P<0.001) and significantly more in grazed treatment plants (P<0.001).

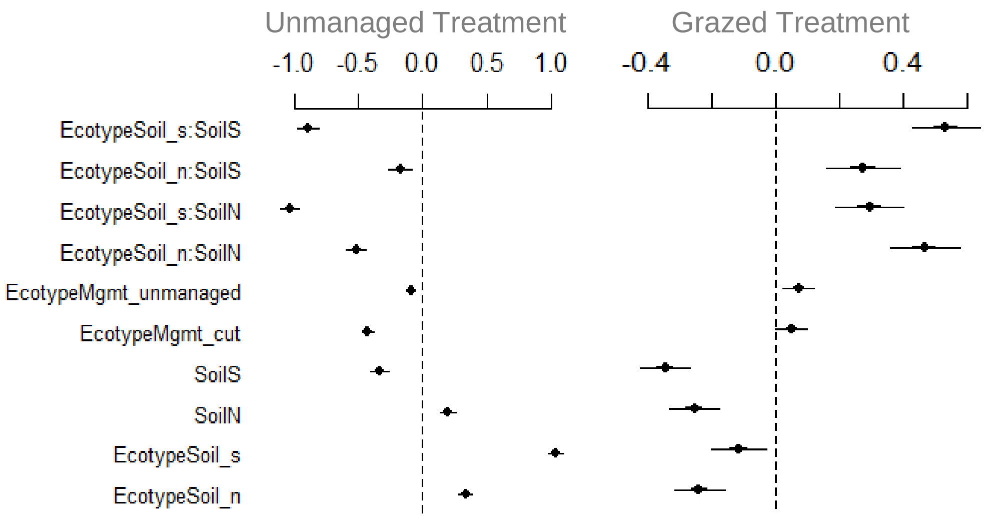


Figure 42. Effects plot of leaflet number per main stem GLMM split with interactions between ecotype and treatment, split between management treatments. Factors with capital letter (S, N) are treatments, those with lower case letters (s, n, unmanaged, cut) are ecotype factors. (Unmanaged Treatment significance: Ecotype soil P=1.000, Ecotype mgmt. P<0.001, Treatment soil P=1.000, Interaction P<0.001. Grazed Treatment significance: Ecotype soil P<0.001, Ecotype mgmt. P=0.006, Treatment soil P<0.001, Interaction P<0.001).

12.2.3 Spatial Autocorrelation

The models already conducted were repeated using the R command nlme, from these results variograms were created for main stem length, stems per plant and leaflets per main stem (harvest and soil & management datasets for each). These can be found in Appendix XI. AIC numbers from ANOVA comparison with the non-spatial model, along with P values ruled out any significant effect on the model from spatial distance between ecotype sites.

12.2.4 Summary of Results – Harvest Measurements (Vegetation)

Main stem length: Calcareous loam treatment soil produced plants with significantly long main stem length.

Stems per plant: Significantly more stems on plants in calcareous loam treatment soil and those with grazed treatment. Sand ecotypes had significantly more stems, though only in Berrow Dunes did in unmanaged treatment plants. Calcareous loam ecotypes in matching soil type are lower than all other interactions in unmanaged treatment plants yet higher than most (except sand ecotypes in matching soil type) in grazed treatment plants.

Leaflets per main stem: Plants grown in calcareous loam soil had significantly higher leaflet number, as did unmanaged treatment plants and sand ecotypes. Cut ecotypes had lower leaflet number. Calcareous loam ecotypes in matching soil type had significantly more leaflets than other interactions in unmanaged treatment plants and significantly less in grazed treatment plants.

Branches per main stem: Plants grown in calcareous loam soil had significantly more branches per main stem.

Longest branch per main stem: No significant differences were recorded.

12.3 Plant Fitness Results: Fecundity

12.3.1 First Seed Pod Formation

Month of first seed pod formation was recorded for each plant, and means calculated, tabulated results are shown in Appendix XIII.

Stacked barcharts were formed for first seed pod formation, the first (Figure 43) is grouped by ecotype.

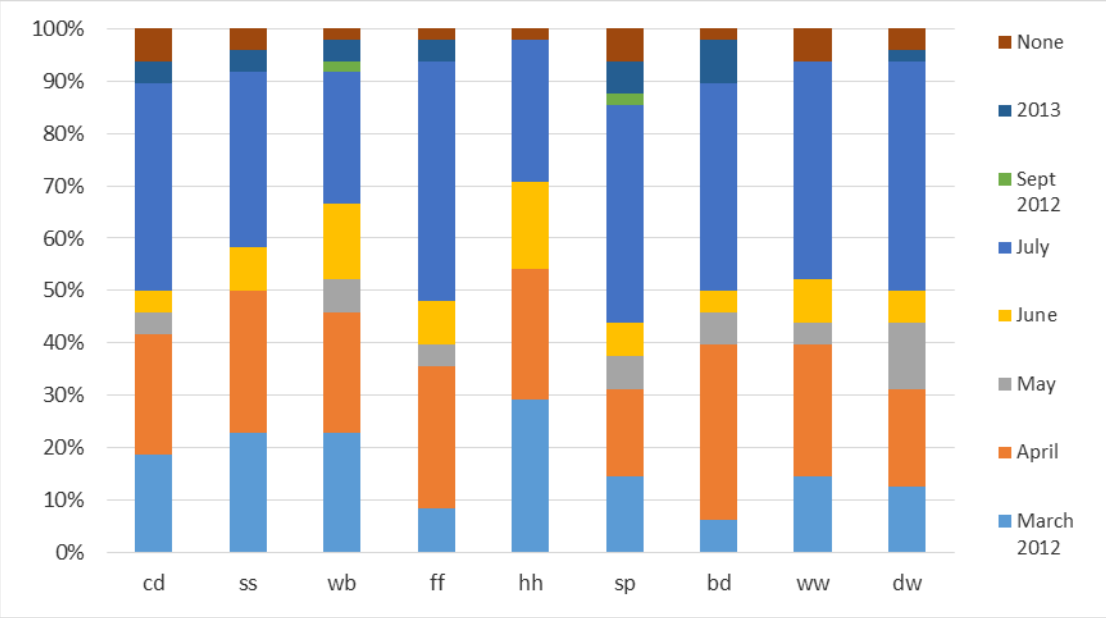


Figure 43. First seed pod formation, grouped by ecotype (*n*=48, Kruskall-Wallis P=0.459). See Table 17 for ecotype and treatment key.

There was seen to be no significant variation between seed pod formation of ecotypes (Figure 43). Woodborough and Salisbury Plain were the only two to form first seed pods in the month following harvest (September).

There was no significant variation found when seed pod formation was split between ecotype groups (Figure 44).

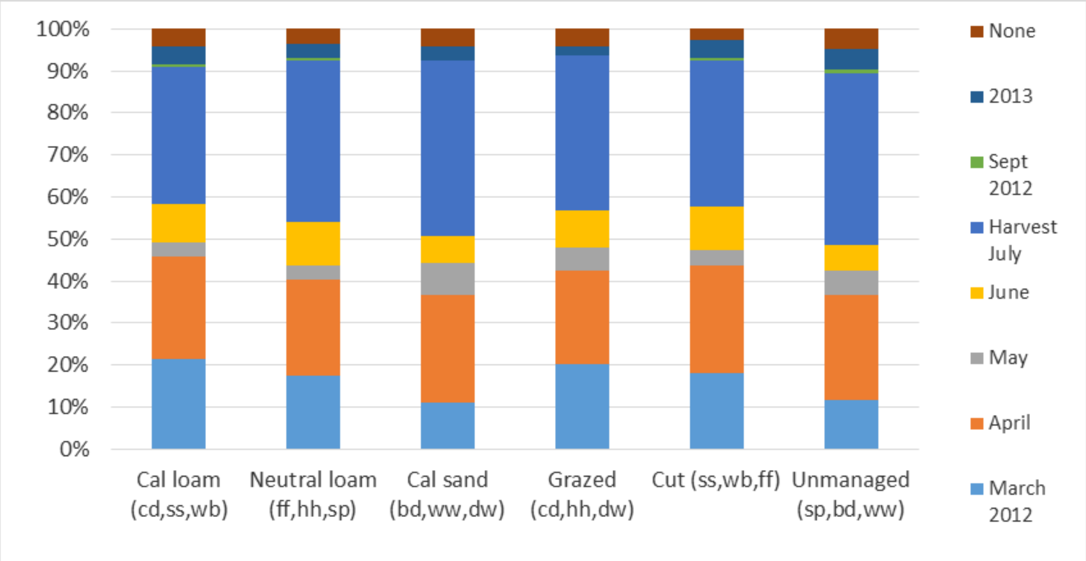


Figure 44. Month of first pod formation, grouped by ecotype home-site soil type (*n*=144, Kruskall-Wallis P=0.159) and management (*n*=144, Kruskall-Wallis P=0.717). See Table 17 for ecotype key.

When seed pod formation was split between treatments in Figure 45, significant variation was found between managements (P<0.001). Grazed treatment plants had lower amount of plants forming early seed pods (March) and more plants with no seed pod formation (by the end of 2013) compared to unmanaged treatment. No significant differences were found between soil treatments.

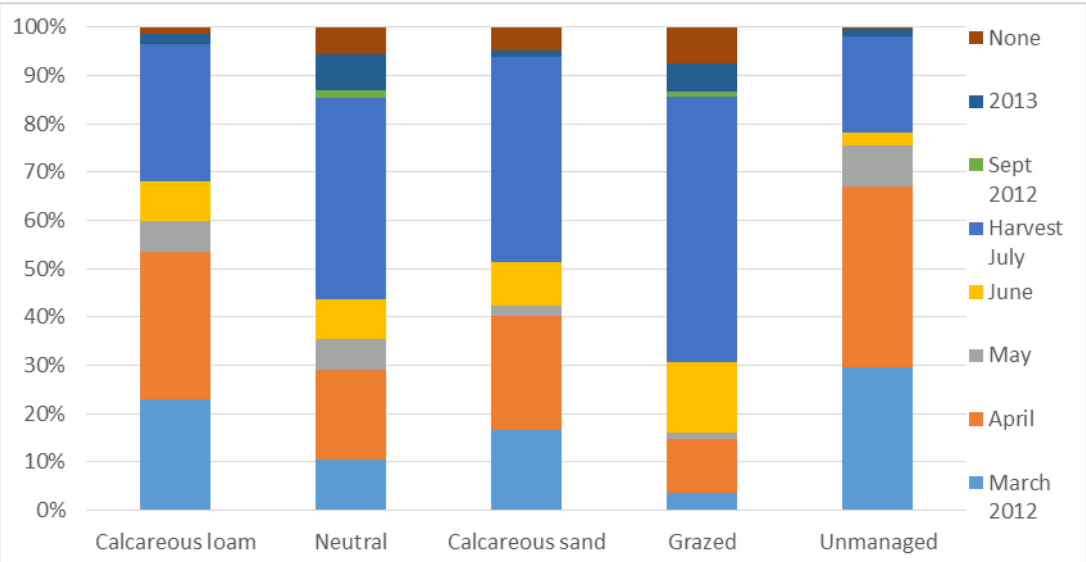


Figure 45. Month of first seed pod formation, grouped by treatment (Soil treatment *n*=144 Kruskall-Wallis P=0.114, management treatment *n*=216, P<0.001).

The time in weeks from the first flower formation in January to first seed pod for each plant was placed into the GLMM, and also a split GLMM between management treatments due to such variation shown in Table 20.

Table 20. General Linear Mixed Effects Regression (GLMM) model of time taken for first seed pod formation. Cockey Down in calcareous loam + grazed treatment is used as the standard, 'significant fast' and 'significant slow' columns refer to significant differences in relation to this standard. See Appendix X for P values and Table 21 for ecotype and treatment key. Underlined ecotypes are those with greatest influence on the model.

		Ecotype		**Soil Treatment**		**Management Treatment**	
	Management Treatment Split	Significant Fast	Significant Slow	Significant Fast	Significant Slow	Significant Fast	Significant Slow
Time taken for first pod formation	**All**	*nsd*	ss, wb, bd, <u>ww, dw</u>	*nsd*	*nsd*	G	U
	Grazed	*nsd*	ff	N	*nsd*		
	Unmanaged	*nsd*	bd	C	N,S		

Grazed treatment plants (Table 20) were significantly faster to form seed pods than unmanaged treatment plants (P<0.001). Berrow Dunes, Woolacombe Warren, Dawlish Warren, Woodborough and Southstoke were significantly slower to form seed pods than the standard (P=0.001).

The model was repeated with addition of ecotype groupings and interaction between treatment and ecotype (Figure 46). The only interaction is shown in grazed treatment plants, neutral loam ecotypes grown in neutral loam soil are significantly faster to form seed pods (P=0.005).

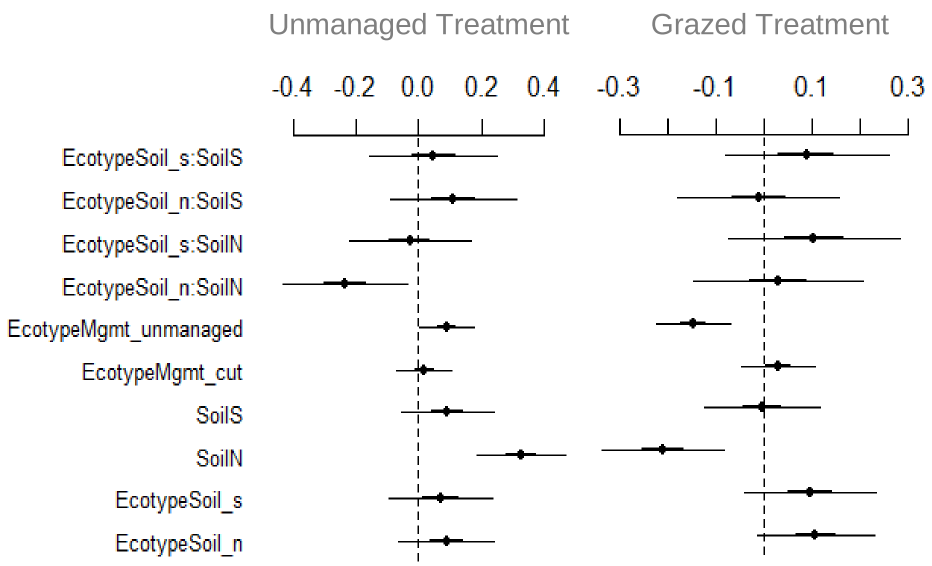


Figure 46. GLMM of time taken to first seed pod, with interaction between ecotype and treatment. Factors with capital letter (S, N) are treatments, those with lower case letters (s, n, unmanaged, cut) are ecotype factors. Cockey Down in calcareous loam + grazed treatment is used as the standard.

12.3.2 Seed Pod Number

Seed pods were counted for each plant during harvest in August 2012 and also at the end of 2013 and means calculated (Table 21). When interpreting the tabulated results, the Kruskal-Wallis test was used to establish which differences were significant.

Most ecotypes produced fewer seed pods in the second year than the first (Table 21), with the exception of Berrow Dunes which produced more in the second year, and Woolacombe Warren which had a mean of one seed pod less in the second year. Grazed treatment plants had a lower mean number of seed pods ($P<0.001$), though some of these would have been peridodically removed during the grazed treatment. Plants grown in calcareous loam soil had the greatest number of seed pods out of the soil treatments ($P=0.002$).

Table 21. Mean seed pod number counted at harvest (August 2012) and October 2013 (Ecotype N=48, Soil N=144, Management N=216). See Table 17 for ecotype key.

		Ecotype									Soil			Management	
		cd	ss	wb	ff	hh	sp	bd	ww	dw	C	N	S	G	U
August 2012		**32**	**36**	**35**	**25**	**35**	**31**	**27**	**31**	**32**	**40**	**26**	**28**	**10**	**53**
	SE	6	6	5	4	5	5	4	5	5	3	3	3	1	3
		P=0.925									**P=0.001*			**P<0.001*	
October 2013		**25**	**25**	**26**	**18**	**33**	**19**	**31**	**30**	**22**	**31**	**25**	**20**	**4**	**47**
	SE	5	4	4	3	6	3	5	4	4	3	3	2	1	2
		P=0.247									**P=0.075*			** P<0.001*	
Both Years		**57**	**61**	**60**	**43**	**68**	**50**	**58**	**61**	**53**	**71**	**51**	**49**	**14**	**100**
	SE	9.6	9.0	8.6	6.3	10.1	7.4	8.7	8.4	8.0	5.5	4.5	4.6	0.9	3.9
		P=0.918									**P=0.002*			** P<0.001*	

Although no initial ecotype significant differences were found in Table 21 it was decided that further analysis should be carried out as seed pod number is a good indicator of fecundity and pollination, both important in this experiment.

The scattergraph matrix [of results from each year and total over both years] identified a strong correlation (Appendix XVII) between Pods_2012 and Pods_2013 with Pods_all, the total of both years (Pods_all) was used for further analysis.

Total seed pod number results from the two years was placed in the GLMM model, with additional models for management split as there was such a large difference, results are shown in Table 22, effects plots are in Appendix X. The largest percent of variance in this model is explained by management regime (P<0.001) with grazed treatment plants having had significantly lower numbers of seed pods, probably due to the grazed treatment clippings being taken away. Plants grown in calcareous loam had significantly more seed pods than the other soil treatments (P<0.001). Salisbury Plain and Folly Farm had significantly lower seed pod number and Hellenge Hill had significanlty higher (P<0.001).

Table 22. Generalized Linear Mixed Effects Regression (GLMM) model of seed pod number over both years. Cockey Down in calcareous loam + grazed treatment is used as the standard, 'significant higher' and 'significant lower' columns refer to significant differences in relation to this standard. See Appendix X for P values and Table 21 for ecotype and treatment key.

	Management Treatment Split	Ecotype Significant Lower	Ecotype Significant Higher	Soil Treatment Significant Lower	Soil Treatment Significant Higher	Management Treatment Significant Lower	Management Treatment Significant Higher
Seed pod number - both years	**All**	ff, sp	hh	N,S	C	G	U
	Grazed	*nsd*	wb, ss, ww, dw	*nsd*	*nsd*		
	Unmanaged	ff, sp, dw	hh	N,S	*nsd*		

To identify ecotype grouping differences or any interactions between treatment and ecotypes for seed pod number a further GLMM was conducted, with the management split (Figure 47). The standard (which is a grazed, calcareous loam ecotype in calcareous loam treatment soil), had significantly more seed pods in grazed treatment than all other ecotype/soil combinations apart from sand ecotypes in sand treatment (and vice versa in unmanaged treatment) (P<0.001). This plot also shows the calcareous loam ecotype had significanly more seed pods than neutral loam or sand ecotypes in the unmanaged treatment.

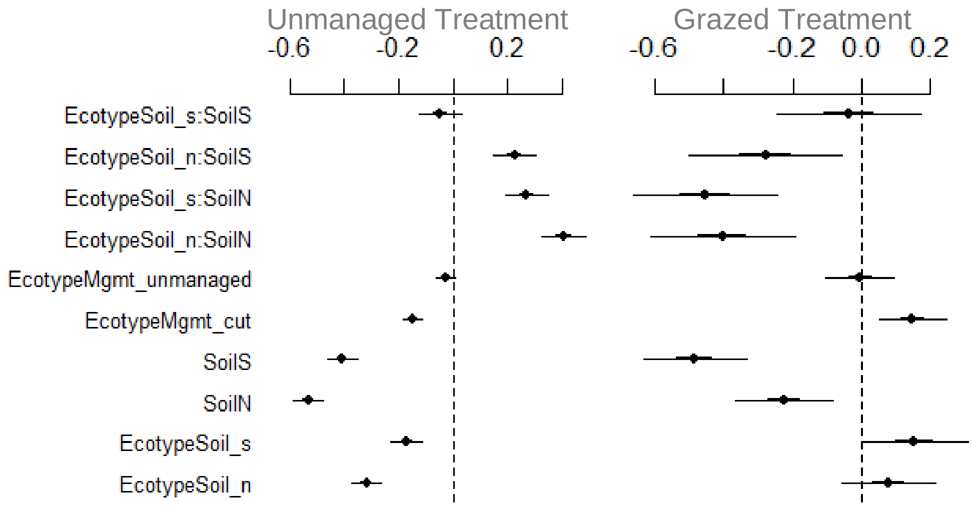


Figure 47. GLMM of seed pod number with interactions between ecotype and treatment. Factors with capital letter (S, N) are treatments, those with lower case letters (s, n, unmanaged, cut) are ecotype factors. Cockey Down in calcareous loam + grazed treatment is used as the standard.

12.3.3 Seed Pod Biomass

Seed pods were collected and weighed on the day of harvest then oven-dried and reweighed (Table 23). When interpreting the tabulated results, the Kruskal-Wallis test was used to establish which differences were significant.

Table 23. Mean harvest weights of seed pods (Ecotype n=48, Soil n=144, Management, n=216). P=Kruskall-Wallis test values. See Table 17 for ecotype and treatment key.

	Ecotype									Soil			Manage-ment	
	cd	ss	wb	ff	hh	sp	bd	ww	dw	C	N	S	G	U
Fresh weight	**2.20**	**2.22**	**2.36**	**1.67**	**2.13**	**2.14**	**1.82**	**1.97**	**1.97**	**2.71**	**1.73**	**1.73**	**0.75**	**3.36**
SE	0.37	0.32	0.32	0.28	0.33	0.34	0.34	0.28	0.34	0.24	0.21	0.17	0.05	0.23
	P=0.764									*P=0.001			*P<0.001	
Dry Weight	**0.70**	**0.86**	**0.96**	**0.53**	**0.81**	**0.65**	**0.60**	**0.69**	**0.71**	**0.97**	**0.57**	**0.63**	**0.15**	**1.29**
SE	0.15	0.16	0.17	0.10	0.15	0.12	0.12	0.11	0.14	0.10	0.07	0.09	0.01	0.07
	P=0.650									*P=0.001			* P<0.001	
Difference (moisture)	**1.50**	**1.36**	**1.40**	**1.15**	**1.33**	**1.48**	**1.22**	**1.29**	**1.26**	**1.74**	**1.16**	**1.09**	**0.60**	**2.07**
SE	0.23	0.19	0.20	0.20	0.20	0.24	0.24	0.18	0.22	0.16	0.15	0.10	0.05	0.11
	P=0.848									*P=0.001			*P<0.001	
Relative moisture content (%)	**74.06**	**68.32**	**64.27**	**68.24**	**65.92**	**68.99**	**69.01**	**64.45**	**68.13**	**66.46**	**67.96**	**69.37**	**76.86**	**60.23**
SE	1.52	2.47	2.97	2.61	2.75	3.24	2.67	2.64	2.05	1.79	1.83	1.45	0.91	1.24
	P=0.165									P=0.564			* P<0.001	

Calcareous loam treatment soil produces plants with the highest dry weight (P=0.001). Sand treatment soil produces seed pods with the lowest moisture content (P=0.001).

As the initial Kruskall-Wallis tests from Table 23 only indicated significant differences between management and treatment (and only management for relative moisture content (%)), and due to seed pod number showing strong correlation with fresh and dry seed pod biomass in the scattergraph matrix (Appendix XVII), it was decided that further analysis of seed pod biomass was unnecessary as seed pod number results could be used as a proxy.

12.3.4 Seeds

Seed was counted from six pods of each plant and means of each ecotype and treatment are displayed in Table 24. As not all plants had six seed pods available at the time of counting, a record was kept of how many out of the six each plant had to rule out bias results from fewer seed pods, this is named 'Mean number of seed pods sampled' and is also displayed in the seed table. When interpreting the tabulated results, the Kruskal-Wallis test was used to establish which differences were significant.

Table 24. Seed counts from sampled seed pods (up to six per plant, subject to availability). Results are shown of means of ecotypes (*n*=48), soil treatment (*n*=144) and management treatment (*n*=216).

		Ecotype									Soil			Management	
		cd	**ss**	**wb**	**ff**	**hh**	**sp**	**bd**	**ww**	**dw**	**C**	**N**	**S**	**G**	**U**
Mean number of seed pods sampled		**3**	**4**	**4**	**3**	**3**	**3**	**4**	**4**	**4**	**4**	**3**	**3**	**1**	**6**
	SE	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		P=0.108									*P=0.375*			**P<0.001*	
Mean seeds per pod		**5**	**7**	**8**	**7**	**7**	**6**	**7**	**7**	**8**	**7**	**7**	**6**	**4**	**9**
	SE	1	1	1	1	1	1	1	1	1	0	0	0	0	0
		**P=0.031*									*P=0.586*			**P<0.001*	
Mean seeds per plant 2012		**214**	**360**	**322**	**215**	**302**	**258**	**270**	**244**	**288**	**329**	**236**	**260**	**43**	**507**
	SE	58	72	63	37	57	50	48	46	46	34	31	39	**5**	**28**
		P=0.315									**P=0.026*			**P<0.001*	
Mean seeds per plant 2013		**178**	**261**	**278**	**172**	**318**	**202**	**328**	**189**	**280**	**283**	**246**	**206**	**24**	**466**
	SE	40	51	57	32	60	40	59	35	46	34	31	29	**4**	**24**
		P=0.227									*P=0.217*			** P<0.001*	
Mean seeds per plant from mean pods over both years		**196**	**310**	**300**	**193**	**310**	**230**	**299**	**217**	**284**	**306**	**241**	**233**	**34**	**486**
	SE	45	58	55	32	57	42	51	38	43	32	28	33	4	23
		P=0.323									*P=0.073*			** P<0.001*	

Table 24 shows ecotypes had three to four mean seed pods sampled, there was no significant differences for the seed pods sampled between ecotypes, or between treatment soils, however there is a significant difference (P<0.001) between management treatments with grazed treatment plants having a mean of only one seed pod compared to six seed pods of the unmanaged treatment. Therefore further results of seeds should be treated with caution when investigating management treatment.

The scattergraph matrix (Appendix XVII) showed strong correlation between 'mean seeds per pods sampled' and 'mean seeds per pod', as they came from the same raw dataset. 'Seed pods sampled' showed weak least squares regression correlation and no rebust regression correlation with either of these parameters, confirming the low amount of [grazed treatment plants] seed pods available for sampling hasn't influenced the pattern with seeds counted.

Results of 'Mean seed per pod' was placed into the GLMM, the mean seed pods available to count from was also analysed. As there was significant difference seen between management treatments the GLMM was also split between these. This created 10 effects plots, therefore results have been tabulated for ease of interpretation (Table 25), all effects plots can be seen in Appendix X.

Table 25. Generalized Linear Mixed Effects Regression (GLMM) model of seed pods sampled and mean seeds per pods. Cockey Down in calcareous loam + grazed treatment is used as the standard, 'significant higher' and 'significant lower' columns refer to significant differences in relation to this standard. See Appendix X for P values and Table 21 for ecotype and treatment key.

	Management Treatment Split	**Ecotype**		**Soil Treatment**		**Management Treatment**	
		Significant Lower	**Significant Higher**	**Significant Lower**	**Significant Higher**	**Significant Lower**	**Significant Higher**
Pods Sampled	**All**	cd (standard)	wb, ss, ff, hh, sp, bd, ww, dw	S	*nsd*	G	U
	Grazed	*nsd*	wb, ss, bd, ww, dw, sp	S, N	*nsd*		
	Unmanaged	*nsd*	*nsd*	*nsd*	*nsd*		
Mean Seed per pod	**All**	cd (standard) borderline with sp	wb, ss, hh, ff, bd, ww, dw (sp borderline)	*nsd*	*nsd*	G	U
	Grazed	*nsd*	ff	*nsd*	*nsd*		
	Unmanaged	ff, sp, dw	hh	*nsd*	*nsd*		

There were no significant differences within seed pods sampled in the unmanaged treatment split (Table 25), which is good for subsequent seed estimates. However there were shown to be several differences in the seed pods sampled of grazed treatment plants (P<0.001) which carried over into the dataset of mixed management treatments. Therefore further grazed treatment results should be interpretted with caution.

In the ‘Mean seed per pod’ the Cockey Down standard was significantly lower than most in the mixed management (P<0.001). The significant difference between the two managements though should be ignored in this analysis due to the significant difference found between these in the seed pods sampled.

Mean seed per pod was placed into the interaction model (Figure 48), again, the grazed treatment model needs caution in interpretation due to significant differences of seed pods sampled, from the model in Table 25.

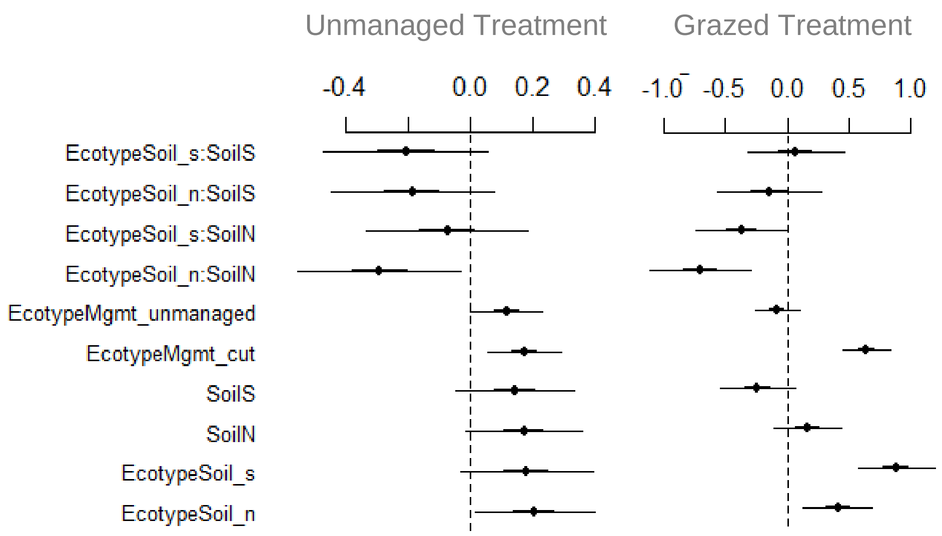


Figure 48. GLMM of mean seeds per pod, with interaction between ecotype and treatment. Factors with capital letter (S, N) are treatments, those with lower case letters (s, n, unmanaged, cut) are ecotype factors. Cockey Down in calcareous loam + grazed treatment is used as the standard.

The interaction model in Figure 48 showed neutral loam ecotypes in neutral loam soil treatment as having significantly less mean seeds per pods in grazed treatment plants (P=0.008). However there was no significant interaction and less variation altogether in the more reliable unmanaged treatment model. The only significant difference in unmanaged treatment plants (which was also shown in grazed treatment plants) was ecotypes from cut home-sites having more mean seed pods (grazed treatment $P=0.007$, unmanaged treatment $P<0.001$).

12.3.5 Summary of Results – Fecundity

First seed pod formation: Unmanaged treatment plants had significantly more seed pods earlier on in the year (in March and April). Grazed treatment plants had formed significantly more seed pods at harvest time and had more that never formed. Model results showed unmanaged ecotypes were significantly faster to form seed pods in the grazed treatment. Ecotypes from Southstoke, Woodborough, Berrow Dunes, Woolacombe Warren and Dawlish Warren were significantly slower in seed pod formation than the standard.

Seed pod number: Plants grown in calcareous loam soil had significantly more seed pods as did those in unmanaged treatment.

Seed pod biomass: Plants grown in calcareous loam soil and those with unmanaged treatment had significantly more biomass. Grazed treatment plants contained significantly greater percentage of moisture.

Seeds per pod: All ecotypes had significantly more seed than the standard though this was also the case with the number of seed pods sampled so should be interpreted with caution. The model indicated Folly Farm, Salisbury Plain and Dawlish Warren had significantly less seed in the unmanaged treatment and Hellenge Hill had significantly more. Cut ecotypes had significantly more seeds [in both management treatments], though all ecotype groups had a mean of seven seeds.

12.4 **Plant Fitness Results: Mortality**

There were 17 mortalities in the first year of growth and a further 7 following the winter after harvest, tabulated results can be found in Appendix XIV.

When interpreting the results, the Kruskal-Wallis test was used to establish which differences were significant. The Kruskal-Wallis results of matching/unmatching ecotype/treatments can also be found in Appendix XIV.

Totals were put into bar charts for clearer representation, the first (Figure 49) is grouped by ecotype.

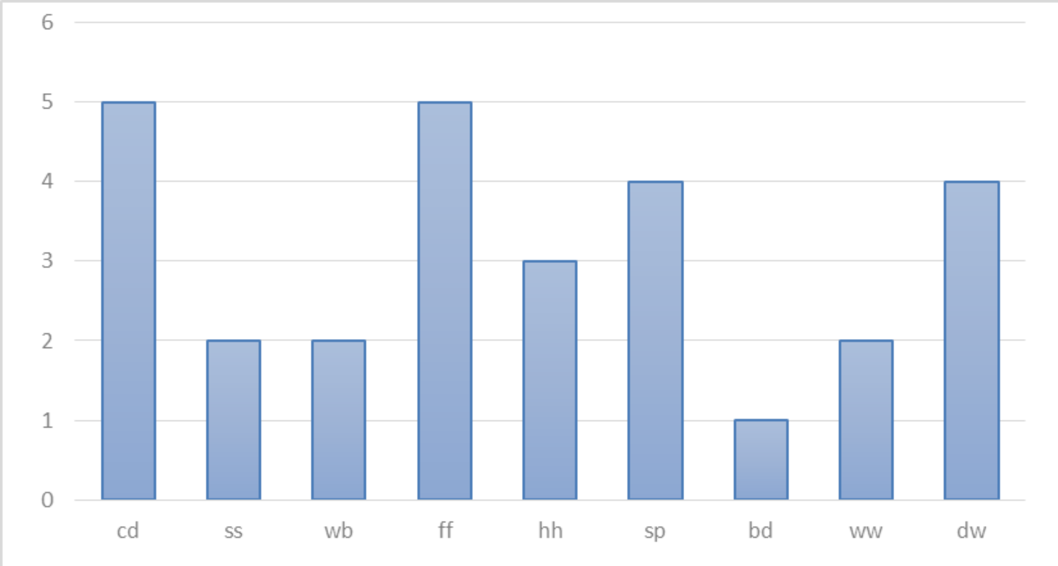


Figure 49. Total number of plant mortalities, grouped by ecotype (Mortalities out of *n*=48). Kruskall-wallis P=0.784. See Table 17 for ecotype key.

There were no significant differences between ecotypes (Figure 49). Mortality ranged from Berrow Dunes with one plant mortality, to Cockey Down and Folly Farm which had five.

Figure 50 shows the plant mortality totals split by treatment.

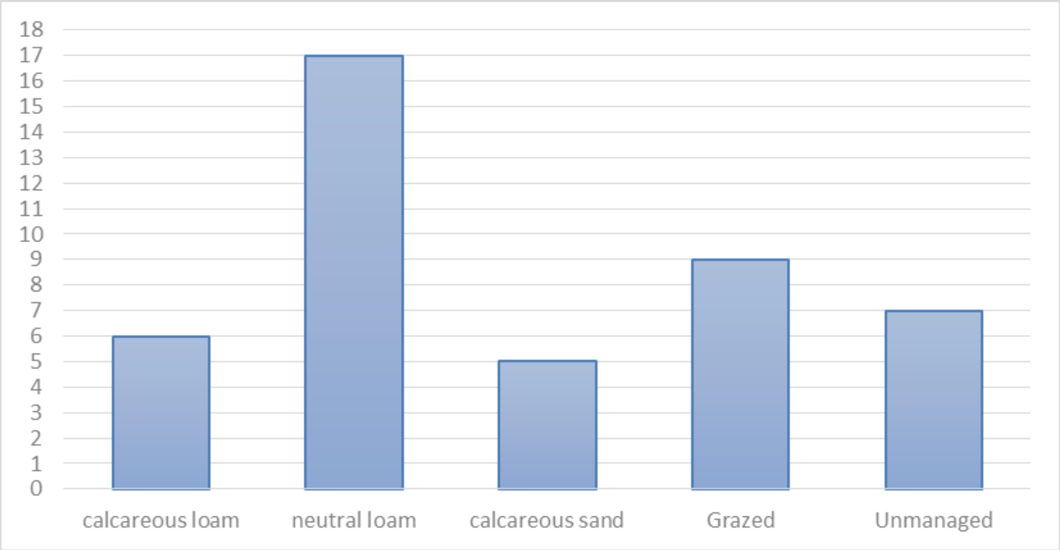


Figure 50. Total number of plant mortalities, grouped by treatment (soil treatment mortality out of *n*=144, management treatment out of *n*=216), Kruskall-wallis, Soil P=0.025; management P=1.000.

As shown in Figure 50, the greatest number of mortalities were of plants grown in neutral loam treatment soil (17 plants) with the least number in sand soils (5 plants) (P=0.025). There was no significant difference between management treatments.

Total plant mortality was lastly split by ecotype home-site soil and management conditions (Figure 51), however, no significant differences were found.

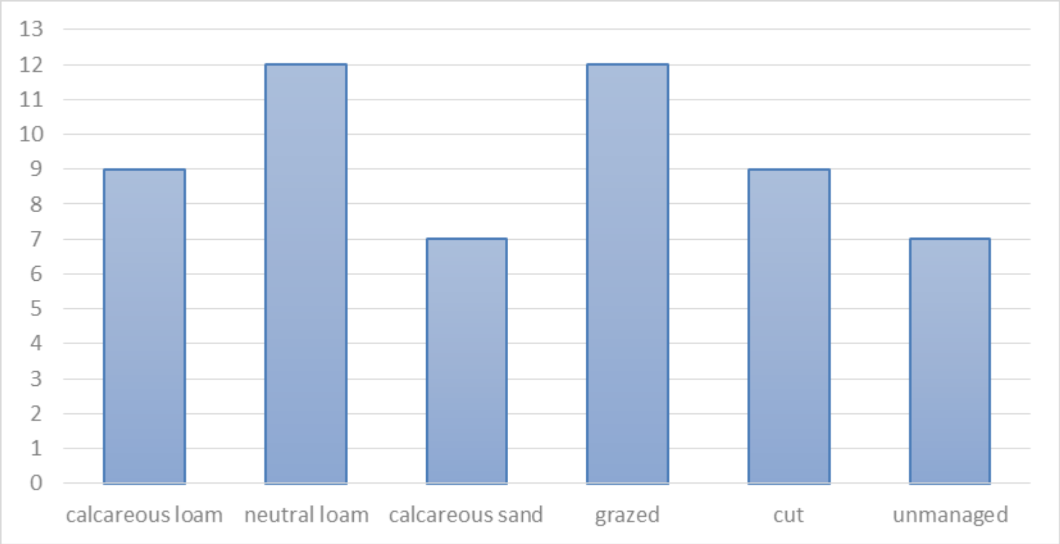


Figure 51. Total number of plant mortalities, grouped by ecotype home-site conditions (mortalities out of *n*=144). Kruskall-wallis, soil P=0.673; management P=0.397).

12.4.1 Summary of Results – Mortality

There were 28 plant mortalities in total throughout the experiment. There was significantly more plant mortality when grown in the neutral loam soil treatment.

Ecotypes did not have significantly higher survival rate when planted within matching soil type or management regime.

12.5 **Herbivore Requirements Results: Flowering Phenology and Flower Production**

12.5.1 First Flower Formation

First month when flowering occurred was recorded for each plant, means of these results are presented in Appendix XV.

Stacked bar charts were created to illustrate month of first flowering, the first is grouped by ecotype (Figure 52). There was wide variety between month of first flowering for ecotypes, Woodborough, Hellenge Hill, Woolacombe Warren and Dawlish Warren were the first to flower, in January.

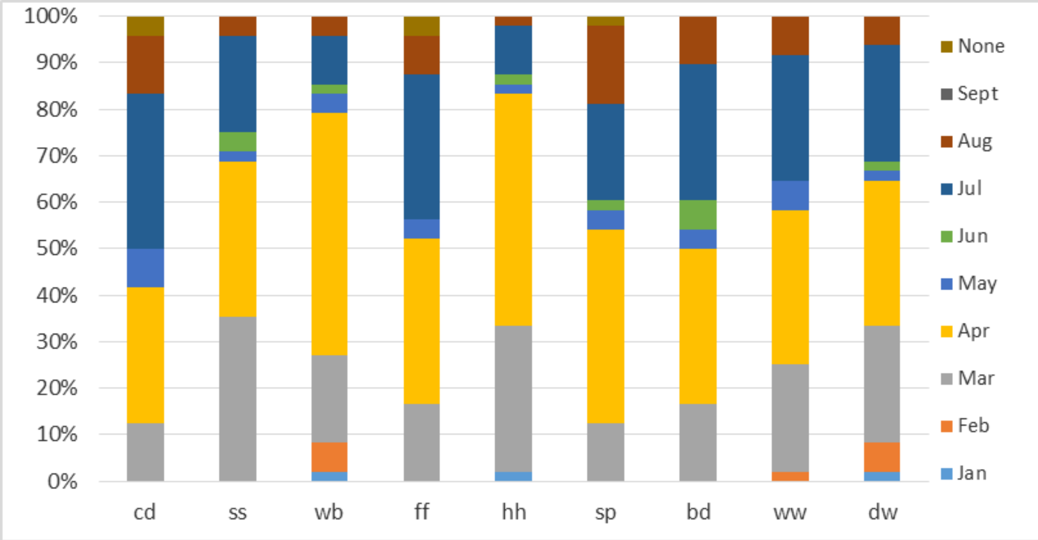


Figure 52. First month of flowering means (2012), grouped by ecotype (Ecotype *n*=48, Kruskall-Wallis *P=0.001*). See Table 17 for ecotype key.

When ecotypes are grouped by home-site conditions (Figure 53), the split between managements was significantly different (P=0.020), with unmanaged ecotypes first flowering later (March) than cut or grazed ecotypes (January/February), and having more plants still first flowering later in the year (August). There were no significant differences between ecotypes split by home-site soil type.

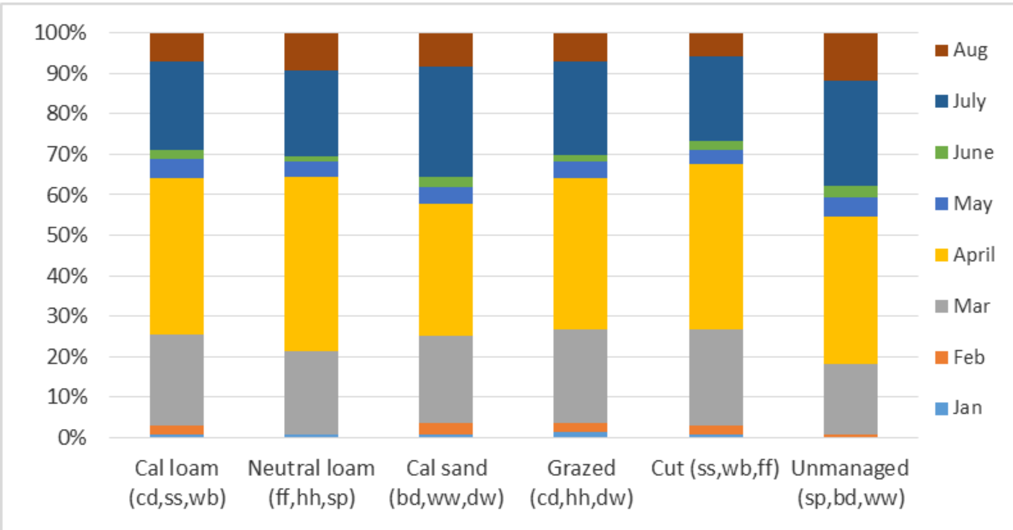


Figure 53. First month of flowering means (2012) grouped by ecotype home-site conditions (*n*=144, Kruskall-Wallis: Ecotype soil *P*=0.600, Ecotype Management *P*=0.020). See Table 17 for ecotype and treatment key.

More variation was seen when the data were split between treatments (Figure 54). Calcareous loam and neutral loam soil treatments had similar first flowering results, but sand treatment was significantly different (P<0.001), with most of the earliest (January) flowering, but less in the spring months (March to May) and more in the later months (July and August), this treatment also had the most unflowered plants. The management treatments were significantly different (P=0.001), with grazed treatment showing a similar pattern to the sand, and unmanaged treatment instead more similar to the other two soil treatments.

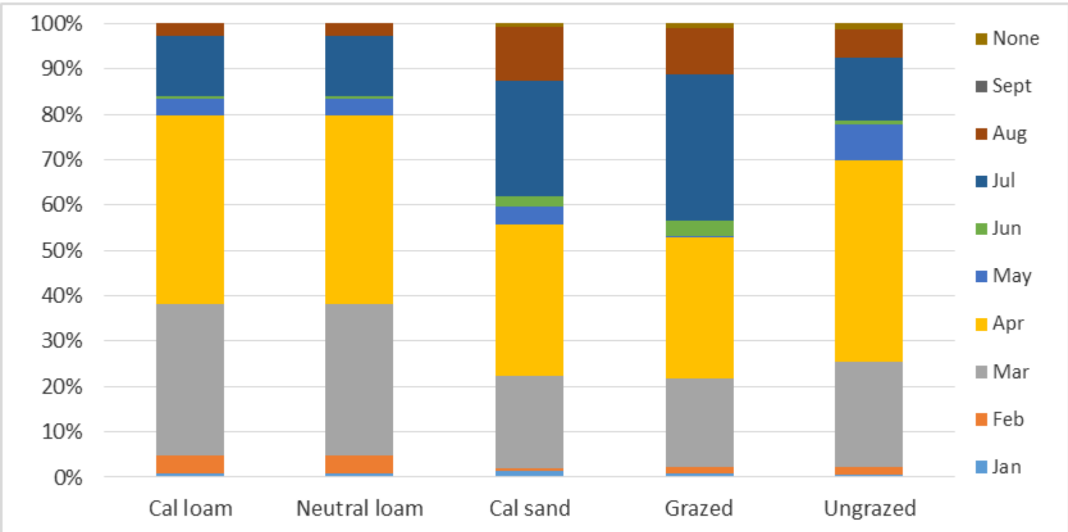


Figure 54. First month of flowering means (2012), grouped by treatment (soil treatment *n*=144, Kruskall-Wallis *P<0.001*, management treatment *n*=216, Kruskall-Wallis *P*=0.001)

When time taken to first flower was placed into the GLMM (Table 24) most variation was seen for soil treatment (P<0.001) and management treatment (P<0.001) with earlier flowering of plants grown in calcareous loam soil and unmanaged treatment. There was also some significant variation within ecotypes, with significantly shorter flowering time for Hellenge Hill and Woodborough.

Table 26. Generalized Linear Mixed Effects Regression (GLMM) model for time taken to first flower formation. Cockey Down in calcareous loam + grazed treatment is used as the standard, 'significant higher' and 'significant low' columns refer to significant differences in relation to this standard. See Appendix X for P values and Table 21 for ecotype and treatment key.

Management Treatment Split	**Ecotype**		**Soil Treatment**		**Management Treatment**	
	Significant shorter time	**Significant longer time**	**Significant shorter time**	**Significant longer time**	**Significant shorter time**	**Significant longer time**
All	hh, wb	*nsd*	C	S, N	U	G
Grazed	*nsd*	*nsd*	C	S, N		
Unmanaged	*nsd*	*nsd*	C	S, N		

Further GLMM models were conducted for time to first flower formation to identify if there were any interactions between ecotypes and treatments or any donor site soil/management differences. These showed no significant interactions were present, the effects plots can be found in Appendix X.

12.5.2 Flower Pattern and Number

Flower number was counted monthly from when they first appeared during 2012 and was recommenced during the flowering period of 2013, the means of which are tabulated and shown in Appendix XV.

Flower means have been plotted into a series of line graphs to identify patterns, the first is grouped by treatment and ecotype (Figure 55), the second (Figure 56) shows ecotype grouping. When interpreting the results, the Standard Error bars and Kruskal-Wallis test was used to establish which differences were significant.

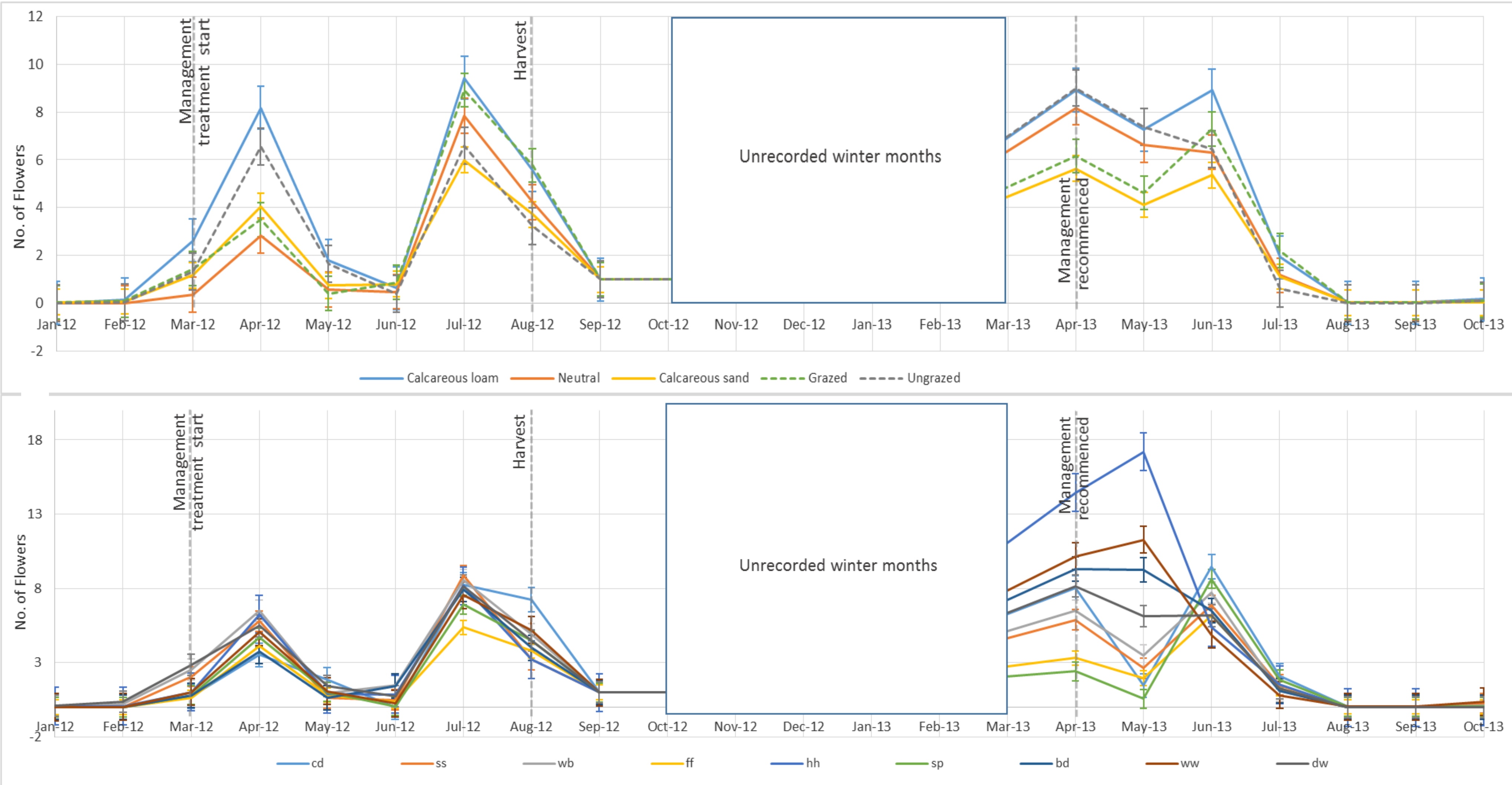


Figure 55. Flower counts over 22 months, upper graph grouped by treatment, lower graph is grouped by ecotype. No count was taken between October 2012 to March 2013, though absence of flowers over winter was noted. Soil treatment n=144, management treatment n=216, ecotype n=48. See Table 21 for key.

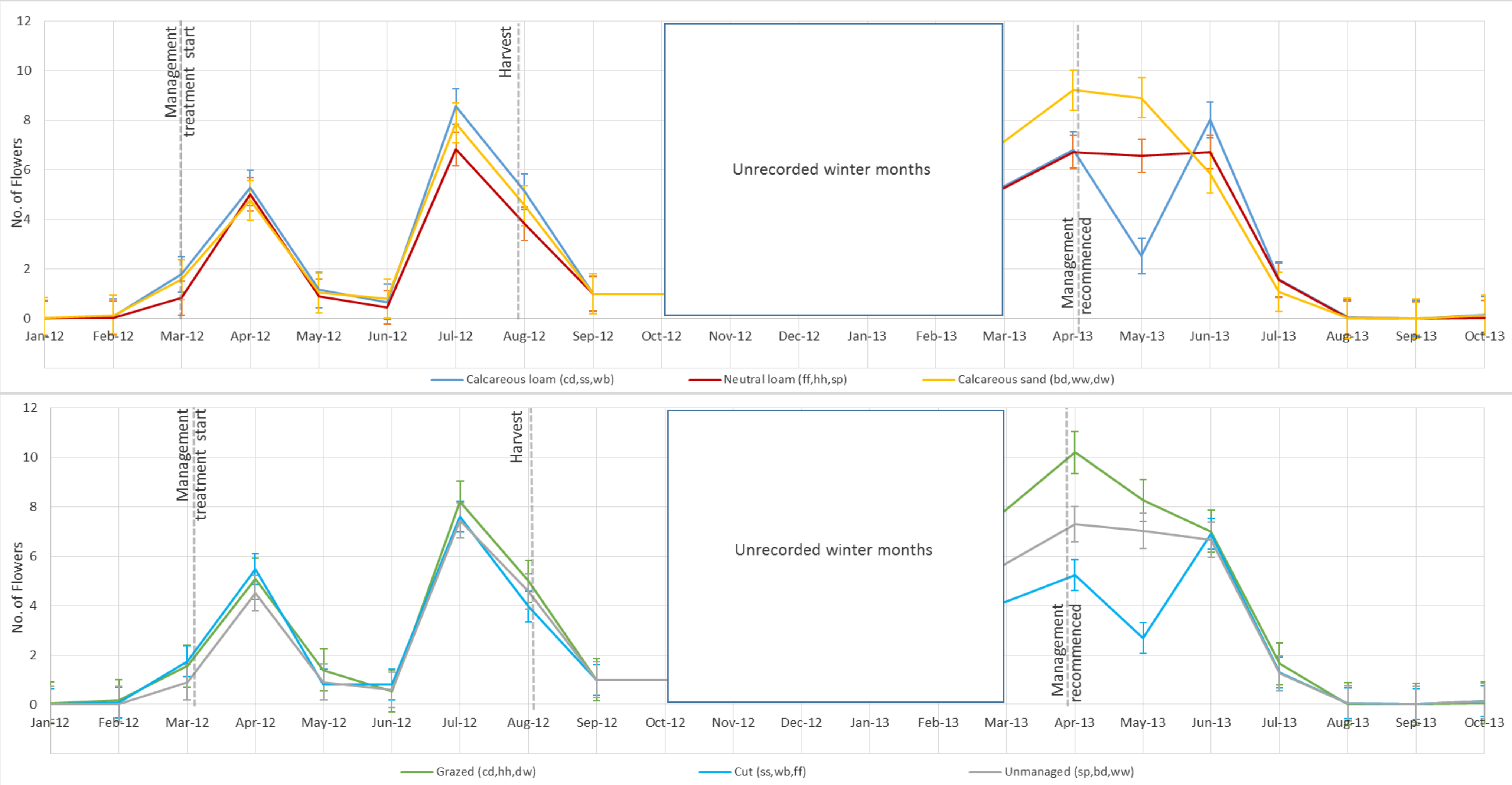


Figure 56. Flower counts over 22 months, upper graph grouped by ecotype site soils, lower graph grouped by ecotype site managements. No count was taken between October 2012 to March 2013, though absence of flowers over winter was noted, *n*=144. See Table 21 for ecotype key.

Apart from June 2012, calcareous loam soil produced most flowers throughout both years (Figure 55) (significant in April 2012 and June 2013). Neutral loam soil produced the least until June 2012 where it produced more flowering plants than sand, sand again produced the least flowers throughout the second year (significant in April and May 2013). It appears that grazed treatment may have induced a lower flower number initially in both years but then encouraged greater flower production later in the year between June to August.

The two peak flowering times of 2012 are shown clearly in Figure 55. Flowering pattern in 2013 differed to that in 2012 this time with most peaking once in May or (most) in June. Cockey Down appears to have an earlier small peak in April before a larger one in June, Southstoke, Woodborough, Folly Farm and Salisbury Plain had this same pattern but more tapered. Hellenge Hill, Berrow Dunes and Woolacombe Warren all have peak flowering in May then taper off.

There was little variation seen between ecotype groups split by home-site, soil type and management, though those in calcareous loam soil usually had most flowers. All ecotype groupings followed a similar two-peak pattern in the first year (2012), by the second year (2013) this changed. Calcareous loam and grazed ecotypes continued to have two obvious peaks, the rest of the ecotype groups peaked once in April then tapered off. Neutral loam soil ecotypes had a steady peak lasting from April until June. Differences between treatments were greater in this second year.

Total flower production from 2012, 2013 and total of both years were plotted into a scattergraph matrix to help identify correlation between datasets (Appendix XVII). This showed All flower production (from both years) to be strongly correlated with both flower production from 2012 and those from 2013. There was also seen to be moderate correlation between flower production of the first year with the second. Therefore all further analysis was carried out on total flower production over both years (All flowers) only.

The subsequent effects plot (Figure 57) of GLMM results for flower number [over both years] indicated there was significant variation between ecotypes (P<0.001) and soil treatments (P<0.001). With significantly lower flower production for plants grown in sand treatment soil and also for the ecotypes Southstoke, Folly Farm, Hellenge Hill, Salisbury Plain and Dawlish Warren.

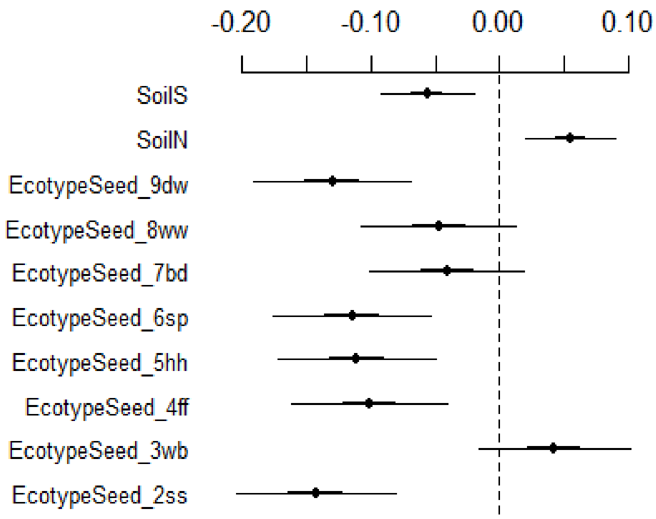


Figure 57. GLMM results effects plot of total flower production during 2012 and 2013

The interaction model (Figure 58) for flower production [over both years] identified sand ecotypes in matching soil type had significantly greater flower production (P<0.001) than other interactions and had the greatest effect on the model. Unmanaged ecotypes receiving unmanaged treatment had significantly greater flower production than the standard (P=0.002) though accounted for less effect on the model. In addition, calcareous loam ecotypes had significantly higher flower production (P<0.001).

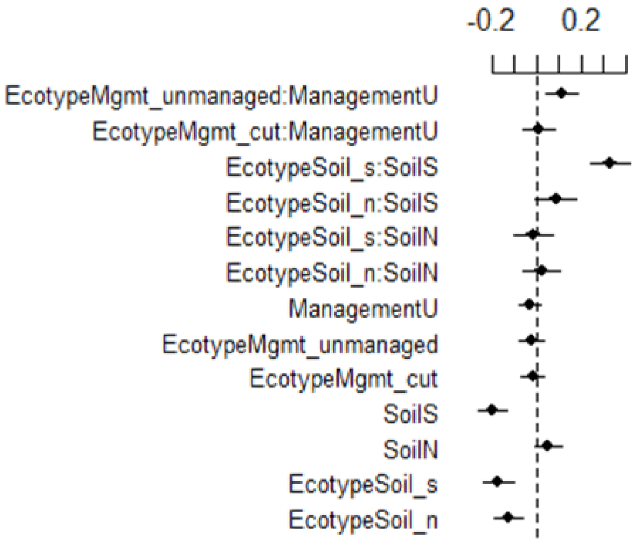


Figure 58. GLMM results effects plot of total flower production during 2012 and 2013 with interaction between treatment and ecotype. Factors with capital letter (S, N,U) are treatments, those with lower case letters (s, n, unmanaged, cut) are ecotype factors. Cockey Down in Calcareous loam + grazed treatment is used as the standard.

12.5.3 Flower Biomass at Harvest

Flowers present at harvest in August 2012, were weighed. Fresh and dry biomass as well as the difference between the two (moisture content) and relative moisture content (%) are tabulated in Table 27. When interpreting the tabulated results, the Kruskal-Wallis test was used to establish which differences were significant.

Table 27. Means of flower biomass at harvest (August 2012) (Ecotype N=48, Soil N=144, Management N=216). See Table 17 for ecotype and treatment key.

Flower harvest		Ecotype									Soil			Management	
		cd	ss	wb	ff	hh	sp	bd	ww	dw	C	N	S	G	U
Fresh weight (g)		**0.41**	**0.20**	**0.31**	**0.18**	**0.18**	**0.26**	**0.20**	**0.26**	**0.22**	**0.33**	**0.21**	**0.20**	**0.32**	**0.17**
	SE	0.06	0.04	0.05	0.03	0.03	0.05	0.03	0.04	0.04	0.04	0.03	0.02	0.02	0.01
		**P=0.016*									**P=0.016*			**P<0.001*	
Dry Weight (g)		**0.08**	**0.04**	**0.06**	**0.04**	**0.03**	**0.05**	**0.04**	**0.05**	**0.04**	**0.06**	**0.04**	**0.04**	**0.06**	**0.04**
	SE	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.00	0.00
		**P=0.012*									**P=0.003*			**P<0.001*	
Difference (Moisture) (g)		**0.38**	**0.22**	**0.32**	**0.20**	**0.21**	**0.30**	**0.20**	**0.25**	**0.24**	**0.33**	**0.24**	**0.21**	**0.29**	**0.21**
	SE	0.05	0.04	0.04	0.02	0.02	0.04	0.02	0.04	0.03	0.03	0.02	0.02	0.02	0.02
		**P=0.004*									**P=0.004*			**P<0.001*	
Relative moisture content (%)		**79.62**	**79.29**	**80.50**	**76.80**	**79.15**	**79.56**	**75.87**	**78.50**	**81.19**	**78.83**	**80.08**	**77.96**	**79.40**	**78.30**
	SE	0.72	0.76	0.82	0.59	0.56	1.46	0.49	1.47	1.05	0.77	0.47	1.33	0.54	0.83
		P=0.543									*P=0.995*			*P=0.165*	

Dry flower weights were similar (Table 27) though Cockey Down was the heaviest at 0.08g (P=0.012). Calcareous loam treatment soil (P=0.003) and grazed treatment (P<0.001) produced the highest dry biomass. The highest relative moisture content (%) between ecotypes was Dawlish Warren and the lowest was found in Berrow Dune flowers (P=0.543).

A strong relationship was seen in the scattergraph matrix (Appendix XVII) between fresh and dry biomass and also, between these and ‘difference’ (moisture). There was no correlation (seen) between biomass or ‘difference’ with relative moisture content (%) or with the previously analysed flower number.

It was decided that there would be no need for further analysis of percent water as the Kruskall-Wallis test (Table 27) did not pick up any initial significance within ecotypes or treatments. As dry flower biomass (and therefore fresh biomass and ‘difference’ with which it was correlated) offers only a single ‘snapshot’ of flowers at harvest and isn’t representative of flower production througout the experiment, it was decided that no further analysis needed conducting on this parameter either.

12.5.4 Flower Scent

The two datasets for flower scent ‘bagged flower scent’ and ‘pre-harvest flower scent’ were tabulated (Table 28). Unmanaged treatment plants of both datasets were shown to produce significantly stronger flower scent than grazed treatment. Ecotype differences for hirsuteness were significant therefore the split between homesites was also added to the table.

Table 28. Flower scent values from pre-harvest scent test and post-harvest bagged flower scent test. *P*=Kruskall-Wallis values. See Table 17 for ecotype and treatment key. Ecotype n=48, Soil n=144, Management n=216).

	Ecotype									**Soil Treatment**			**Management Treatment**	
	cd	**ss**	**wb**	**ff**	**hh**	**sp**	**bd**	**ww**	**dw**	**C**	**N**	**S**	**G**	**U**
Pre-harvest flower scent	**1.83**	**1.39**	**1.24**	**1.62**	**1.66**	**0.98**	**0.98**	**0.82**	**1.13**	**1.34**	**1.34**	**1.23**	**1.18**	**1.42**
SE	0.13	0.12	0.13	0.15	0.13	0.13	0.14	0.15	0.15	0.09	0.09	0.10	0.07	0.07
	**P=0.012*									*P=0.217*			**P=0.018*	
	Ecotype soil					**Ecotype Management**								
	Cal loam		**Neutral loam**	**Sand**		**Grazed**	**Cut**		**Unmgd**					
	1.49		1.42	0.98		1.54	1.42		0.93					
SE	0.06		0.03	0.01		0.02	0.01		0.01					
	**P=0.008*					**P=0.043*								
Bagged flower scent	**0.38**	**0.03**	**0.21**	**0.95**	**0.56**	**0.22**	**0.83**	**1.09**	**0.24**	**0.43**	**0.54**	**0.53**	**0.48**	**0.51**
SE	0.08	0.02	0.13	0.19	0.19	0.09	0.21	0.22	0.11	0.10	0.11	0.10	0.07	0.08
	P=0.131									*P=0.071*			**P=0.049*	

As initial Kruskall-Wallis values indicated significant differences between ecotypes for pre-harvest flower scent, this dataset was placed into the GLMM model (P values in Appendix X) (Figure 59). As with the Kruskall-Wallis test, there was significant difference shown between management treatments (P=0.049).

The interaction effects plot showed no significant differences and none from ecotype groups.

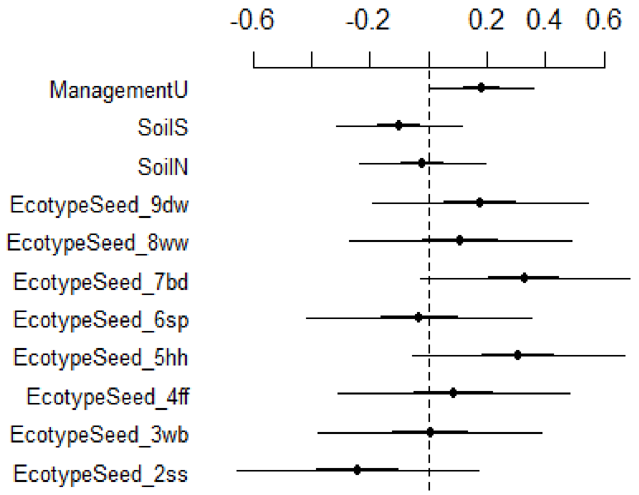


Figure 59. GLMM results effects plot of pre harvest flower scent

12.5.5 Summary of Results – Flowering Phenology and Production

First flower: Calcareous loam soil had significantly shorter time to flowering, as did plants in the unmanaged treatment. There was significant difference between ecotype managements with more unmanaged ecotypes delayed in flowering. Hellenge Hill and Woodborough were significantly earlier to flower in the combined model.

Flower pattern: Two clear peaks (April and July) were seen in the first year but different patterns occurred in the second year, some ecotypes having just one peak. Only cut ecotypes and calcareous loam ecotypes have two distinct peaks again in the second year (April and June). All flowering finished earlier in the second year than the first.

Flower number: Southstoke, Folly Farm, Hellenge Hill, Salisbury Plain and Dawlish Warren had significantly less flowers at harvest. Plants grown in neutral loam soil had significantly more flowers. The calcareous loam ecotype had significantly more flowering, and there was significant interaction between the sand ecotype in matching soil type.

Flower scent: There was significantly stronger scent in unmanaged treatment for both pre-harvest flower scent and bagged flower scent. Sand ecotypes had significantly lower scent than calcareous loam ecotypes, and unmanaged ecotypes had significantly lower scent than grazed ecotypes.

12.6 Herbivore Requirements Results: Growth Habit and Hirsuteness

Growth habit and hirsuteness were recorded prior to harvest, Table 29 shows mean results found. When interpreting the tabulated results, the Kruskal-Wallis test was used to establish if differences were significant.

Table 29. Growth observations at harvest (Ecotype n=48, Soil n=144, Management n=216). Growth habit is graded by 0= prostrate, 1=decumbent, 2=erect. Hirsuteness is graded as: 0= glabrous, 1=very sparsely hirsute, 2=sparsely hirsute, 3=hirsute. P numbers are generated using Kruskall-Wallis. See Table 17 for ecotype and treatment key.

		Ecotype									Soil Treatment			Management Treatment	
		cd	**ss**	**wb**	**ff**	**hh**	**sp**	**bd**	**ww**	**dw**	**C**	**N**	**S**	**G**	**U**
Growth Habit		**0.9**	**1.1**	**1.0**	**1.0**	**1.0**	**1.1**	**1.1**	**1.0**	**1.1**	**1.0**	**1.1**	**1.0**	**1.0**	**1.1**
	SE	0.0	0.1	0.1	0.1	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
		P=0.124									**P=0.022*			**P=0.015*	
Hirsuteness		**1.6**	**1.8**	**1.9**	**2.1**	**1.9**	**2.0**	**1.8**	**2.1**	**1.9**	**1.7**	**2.1**	**1.9**	**1.9**	**1.9**
	SE	0.2	0.2	0.1	0.1	0.2	0.2	0.2	0.1	0.2	0.1	0.1	0.1	0.1	0.1
		**P<0.001*									**P=0.001*			*P=0.155*	

		Ecotype Soil			Ecotype Management		
		Cal loam	**Neutral loam**	**Sand**	**Cut**	**Grazed**	**Unmgd**
Hirsuteness		1.8	2.0	1.9	1.9	1.8	2.0
	SE	0.06	0.01	0.01	0.01	0.01	0.01
		**P<0.001*			**P<0.001*		

If results (Table 29) were rounded to a single figure it would appear all ecotypes and treatment means were decumbent (1). Plants grown in neutral loam soil show the most prostrate growth habit (P=0.022) and also those receiving unmanaged treatment (P=0.015). Due to limited variation of growth habit and no significant differences between ecotypes, it was decided there was no need for further analysis.

Folly Farm and Woolacombe Warren were shown to be the most hirsute ecotypes (P<0.001). Plants grown in calcareous loam soil were the least hirsute and those in neutral loam soil the most (P=0.001), though again, all ecotypes and treatments if rounded to a single number would appear to be sparsley hirsute (2). There were no significant differences between management treatments.

Although little variation was seen in Table 29 for hirsuteness, the significant value between ecotypes as well as soil treatment gave reason to analyse the results in the GLMM (Figure 60) and in the second GLMM with interactions between treatments and ecotypes (Figure 61).

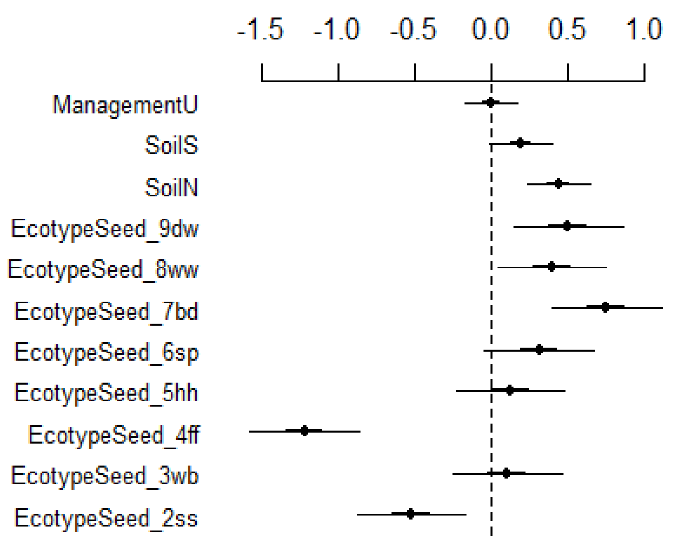


Figure 60. GLMM effects plot for hirsuteness (results grading; 0=glabrous, 1=very sparsely hairy, 2=sparsely hairy or 3=hirsute). Cockey Down in calcareous loam + grazed treatment is used as the standard.

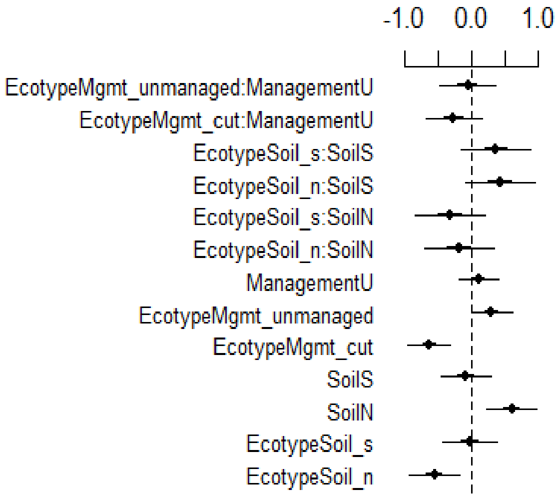


Figure 61. GLMM effects plot for hirsuteness (results graded by 0=prostrate, 1=decumbent, 2=erect). Split between management treatments with interactions between ecotype and treatment. Factors with capital letter (S, N, U) are treatments, those with lower case letters (s, n, unmanaged, cut) are ecotype factors. Cockey Down in calcareous loam + grazed treatment is used as the standard.

The effects plots in Figure 60 shows the three ecotypes from sand sites (Berrow Dunes, Woolacombe Warren and Dawlish Warren) were significantly more hirsute than Folly Farm, Southsoke and Cockey Down (P<0.001). Although no interactions were shown to occur in Figure 61, Ecotypes from neutral loam soil sites (P=0.001), and those from cut sites (P<0.001) were significantly more glabrous.

12.6.1 Summary of Results – Growth Habit and Hirsuteness

Growth habit: No significant differences.

Hirsuteness: The neutral loam ecotypes were more hirsute than the calcareous loam, and unmanaged ecotypes more hirsute than grazed. However, all means rounded to 2 (slightly hirsute). The model identified that ecotypes from neutral loam sites and those from cut sites had significantly more plants which were glabrous compared to the standard.

12.7 **Herbivore Requirements Results: Vegetation Biomass**

12.7.1 Grazed Clippings Biomass

The grazed treatment commenced in March 2012, immediately after measurement recording, and was repeated each month for five months. The clippings of each plant were oven-dried and weighed. Mean results have been tabulated in Appendix XVI. A post-winter grazed treatment weight was also taken in April 2013.

A line graph was created from the ecotype results (Figure 62). Only weights from grazed treatment (cut material) between March and July were shown in the graph due to the April 2013 grazing being after a break in management for winter which may have distorted the graph results. Two patterns could be identified from the grazed treatment clippings biomass; The first group (Hellenge Hill, Berrow Dunes, Folly Farm and Woodborough) seemed to be unaffected by the first grazed treatment, keeping a level weight by the second grazed treatment, then declining after April and growth not recuperating for the remainder of the year. The second group (Southstoke, Dawlish Warren, Cockey Down, Salisbury Plain and Woolacombe Warren) were affected by the first grazed treatment with the lowest trough in May and then a steady incline to a heavier biomass than the first in July.

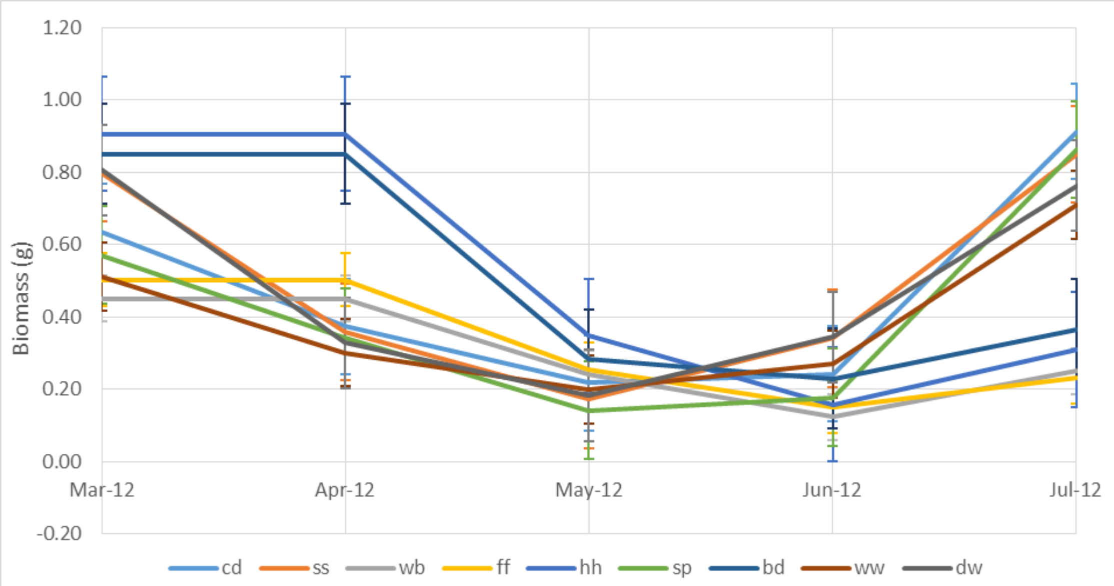


Figure 62. Grazed treatment clippings dry biomass grouped by ecotype. Results from means (+ 1 SE) *n*=24. See Table 17 for ecotype and treatment key.

The results were split between soil type treatments (Figure 63), this illustrated the greater growth rate of plants grown in calcareous loam treatment soil with neutral loam treatment soil regrowth only overtaking those grown in sand by July.

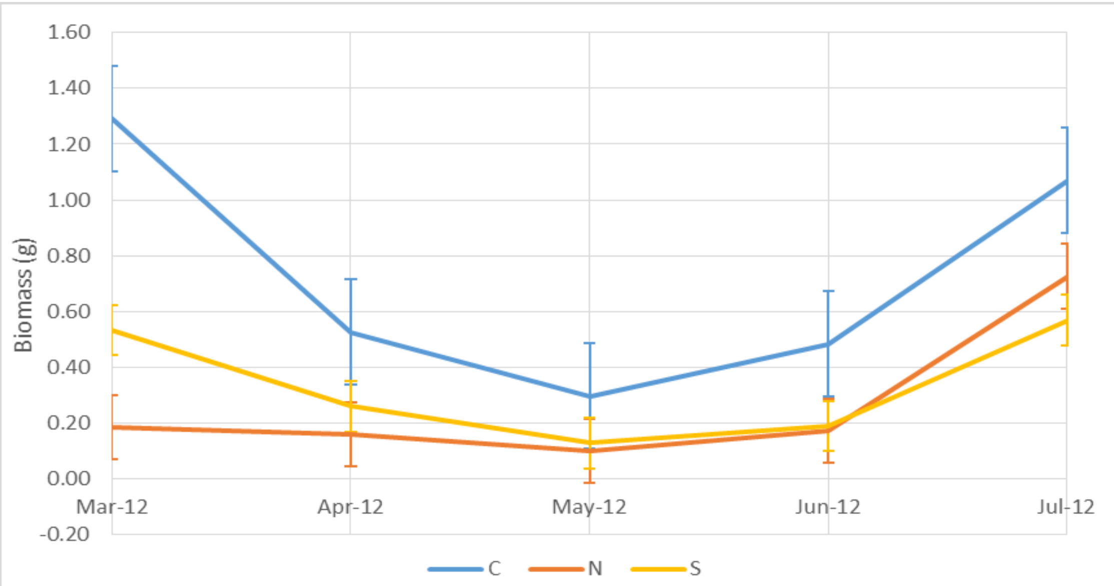


Figure 63. Grazed treatment clippings dry biomass, grouped by soil type treatment. Results taken from means (+ 1 SE) *n*=72. See Table 17 for ecotype and treatment key.

Results from the GLM model of grazed treatment clippings (Figure 64) indicate the most important factor in difference was the treatment soil, with significantly more regrowth found in plants grown in the calcareous loam treatment soil (P<0.001). Woodborough had least regrowth of all ecotypes (P=0.035). The data were placed into a further model to identify any interactions between ecotypes and treatments, no significant interactions or ecotype groupings were found.

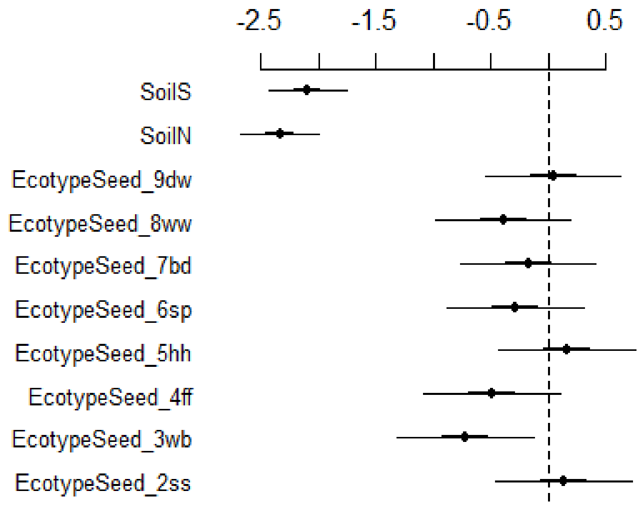


Figure 64. GLM of grazed treatment clippings dry biomass. SoilS and are treatment soils. Cockey Down in calcareous loam + grazed treatment is used as the standard.

12.7.2 Harvest Biomass

Dry and fresh vegetative harvest weights, and the moisture content (which was the difference between the two weights) were tabulated (Table 30). When interpreting the tabulated results, ANOVA was applied to dry biomass, difference and relative moisture content (%) data to establish which differences were significant, and Kruskal-Wallis was used for fresh weights.

Table 30. Vegetative harvest biomass, 'Difference' is the difference between fresh and dry biomass (Ecotype n=48, Soil n=144, Management n=216). P=ANOVA for Dry Biomass, Difference and Relative moisture content (%) and Kruskall-Wallis for Fresh biomass. See Table 17 for ecotype and treatment key.

		Ecotype									Soil			Management	
		cd	**ss**	**wb**	**ff**	**hh**	**sp**	**bd**	**ww**	**dw**	**C**	**N**	**S**	**G**	**U**
Fresh Biomass (g)		**11.2**	**9.2**	**7.4**	**9.8**	**9.1**	**8.4**	**10.3**	**10.5**	**9.7**	**13.4**	**7.4**	**7.8**	**6.8**	**12.3**
	SE	1.2	0.9	0.8	1.0	0.9	0.8	0.9	1.0	0.9	0.6	0.4	0.6	0.3	0.5
		P=0.185									*P<0.001			*P<0.001	
Dry biomass (g)		**3.1**	**2.8**	**2.3**	**2.9**	**2.7**	**2.5**	**3.0**	**3.0**	**2.9**	**3.9**	**2.1**	**2.4**	**1.8**	**3.7**
	SE	0.3	0.3	0.3	0.3	0.3	0.2	0.3	0.3	0.3	0.2	0.1	0.2	0.1	0.2
		P=0.639									*P<0.001			*P<0.001	
Difference (moisture)		**8.1**	**6.4**	**5.1**	**6.9**	**6.4**	**5.9**	**7.4**	**7.5**	**6.8**	**9.5**	**5.3**	**5.4**	**4.9**	**8.5**
	SE	0.9	0.7	0.5	0.7	0.6	0.6	0.6	0.8	0.6	0.5	0.3	0.4	0.2	0.4
		P=0.082									*P<0.001			*P<0.001	
Relative moisture content (%)		**71.3**	**69.3**	**68.8**	**70.3**	**70.3**	**68.6**	**71.0**	**69.8**	**69.1**	**70.1**	**69.9**	**69.5**	**71.2**	**68.5**
	SE	0.7	0.8	0.8	0.6	0.6	1.5	0.5	1.5	1.1	0.8	0.7	0.4	0.5	0.4
		P=0.443									P=0.745			*P<0.001	

Vegetative harvest weights (Table 30) show calcareous loam soil and unmanaged treatment produced the highest dry biomass (Soil P<0.001, Management P<0.001), fresh biomass (Soil P<0.001, Management P<0.001) and moisture content (Soil P<0.001, Management P<0.001), though relative moisture content (%) was very similar in each soil.

As there was no significant variation identified between ecotypes in (Table 30), it was decided that further analysis was not required.

12.7.3 Summary of Results – Biomass

Grazed treatment clippings: Higher biomass was found for plants grown in calcareous loam soil.

Harvest biomass: Plants grown in calcareous loam soil had significantly higher biomass, as did unmanaged treatment plants.

Harvest relative moisture content (%): Grazed treatment plants had higher relative moisture content (%).

12.8 Herbivore Requirements Results: Leaf Chemical Analysis

12.8.1 Leaf-nitrogen

The mean results of leaf-nitrogen levels were calculated and tabulated (Table 31). When interpreting the results, ANOVA was used to establish which differences were significant.

Table 31. Leaf-nitrogen (Ecotype *n*=48, Soil *n*=144, Management *n*=216), *P*=ANOVA. See Table 21 for ecotype and treatment key.

	Ecotype									Soil			Management	
	cd	ss	wb	ff	hh	sp	bd	ww	dw	C	N	S	G	U
N (%)	**0.66**	**0.68**	**0.67**	**0.68**	**0.67**	**0.68**	**0.68**	**0.62**	**0.63**	**0.68**	**0.69**	**0.62**	**0.71**	**0.62**
SE	0.02	0.02	0.02	0.02	0.02	0.02	0.01	0.02	0.02	0.01	0.01	0.01	0.01	0.01
	P=0.064									**P<0.001*			**P<0.001*	

Nitrogen levels (Table 31) were significantly lower in plants grown in sand soil (P<0.001) and unmanaged treatment (P<0.001). Lowest nitrogen levels were found in Woolacombe Warren ecotypes (almost significant P=0.064).

Although no significant ecotype results were identified in Table 32 it was still considered necessary to process further statistical analysis on nitrogen, being one of the more important variables to herbivory, and as the P value for ecotype was almost low enough to be significant.

GLMM was carried out for nitrogen results, as a large significant difference was found between the two management treatments the model was repeated separately for each (Table 32).

Table 32. Generalized Linear Mixed Effects Regression (GLMM) model of leaf-nitrogen. Cockey Down in calcareous loam + grazed treatment is used as the standard, 'significant higher' and 'significant lower' columns refer to significant differences in relation to this standard. See Appendix X for P values and Table 21 for ecotype and treatment key.

	Ecotype		**Soil Treatment**		**Management Treatment**	
Management Treatment Split	**Significant Lower**	**Significant Higher**	**Significant Lower**	**Significant Higher**	**Significant Lower**	**Significant Higher**
All	*nsd*	*nsd*	S	*nsd*	U	G
Grazed	*nsd*	*nsd*	S	*nsd*		
Unmanaged	dw	*nsd*	*nsd*	*nsd*		

Most variation (Table 32) was seen between treatments with significantly lower nitrogen in plants grown in sand soil (P<0.001) and those under unmanaged treatment (P<0.001). The grazed treatment may have influenced the sand treatments decrease in nitrogen, a factor which could be genetic as the sand ecotype Dawlish Warren was lower in unmanaged treatment (P=0.007).

A further GLMM was conducted to identify any interactions between ecotype and treatment, none of the interactions or ecotype groupings were found to be significant.

12.8.2 Leaf Hydrogen Cyanide (HCN)

Means of qualitative and quantitative hydrogen cyanide (HCN) results of the leaves were tabulated (Table 33). When interpreting the tabulated results, the Kruskal-Wallis test was used to establish which differences were significant. As there were only significant differences between ecotypes, means were calculated for each ecotype grouping of quantitative leaf-HCN to explore the dataset.

Table 33. Leaf hydrogen cyanide (HCN) (Ecotype *n*=48, Soil *n*=144, Management *n*=216). See Table 17 for ecotype and treatment key. *P*=Kruskall-Wallis. Units= 'ranked' - from 0 (no HCN) to 4 (high HCN), 'degrees of colour' - green hue colour absence of picrate papers (higher degrees represent greater HCN presence).

		Ecotype									Soil Treatment			Management Treatment	
		cd	**ss**	**wb**	**ff**	**hh**	**sp**	**bd**	**ww**	**dw**	**C**	**N**	**S**	**G**	**U**
Qualitative HCN	**Ranked**	**0.42**	**1.79**	**1.35**	**1.94**	**0.58**	**0.04**	**0.83**	**0.58**	**1.17**	**1.08**	**0.95**	**0.86**	**0.97**	**0.96**
	SE	0.09	0.11	0.13	0.13	0.10	0.04	0.14	0.10	0.14	0.09	0.08	0.07	0.07	0.07
		**P<0.001*									*P=0.257*			*P=0.923*	
	Present	17	47	39	47	22	1	25	23	33	86	85	83	129	125
	Absent	31	1	9	1	26	47	23	25	15	58	59	61	87	91
Quantitative HCN	**Degrees of colour**	**12.13**	**35.67**	**25.40**	**37.53**	**14.81**	**0.73**	**17.33**	**10.48**	**22.06**	**22.45**	**18.28**	**19.10**	**19.06**	**20.64**
	SE	2.14	2.43	2.85	2.56	2.32	1.19	2.65	2.43	2.71	1.88	1.64	1.49	1.43	1.31
		**P<0.001*									*P=0.483*			*P=0.791*	

	Ecotype Soil type			Ecotype Management		
	Calcareous loam	**Neutral loam**	**Sand**	**Grazed**	**Cut**	**Unmgd**
	53.07	24.4	16.6	16.3	32.9	9.5
SE	1.65	1.75	1.55	0.35	0.44	0.57
	**P=0.003*			**P<0.001*		

Folly Farm, Woodborough and Southstoke (cut) ecotypes had the highest amount of leaf-HCN, and Salisbury Plain the lowest (QL&QN P<0.001) (Table 33). Apart from Cockey Down, the lowest amounts were from ecotypes of unmanaged home-sites. There were no significant differences between treatments. All treatments and ecotypes contained both acyanogenic and cyanogenic forms, though only one was cyanogenic for Salisbury Plain and only one acyanogenic for Southstoke and Folly Farm.

The scattergraph matrix (Appendix XVII) showed a strong correlation between the two datasets, therefore further analysis was conducted on the quantitative leaf-HCN results only.

Results from the quantitative leaf-HCN GLMM model (Figure 65) show significant variation between ecotypes (P<0.001), the only low result is for Salisbury Plain ecotype, whereas Dawlish Warren, Folly Farm, Woodborough and Southstoke are all high.

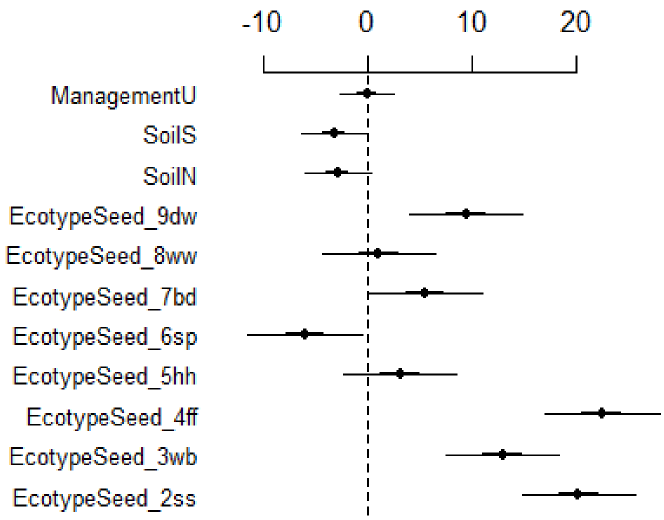


Figure 65. GLMM for quantitative leaf-HCN results. ManagementU, SoilS and SoilN are treatments, Seeds are ecotypes. Cockey Down ecotype in calcareous loam + grazed treatment are used as the standard.

The interaction model (Figure 66) of leaf-HCN identified no significant interactions. The grazed ecotypes had significantly more leaf-HCN than unmanaged ecotypes and significantly less than cut ecotypes (P<0.001).

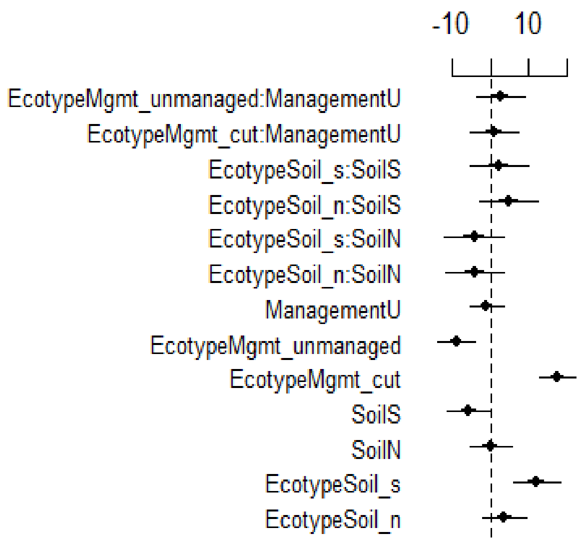


Figure 66. GLMM effects plot for quantitative leaf-HCN results, with interactions between ecotype and treatment. Factors with capital letter (S, N, U) are treatments, those with lower case letters (s, n, unmanaged, cut) are ecotype factors. Cockey Down in calcareous loam + grazed treatment is used as the standard.

12.8.3 Summary of Results – Leaf Chemical Analysis

Leaf-nitrogen: Plants receiving unmanaged treatment had significantly lower leaf-nitrogen. Significantly lower nitrogen was also found in plants grown in sand (in grazed treatment plants only). Dawlish Warren showed significantly lower leaf-nitrogen in unmanaged treatment compared to the model standard.

Leaf-HCN: Only Ecotype differences were significant; Salisbury Plain HCN was the lowest (significant) with only 1 plant out of 48 being cyanogenic. In the ecotype groupings those from cut sites were significantly higher [in HCN] than unmanaged ecotypes, also calcareous loam ecotypes were significantly higher than sand ecotypes. Southstoke, Woodborough, Folly Farm and Dawlish Warren had significantly higher HCN than the model standard.

13 DISCUSSION (STUDIES 1 & 2)

Please note that within this chapter, calcareous sand is referred to as ‘sand’ and cut with aftermath grazing is referred to as ‘cut’. ‘Grazing treatment’ refers to simulated grazing from cutting by hand.

13.1 Plant Fitness

13.1.1 Growth Parameters and Growth Pattern

The most significant variations in growth parameters were shown to be stem number per plant and leaflet number per main stem, which were significantly influenced by ecotype and treatments (Table 18). The three sand ecotypes had greatest number of stems [of ecotypes] after March which was when management commenced, this difference became more pronounced by harvest (August) (mean 44, $P<0.001$). Additional stems (per plant) may be an adaptation all three ecotypes have gained at their home-site that reduces the threat of sand burial (Gilbert & Ripley, 2008), a risk which is thought can kill plants through lack of oxygen (Maun, 1994) or absence of light (Sykes & Bastow Wilson, 1990). Zhang & Maun (1990) also found burial of young *Agropyron psammophilum* Gillett & Senn seedlings encouraged tiller production. Xu *et al*. (2013) found burial of *Caragana intermedi* Kuang & H.C.Fu induced a plastic response of elongated stems, and Gilbert & Ripley (2008) found stem production of *Scaevola plumieri* L., was increased by biomass allocation shift for compensatory growth. The greater stem number could also be a result of adaptation to low-nutrient status home-sites (Hawke & Maun 1988) as phenotypic plasticity has been found to exist in sand dune plants tolerant of low-nutrient availability (Houle, 1997), particularly in terms of number of tillers and lateral shoots (Basra, 1994). When the stem number model was split between management treatments (Table 19) all three sand ecotypes had significantly greater stem numbers in the grazed treatment ($P<0.001$) yet only Berrow Dunes had a significantly higher result ($P<0.001$) in the unmanaged treatment. This difference indicates that the grazed treatment may have stimulated growth of these particular ecotypes. This adaptation is important for plant establishment and longevity when translocating plants to such stressed environments (Sykes & Bastow Wilson, 1990; Zhang & Maun, 1990; Maun, 1994; Xu *et al*., 2013; Zhu *et al*., 2014) and highlights the need to match stressed environments in donor seed choice.

Sand ecotypes also had significantly greater numbers of leaflets per main stem (mean 118, $P<0.001$). Similar to stem number, this shows a possible adaptation to the home-site as sand burial has been found to stimulate leaf production (Zhang & Maun, 1990; Shi *et al*., 2004). This is a recurrent event thought to influence evolution and adaptation in sand dune plants (Maun, 2009). This response doesn't seem to reflect phenotypic plasticity generally as higher leaflet number (per main stem) in sand ecotypes was evident throughout all soil treatments. Plasticity is shown for plants growing in sand treatment, presenting lower leaflet number (per main stem) possibly due to higher desiccation and nutrient leaching of this soil (International Plant Nutrition Institute (IPNI), 2013) in comparison with the two loam treatment soils. It has been found that leaf number and biomass often respond positively to soil nutrients and soil water (Bazzaz & Harper, 1977; Cornelissen *et al.*, 2003). Another factor, this time shown in 'Soil&Mgmt' leaflet (number per main stem) results, was the negative values indicating the number of leaflets shed following commencement of management (in March). The leaf shedding results were lower for two of the sand ecotypes (Berrow Dunes and Woolacombe Warren with figures of -20 and +10 respectively), whereas the other ecotypes ranged from -42 (Cockey Down) to -61 (Woodborough). Greater leaf longevity is thought to be a trait of species able to occupy nutrient-poor soils (Cornelissen *et al*., 2003) and loss of leaves can occur in response to high salinity (Munns, 2002). The findings on leaflet number (per main stem) for sand ecotypes therefore also suggests an adaptation to the edaphic stresses of a sand dune site.

The calcareous loam ecotypes had significantly smaller leaflet number (per main stem) in unmanaged treatment ($P<0.001$), and two of these (Southstoke and Woodborough) also had significantly greater in grazed treatment ($P<0.001$), suggesting these ecotypes are more sensitive to the management treatment than the others. As these three ecotypes were from sites that were cut or grazed (with none from unmanaged sites), this difference could indicate adaptation to home-site management. Similarly, a study using clover found less energy was invested into lamina ratio in the most lenient grazing height (9cm) of three studied (Parsons *et al*., 1991). Panagiota *et al*. (2014) concluded that morphological differences between their studied *Lotus corniculatus* populations could be largely attributed to grazing intensity (results included finding smaller leaves in heavily grazed management).

Hellenge Hill also had significantly higher leaflet number (per main stem) throughout both treatments (though less pronounced than sand ecotypes). As there were no similarities in soil type parameters measured and due to Hellenge Hill's closer geographical proximity to the sand ecotypes it was suggested this could be a climatic influence. However, there was no significant effect of spatial distance between ecotypes in the model.

Stem number (per plant) and leaflet number (per main stem) were both significantly greater in calcareous loam treatment soil. As well as previously seen effects from stressed home-site conditions, plant growth is supported by greater nitrogen availability (Prine & Burton, 1956; McLeod & Murphy, 1983; McNaughton *et al*., 1983*;* IPNI, 2013), and this treatment had significantly greater amounts of nitrate-nitrogen compared to other soil treatments ($P<0.050$) (Table 11). The grazed treatment resulted in significantly greater stem numbers, thought to be a response that overcomes the removal of top vegetation by producing greater growth lower to the soil (Parsons *et al*., 1991). Increased cutting (from 12 to 6 week intervals) improved yield of Bermuda grass (Burton *et al*., 1963), and NcNaughton *et al*. (1983), also found compensatory growth in response to moderate material removal. This treatment also produced fewer leaflets (per main stem) ($P<0.001$), although this could be leaflets lost to plant matter removed.

The pattern of main stem length (Figure 33, Figure 34) showed a fall in the second year, this may have been due to the pot-grown experiment causing root restriction, leaching and also salt build up due to the capillary bench system of irrigation used (Bunt, 1983). Plant height has been found to be positively correlated with organic matter, and therefore leachable soil nutrients such as nitrate and phosphorus (Fu *et al.,* 2015).

Plants grown in calcareous loam soil produced significantly longer main stems (mean 25.4cm, $P<0.001$) (Table 19). Although main stem length is not directly linked to invertebrate diet, it is a good indicator of competitive vigour, potential fecundity and therefore plant longevity (Cornelissen *et al*., 2003) and indicates where there may be problems in the long term growth for *L. corniculatus* grown in neutral loam or sand soil and those not adapted to nutrient-poor soil.

Longest branch (per main stem) length (Table 18) did not differ significantly between ecotypes or treatments, there were only treatment differences for branches per main stem, and little variation was found between ecotypes for main stem length, which differs to previous research which found ecotypic variation in *L. corniculatus* for main stem length, branch number and branch size (Kelman *et al.*, 1997; Sareen & Dev, 2003). The lack of evidence of ecotypic adaptation carrying over to the receptor site shown for variables such as this is encouraging as it suggests neither geographical distance nor soil type or management adaptations produce significantly differing results for a range of likely receptor sites. An unexpected outcome from this research was that unmanaged treatment produced significantly more branching whereas it has been suggested in other research that herbivore damage/grazing or cutting may induce branching (Naber & Aarssen, 1998).

13.1.2 Fecundity

Model results indicated the two cut ecotypes (Southstoke and Woodborough) exhibited seed pods (Table 21) which took significantly longer to form ($P=0.001$) than the standard, and cut ecotypes as a whole produced significantly fewer seed pods (than the standard) in the unmanaged treatment ($P=0.001$). As defoliation is thought to reduce plant fecundity (Stephenson, 1980; Hare, 1983) due to removal of reproductive organs, stunting flowering (Vignolio *et al*., 2010) and reducing resource availability, such as carbohydrate (Eaton & Ergle, 1954; Trlica & Cook, 1971; Crawley, 1983), these results could be explained by the significantly greater amount of seeds per pod found for cut ecotypes in both treatments, indicating that these ecotypes have evolved to produce fewer, larger seed pods. Although less, these would only need to ripen by cutting-stage at July/August. This would enable a greater degree of seed return rather than allocating resource to a larger number of smaller seed pods, which after the first cut may not develop fully due to aftermath grazing and therefore reduced carbohydrate availability (Eaton & Ergle, 1954; Trlica & Cook, 1971; Crawley 1983). There may also be some plasticity for fecundity where cut ecotypes produced significantly more seed pods in the grazed treatment, and unmanaged ecotypes were significantly fast to form seed pods in the grazed treatment. However, these results should be interpreted to field use cautiously as mean values of seeds per pod rounded to seven seeds for all ecotype and treatment groupings.

Berrow Dunes, Woolacombe Warren and Dawlish Warren were the other ecotypes found to be significantly delayed (in the model) in initiating seed pod formation (P=0.001). Although this delay could be due to factors such as reduced number of umbels (Vignolio *et al*., 2010) and early flower abortion (Siddique & Sedgley, 1986), it may also be due to these three ecotypes being from sand soils which could have given them an adaptation to slower reproductive growth in a harsher environment. For example, the surface of freely-draining sand heats up quickly (Maun, 2009), this can be hazardous to young seedlings, and can induce germination delay and increased seed dormancy when high temperatures cause desiccation (Grime, 1979; Maun, 2009; Zhu *et al.,* 2014). Supporting this, were the germination results (Chapter 7) which were lower for sand ecotypes.

Therefore, later seed dispersal and preference of spring germination may be another possible adaptation causing [the] slower seed pod formation. Such germination delay has been found in *Polygonum aviculare* L. (Courtney, 1968) and *Leymus secalinus* Georgi (Zhu *et al*., 2014), where it was thought that high temperatures and dehydration had induced dormancy. As previous research by Carter *et al*. (1997) found drought stress could reduce reproduction of *L. corniculatus* and a study by Stephenson (1984) found soil nutrient limitations explained decreased reproductive output, this could be indicative of an adaptation that these sand ecotypes have developed to a droughty medium.

All ecotypes had significantly more seeds per pod (Table 24) than the Cockey Down standard (mean 5, P<0.001). The grazed treatment seed model showed similarities to seed pods sampled so should be treated with caution in interpretation as there were fewer pods available to sample in these. Instead looking at unmanaged treatment, Folly Farm, Dawlish Warren and Salisbury Plain had significantly fewer seeds per pod (P=0.004) and Hellenge Hill had significantly more (P=0.004), which for the latter two of those ecotypes (Salisbury Plain and Hellenge Hill) was surprising as they showed reduced seed production when receiving similar management to their home-sites. However, lower seed production could be an indicator of high home-site competition as plants from high density stands can bear fewer seeds per plant through intraspecific and interspecific competition, both directly (Weiner, 1985), and indirectly, through depressing plant size (Borowicz, 1993; Ollerton & Lack, 1998; Forrest, 2014).

Treatments led to significant variation in fecundity which is likely to be due to the resource limitations as shown in Stephenson (1984)'s study where fertilized soil and partial defoliation explained reproductive output more than pollination did. The calcareous loam soil treatment led to significantly more seed pods (Table 21) (P=0.002) and significantly greater seed pod biomass per plant (Table 23) (P=0.001), as did the unmanaged treatment (P<0.001 for both). Unmanaged [treatment] seed pods also contained significantly more seeds per pod (P<0.001), possibly where these were given longer to mature before defoliation at harvest. In the grazed treatment, significantly more plants formed seed pods later in the growing season and more plants in this treatment never formed seed pods before harvest. These [grazed treatment] seed pods also contained significantly higher moisture content (possibly due to more being unripe at harvest).

Mean seeds per plant (Table 24) only showed variation between management treatments, with significantly more mean seeds per plant [over both years] in the unmanaged treatment (mean 486, compared to 34 in grazed treatment, P<0.001).

13.1.3 Mortality

There were 28 plant mortalities throughout the experiment (Figure 49). Significantly more of these mortalities occured when grown in neutral loam soil treatment (P=0.025). This may suggest a soil type which *L. corniculatus* is less able to initially colonise successfully, possibly due to the low calcium and lower than 6.2 pH content (Rhykerd, 2007). So greater relative proportion of *L.corniculatus* seed should be considered in mixes, for neutral grassland restoration.

13.2 **Herbivore Requirements**

13.2.1 Flowering Phenology and Production

Hellenge Hill and Woodborough ecotypes began flowering significantly earlier than the other ecotypes (Figure 55), this may be a reflection of these two sites having the highest altitudes in the study (160m and 190m respectively) (Table 7) as flowering time variation has been found over an altitudal gradient in a clinal study (Suter *et al.,* 2014).

Two clear flowering peaks were seen in the first year (April and July) but different patterns occurred in the second, some ecotypes having just one peak and all finishing earlier (which could explain the lower seed pod count for the second year). Springate and Kover (2014) found elevated temperatures accelerated flowering time and Meineri *et al*. (2014) found it to increase flowering production, both of which could be the case here, as Figure 26 shows higher monthly temperatures in this year [to the previous year] for June to October. However, in contrast, Frei *et al*. (2014) found elevated temperature did not affect flower number of *Trifolium montanum* L., *Ranunculus bulbosus* L. and *Briza media* L. in their study and Reisch & Poschlod (2009) instead found genetic differentiation of flowering phenology was stronger between land managements than it was between geographic regions. Looking again at the flowering pattern, only cut and calcareous loam ecotypes still had two distinct peaks (in April and June) again during the second year (Figure 56). As all calcareous loam ecotypes were either from grazed or cut sites, this could be an adaptation to the timing of seed pod loss through biomass removal at home-sites. Research on *Scabiosa columbaria* L. (Reisch & Poschlod, 2009) found genetic variation of floral display was clearly linked with land-use, shown by populations of mown sites flowering significantly earlier than those from grazed, and Warren & Billington (2005) also found hay meadows flowered earlier than other grasslands.

The calcareous loam ecotype had produced significantly more flowers by harvest and there was significant positive interaction between the sand ecotype in matching soil type, and unmanaged ecotype in unmanaged treatment. This could indicate more productive ecotypes and/or a matching soil type or management advantage (and adaptation). Indeed, Stephenson (1984) concluded that *L.corniculatus* flower number was regulated by soil resource availability, Ollerton & Lack (1998) determined larger plant size to be correlated with a longer flowering period in *L.corniculatus* and Forrest (2014) stated that in many plant populations early flowering and improved fecundity are positively correlated with larger plant size which [size] could also be indicating greater fitness here, potentially from factors such soil resources.

Treatments led to significant differences in all flower parameters; plants grown in calcareous loam soil had significantly shorter time to first flowering, supporting Ollerton and Lack's (1998) and Forrest's (2014) theory of larger plants flowering earlier. Plants grown in neutral loam treatment soil produced significantly more flowers at harvest and plants of unmanaged treatment had signifiantly stronger flower scent in both pre-harvest flower scent and bagged flower scent tests. Such differences in flower phenology should cause negligible implication for herbivory as this plasticity would presumably be constant at the herbivore home-site due to the treatments being the cause rather than the ecotypes.

13.2.2 Growth Habit and Hirsuteness

There was no management treatment difference within hirsuteness though calcareous loam treatment soil produced significantly more glabrous plants ($P<0.001$) (Table 29). Ecotypic variation was also seen for hirsuteness with the three sand ecotypes (Berrow Dunes, Woolacombe Warren and Dawlish Warren) significantly more hirsute than the Cockey Down standard ($P<0.001$).

These differences in hirsuteness could be another adaptation of the sand ecotypes to a dry environment as leaf surface hairs can reduce moisture loss (Maun, 2009). When grouped together, ecotypes from neutral loam sites and those from cut sites were significantly more glabrous than the standard. This could have fitness implications in terms of plant longevity, in a mismatched receptor site, a glabrous plant from a loamy meadow translocated to a dry, coastal site with high desiccation may exhibit signs of drought stress. Hirsuteness could also reduce feeding ability, if animals at the receptor site are not adapted to digesting trichomes, often found to be a form of structural defence (Hanley *et al*., 2007). However it should be noted that mean results (for ecotypes and treatments) each rounded to 2 ‘slightly hirsute’.

Contrasting to previous research (Kelman *et al*., 1997), only treatment differences were shown to be significant for growth habit with neutral loam soil and unmanaged treatments producing more erect profiles. Panagiota *et al*. (2014) found *L. corniculatus* had a more prostrate growth habit when heavily grazed and Kelman noticed associations between prostrate growth habits of *L. corniculatus* and condensed tannin levels. However, growth habit here was considered to be phenotypic plasticity only, due to lack of differentiation between ecotypes.

13.2.3 Biomass

Treatments had the greatest effect on biomass, with higher grazed treatment dry clippings (Figure 63) and harvest biomass (Table 30) found for plants grown in calcareous loam treatment soil ($P<0.001$ for both). In other studies, nitrogen has been shown to be the main limiting factor in plant biomass production (Prine & Burton, 1956; McLeod & Murphy, 1983; McNaughton *et al*., 1983; Zhang *et al*., 2015), which may be the case here as sand and neutral loam treatment soils had significantly less nitrate than calcareous loam ($P<0.050$) (Table 11).

Unsurprisingly, unmanaged treatment plants had significantly higher dry harvest weight (mean 3.7g compared to 1.8g for grazed treatment $P<0.001$), likely due to no previous vegetation removal. However, grazed treatment plants had significantly higher relative moisture content (mean 71.2%

compared to 68.5% in unmanaged, $P<0.001$), a result also found in previous research of defoliation effects on a sedge (*Kyllinga nervosa* Steud) (McNaughton *et al.,* 1983), where it was considered plant material lost [through grazing] conserved soil-water and increased relative moisture content of the plant, both of which contributed to increased plant growth after grazing. It could be that harvesting fresh-growth regularly (through the grazed treatment) reduced dry material that would have instead become thickened and lignified from maturity (Engels & Jung, 1998).

The only significant variation between ecotypes for biomass (Figure 62, Table 30) was Woodborough which had significantly lower grazed treatment clippings biomass ($P=0.035$) (Figure 62). As this was the only site differing it could be this ecotype is just a poorer biomass producer than the others.

13.2.4 Chemical Properties

For leaf-nitrogen it was treatment that resulted in greatest variation (Table 31, Table 32). Dawlish Warren was the only ecotype to show significant difference (in the model) with lower leaf-nitrogen than the standard in unmanaged treatment. Dawlish Warren's lower leaf-nitrogen could reflect a reduced capacity to retain this element where the ecotype has become adapted to low amounts at the home-site (Chapin, 1980; IPNI, 2013) [Dawlish Warren had the lowest nitrate content of all ecotype soils at 6.12ppm] (Table 3), this possible adaptive feature is reflected in the treatment differences. It has also been found that low plant nitrate content (and therefore low nutritional value) can be used as a form of herbivore defence when low dietary nitrogen cannot be compensated for by herbivores by infinite eating (Mattson, 1980). However, nitrogen is also needed for production of nitrogen based chemical defences such as HCN (Hermes & Mattson, 1992).

The management treatment was shown to be the most influential factor in the model, with significantly higher leaf-nitrogen shown in the grazed treatment plants (mean 0.71%). Such increased leaf-nitrogen is thought to be at least partly due to induced reaction of secondary metabolite production after herbivory (Bardgett *et al.,* 1998). The sand treatment soil produced significantly lower leaf-nitrogen throughout management (mean 0.62%). It can be assumed that this low leaf-nitrogen reflects that available from the soil (Aerts, 1996; Cornelissen *et al*., 2003). This effect in the sand treatment could be due to leaching of nitrate from the soil, nitrate is a mobile element (IPNI, 2013) and lack of soil organic matter and clay (mean in sand soil = 1.71%) (Figure 20) in this substrate would have created a faster rate of loss and a lower reservoir (normally held in the organic matter) (IPNI, 2013).

Hydrogen cyanide (HCN) was found to be significantly lower in Salisbury Plain plants (mean 0.73 degrees of colour) (Table 33, Figure 65), this could be a factor of the unmanaged condition of this home-site, as HCN is thought to be used as herbivore defence (Morant *et al*., 2008; Pentzold *et al*., 2014). Although historically the Salisbury Plain site would have been grazed by rabbits and sheep it is now one of the sites with the longest sward height of all ecotypes sampled (Appendix V). This site also contained the least cyanogenic plants (1), which contrasts with Ellis (1977) who suggested only highly exposed coastal sites would contain predominantly acyanogenic plants.

The three significantly higher leaf-HCN ecotypes (Southstoke, Woodborough, and Folly Farm) (means respectively 35.67, 25.40, 37.53 degrees of colour) which also contained the least acyanogenic plants of all, were from cut sites. This was supported in the ecotype management grouping model which showed cut ecotypes as having significantly higher leaf-HCN compared to the grazed standard, and higher still than the unmanaged ecotypes. This result suggests an environmental adaptation to a stressed home-site management, corresponding with previous work in which predominantly acyanogenic *L.corniculatus* often occurs in environmental conditions where cost of producing the chemical is outweighed by other factors (Bloom *et al*., 1985; Till-Bottraud & Gouyon, 1992).

Ecotype soil groupings (Table 33) also showed variation with those from calcareous loam sites having significantly more leaf-HCN (mean 53.07 degrees of colour) compared to the sand ecotypes (mean 16.6 degrees of colour). This may be from adaptation to homesite conditions where decreased nutrient uptake of an easily leaching sand soil (Aerts, 1996; Cornelissen *et al*., 2003; IPNI, 2013) prioritises nitrogen availability towards plant growth, whereas excess nitrogen (to that needed in growth) in the loam soils have been allocated to HCN production (Herms & Mattson, 1992), as HCN is found to be higher in plants when nitrogen is in high supply (Gleadow & Møller 2014). This result could also be due to the sand ecotypes close proximity to the sea, and therefore greater exposure to wind-borne salt, where numbers of selective herbivores are found to be lower (Ellis *et al*., 1977). However, in contrast to Ellis *et al.* (1997) Berrow Dunes contained 52% cyanogenic plants, and Dawlish Warren 69% rather than predominantly acyanogenic plants. Ellis *et al.* (1977)'s thoeory of coastal exposure could however, explain differences between the sand ecotypes, as Dawlish Warren is located on the south coast as apposed to the north (more exposed coast) of the other two sand ecotypes this could be a contributing factor to its higher amount of cyanogenic plants.

Although there was no correlation between leaf-nitrogen and leaf-HCN, it is interesting to see Dawlish Warren had significantly higher leaf-HCN, yet significantly lower nitrogen (compared to the model standard), suggesting a resource allocation trade-off between the two with nitrogen being used in HCN production as priority over plant growth (Hermes & Mattson, 1992). It may also suggest this site has had long historical grazing of wild and/or domestic herbivores (Morant *et al*., 2008).

There were no management treatment differences for leaf-HCN, this result was surprising as previous research has pointed towards herbivory (and therefore tissue damage) as activating chemical defences (Briggs, 1991; Morant *et al*., 2008; Pentzold *et al.,* 2014). However, it has also been found that this interaction is more likely to happen in nectar rather than leaves (Alder *et al*., 2006). There were both cyanogenic and acyanogenic forms within each treatment and each ecotype, as found in previous research (Ellis *et al*., 1977). Although leaf-HCN levels did not differ significantly in response to soil or management treatments, significant differences were recorded between ecotypes. Such ecotype differences are thought more likely to be genetic adaptations to home-sites rather than phenotypic plasticity.

14 BEE INTERACTION - STUDY 3

Please note that within this chapter, calcareous sand is referred to as ‘sand’ and cut with aftermath grazing is referred to as ‘cut’. ‘Grazing treatment’ refers to simulated grazing from cutting by hand.

14.1 Pollinators and Bees - Introduction

Insects which visit flowers to collect pollen and nectar for consumption transfer pollen between plants from their bodies, thereby pollinating them (Defra, 2014). Such insects include bees, wasps, hoverflies and butterflies (Defra, 2014). Bees (especially honeybees) have been highly publicised in the media in recent years due to their global decline in numbers (Black, 2010; Carrington, 2012; BBC News UK, 2013; Holland, 2013; Goldenberg, 2014; *Who killed the honeybee?*, 2014), with much research (Henry *et al*., 2012; Tirado *et al.,* 2013; Devoto *et al.,* 2014; Goldenberg, 2014; The Laboratory of Apiculture and Social Insects (LASI), 2015; United States Depatment of Agriculture (USDA), 2015) and a national strategy (Defra, 2014) to help identify and halt the declines. Several factors are thought to be causing bee decline, including habitat destruction decreasing plant species diversity and therefore foraging source (Müller *et al.*, 2006). It has been suggested that the loss of Fabaceae-rich unimproved grassland is of particular importance (Goulson *et al*., 2005), and agricultural intensification has been blamed in part for the decline (Goulson *et al*., 2005; Heard *et al*., 2007; Batáry *et al*., 2010; Defra, 2014). Other pressures include diseases, pathogens, some insecticides, invasive species and climate change (Defra, 2014). The importance of bees for pollination of wild plants and crops worldwide is paramount (Blake *et al*., 2012), over 70% of the major food crops are dependant on pollination provided by bumblebees, honeybees and solitary bees (Blake *et al*., 2012). Therefore research to find best ways of protecting these insects is crucial.

Plant choice to bees is often regulated by proboscis length. Long-tongued species, such as *Bombus pascorum* (Scopoli,1763) (Pywell, 2004) usually visit deep flowers, including Fabaceae (Goulson *et al*., 2005), these are often more specialised than short-tongued species and therefore many, such as *B. ruderatus* (Fabricius, 1775) are among those in decline (Pywell, 2004). However, it appears short-tongued bumble bees are also attracted to *Lotus corniculatus* with the species *B. mixtus* (Cresson, 1878), *B. sitkensis* (Nylander 1848), and *B. occidentalis* (Greene, 1858) found to prefer this plant out of a choice of 21 bee forage herbs in one study (Patien *et al*., 1993). In combination with the bees' physiological differences, flower choice decision is also determined by the bees' olfactory and visual traits (Junker & Parachnowitsch, 2015) to find flowers of greatest nutritional reward. Flower colour was not found to be important in this choice in a study on nectar production in *L. corniculatus* (Murrell *et al*., 1982).

As with other herbivores, nitrogen is vital for bee nutrition, helping with cell structure and fecundity (Mattson, 1980; Crawley, 1983). In addition to nutritional differences, another factor considered in flower quality to bees is high nectar alkaloids (Alder and Irwin, 2005). Previous research has found that bees still use plants with high amounts of such toxic chemicals yet do not spend as much time per flower (Alder & Irwin, 2005). This finding supports (other) research that found bees are not well adapted to avoid plants containing allelochemicals (including cyanogenic glycosides), many causing bee mortality (Detzel & Wink, 1993). Therefore the concentration of these chemicals in nectar are viewed to be of greater importance to herbivores than are nutrients (Bryant & Kuropat, 1980; Howe & Westley, 1988).

Foraging distance for individual bumblebees and their colonies has been shown to be approximately 1km to 1.5km (Osborne *et al*., 2008; Goulson, 2009), the shorter the range of foraging by a bumblebee species reflects the more negative a response to declines in landscape species richness (Osborne *et al*., 2008). In contrast *Apis melifera* L. (Western Honeybee) has been shown to have a wider range, flying distances of between 0.45km – 5.98km (Hagler *et al*., 2011), whereas solitary bees have been estimated to have a smaller foraging range, between 0.15km and 0.60km (Gathmann & Tscharntke, 2002).

14.2 Bee Preference Study - Methods

During the early stages of the glasshouse experiment it was decided that bees would be observed and their plant preferences recorded. Several methods were devised for this including introducing bees to the plants at certain periods, and knowledge was sought from experts in this field (Chapter 16). However, once the plants had started flowering it was noticed that bees were entering the glasshouse and using the plants of their own accord. Due to technical and logistical difficulties with the originally proposed methods, it was decided that the bees already freely using the plants would give good insight into bee preference and should therefore be observed.

During the peak flowering times in 2012 and 2013, bee foraging preferences were observed during 1-hour periods (Goulson & Hanley, 2004). During each survey, individual bees were selected and their activity recorded during a 5-minute period. Where possible, each bee was identified to species level and the identification number of the plant used by the bee was recorded ('bee visits') and total time spent on flowers in a given plant (from landing to departure) was recorded ('time spent') in seconds (Herrera, 1989) using a stopwatch. The total number of plants with flowers visited over the survey period was also recorded (Herrera, 1989). Interference and disturbance of the bee activity by the recorder was avoided by limiting fast movements, avoiding creating shadow onto each bee and keeping at least 1m distance. If plant identification numbers could not be identified at this distance they were checked as soon as the bee vacated it. Notes were made if the bee was only resting on the plant rather than collecting pollen and nectar. Ambient temperature and outside weather conditions were recorded at the start of each survey, and surveys were only undertaken between 10.00 and 17.30, during dry weather when ambient temperature was above 17^{o}C (Heard *et al*., 2007).

Bees which went out of sight either during or after their allotted 5 minutes and then reappeared might be recorded more than once (Fussel and Corbet, 1992).

Survey details and conditions are tabulated in Appendix XVIII.

The Herbivore Requirements Results (Chapter 12.8) were used in conjunction with bee observations to determine whether any interactions existed between these response variables.

14.2.1 Data Analysis

All parameters were first tabulated with means, standard deviations and standard errors calculated. Bar charts were produced in Excel (Microsoft, 2013) (version 15.0.4551.1005) to see initial differences between ecotypes and treatments.

Data were then saved as CSV files for use in R statistical software version 3.1.0 (R Core Team, 2014). Histograms were created using Rcmdr (Fox & Bouchet-Valat, 2014) (version 2.0-4) to help determine the distribution of each data set, as data was found to be of non-parametric distribution the Kruskall-Wallis test was used to determine initial significant differences.

Scattergraph matrices and boxplots were created using Rstudio (RStudio, 2013) (version 0.98.994) to identify similarities and correlation between datasets.

14.2.2 Data Analysis: Generalized Linear Mixed Model (GLMM)

Generalized linear mixed models (GLMM) were used to statistically predict ecotypic distributions with specific sub-sets of response variables, as this statistical technique is good for species-specific models (Guisan *et al.,* 1999). GLMM tuition and written aids were used in the application and interpretation of the models in R (Field *et al.,* 2009; Zuur *et al.,* 2009; Winter, 2013; Smith, 2014) in R Studio (RStudio, 2013) (version 0.98.994). It was originally hoped that one model could be used (with the response variable changed for each dataset). However due to the high number of variables and interactions, the sample size prevented a full model analysis (Smith, 2014). Partial models were therefore processed and resulting effects plots along with the Akaike Information Criterion (AIC) and P values from ANOVA comparisons values used to determine the most important factors for the final model (Winter, 2013;

Smith, 2014). For the first model (Model A) it was hoped to include each ecotype, the treatment soil and management. The partial models outlined in Chapter 11.6 were tried with the following models found to be the most suitable for ‘bee visits’ and ‘time spent’ (the bold text is the response variable which was changed to that being investigated at the time of analysis). The standard used in these models was Cockey Down (a calcareous loam/grazed ecotype), in calcareous loam and grazed treatment.

<-lmer(**response_variable**~EcotypeSeed+soil+management+(1|f.replic), data= **response_ variable**)

This model uses replication as a random effect and ecotype, soil treatment and management treatment as fixed effects.

The interaction and ecotype site model was simpler (with no significant difference) without the interaction factor, therefore the simpler model was used:

<-lmer(**response_variable**~EcotypeSoil+soil+EcotypeMgmt+management+(1/f.replic),data=**reponse_variable**)

As before, these models were repeated with ANOVA comparison of null (partial) models to establish P value significance of factors.

All of the above models were calculated using a Poisson distribution due to the nature of the data (count data and non-parametric data), ‘family = poisson’ was entered into the model equation before ‘data=’.

To identify whether any of the previously measured response variables would act as predictor factors for the bee variables two further models were calculated, these were split between vegetation factors and flower factors. As all factors were numeric the predictor variable was defined as continuous.

Vegetation factors model:

<-lmer(**response_variable**~+ nitrogen + HCN + hirsuteness + vegetation_dry_biomass + (1/f.replic),data=**reponse_variable**)

Flower factors model:

<-lmer(**response_variable**~+ flower_number + flower_dry_biomass + pre_harvest_flower_scent + relative_moisture_content + (1/f.replic),data=**reponse_variable**)

14.2.3 Distance from Bee Home-site (Bath Spa University)

To aid in interpreting the results of the bee data, geographical distances were calculated between the glasshouse location and the bees foraging there (referred to as 'test foraging area'), to each ecotype site (Table 34). These distances were studied in conjunction with results to identify whether there were any bee preferences for sites geographically closer to the glasshouse.

Table 34. Geographical distance (km) between the glasshouse (home-site of bees) and ecotype sites, in ascending order of distance

Ecotype sites	OS Grid refs.	Bath Spa University Glasshouse ST693637
Southstoke	ST737610	5.16
Folly Farm	ST611606	8.77
Hellenge Hill	ST345572	35.4
Berrow Dunes	ST292532	41.45
Woodborough	SU117614	42.46
Salisbury Plain	SU192481	52.28
Cockey Down	SU173320	57.52
Dawlish Warren	SX983789	110.6
Woolacombe Warren	SS455426	125.59

14.3 **Bee Preference Study – Results**

14.3.1 Bee Species Recorded in the Survey

Seven species were timed and recorded during the bee survey, with an extra species (*Halictus tumulorum* L.) also noted using the plants outside of the bee survey times.

Apis mellifera was one of the first bees recorded using the plants (Figure 67). *A. melifera* are active from spring until late autumn, coming from wild colonies or domestic hives (Chinery, 2005).

Three of the species were bumble bees; *Bombus lapidarus* L. (Figure 68), *B. terrestris* L. (Figure 69) and *B. pascorum* (Scopoli,1763) (Figure 70). Both *B. lapidarius* and *B. terrestris* are short-tongued species (Heard *et al*., 2007)) and *B. pascorum* medium/long tongued (Goulson *et al*., 2005; Heard *et al*., 2007). However it is this latter species and *B. lapidarius* which have the more specialised diet of the three with *B. terrestris* using a broad diet.

Figure 67. *Apis mellifera* L.
(Source: Author, 2013)

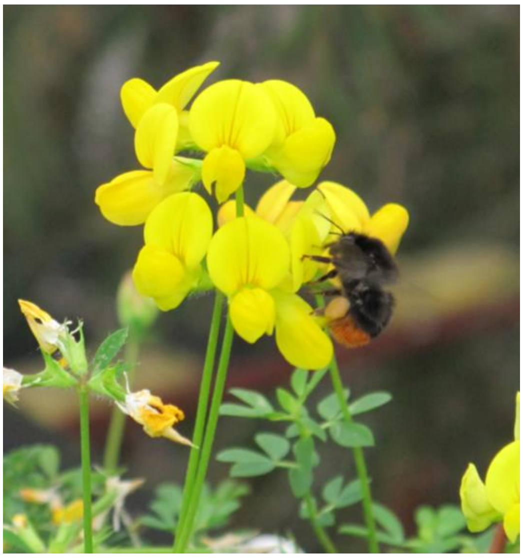


Figure 68. *Bombus lapidarus* L.
(Source: Author, 2013)

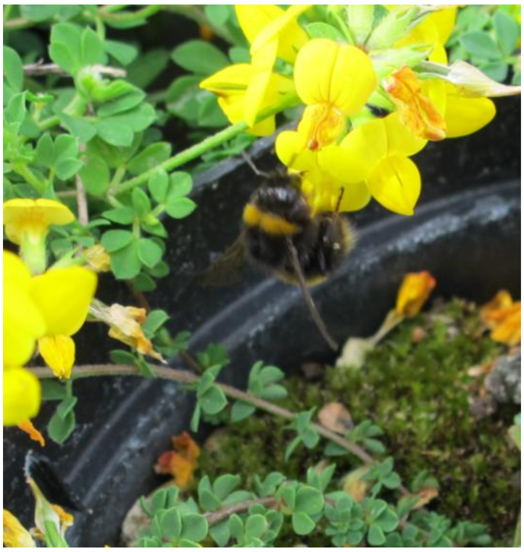


Figure 69. *Bombus terrestris* L.
(Source: Author, 2013)

Figure 70. *Bombus pascuorum* (Scopoli,1763)
(Source: Author, 2013)

In addition to the bumblebees was *Bombus vestalis* (Geoffroy, 1785) (Figure 71), a similar species in appearance, this is a cuckoo bee which is parasitic on bumblebees (Chinery, 2005). This particular species lays its eggs in the nests of *B. terrestris,* which then rear the larvae as their own (Chinery, 2005).

Two solitary bees were also recorded during the surveys, these were both leaf-cutter bees; *Megachile willughbiella* (Kirby, 1802) (Figure 72) and *M. centuncularis* L. (Figure 73). These use their jaws to cut sections out of leaves to create nest cells which line cavities (Bees, Wasps, Ants, Recording Society (BWARS), 2014). Their flight patterns are from mid-June to early and late August (BWARS, 2014).

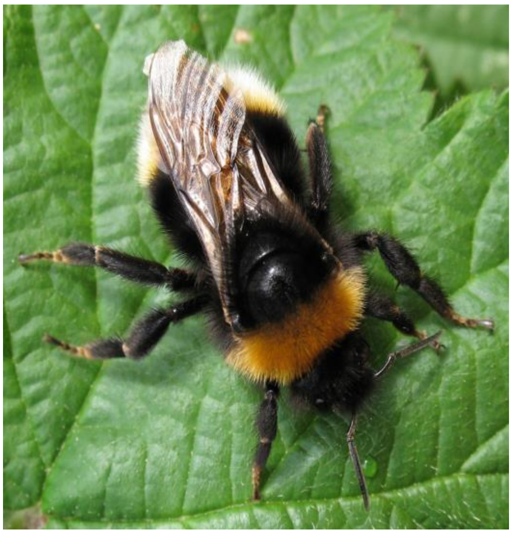


Figure 71. *Bombus vestalis* (Geoffroy, 1785) (Source: Jones, undated)

Figure 72. *Megachile willughbiella* (Kirby, 1802) (Source: Author, 2013)

Halictus tumulorum L. (Figure 74) was observed using the plants but wasn't recorded during the surveys. This species has a short tongue, usually nesting in burrows and is active between April and July (Chinery, 2005).

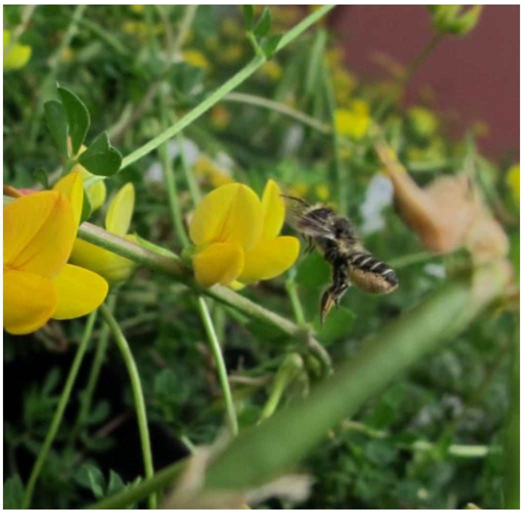


Figure 73. *Megachile centuncularis* L. (Source: Author, 2013)

Figure 74. *Halictus tumulorum* L. (Source: Author, 2013)

14.3.2 Bee Use of Plants in Survey

Mean results were calculated for number of plants visited ('bee visits'), time (in seconds) spent per visit ('time spent') and number of surveys the visits occurred in ('surveys') (Table 35). The only significant variation was seen to be between ecotypes for bee visits and time spent per visit. The shortest time was spent on Hellenge Hill and the most time on Cockey Down.

Table 35. Bee survey results. Time Spent: Seconds spent per plant visit, Bee Visits: number of plant visits per survey, Surveys: Number of surveys the bee occurred. (Ecotype *n*=48, Soil *n*=144, Management *n*=216), *P*=Kruskall Wallis. See Table 21 for ecotype and treatment key.

		Ecotype									Soil			Management	
		cd	ss	wb	ff	hh	sp	bd	ww	dw	C	N	S	G	U
Bee Visits		**2.0**	**1.3**	**2.0**	**1.1**	**1.1**	**1.5**	**1.5**	**1.0**	**1.1**	**1.6**	**1.3**	**1.3**	**1.3**	**1.4**
	SE	0.3	0.2	0.3	0.2	0.1	0.2	0.3	0.2	0.2	0.2	0.1	0.1	0.1	0.1
		**P*=0.017									*P*=0.515			*P*=0.511	
Time Spent		**13.5**	**10.5**	**13.3**	**12.0**	**6.8**	**10.8**	**13.4**	**8.4**	**11.4**	**14.1**	**9.9**	**9.6**	**11.5**	**11.0**
	SE	1.9	1.5	1.9	1.7	1.0	1.6	1.9	1.2	1.7	1.3	0.8	0.8	0.8	0.8
		**P*=0.039									*P*=0.230			*P*=0.648	
Surveys		**1.9**	**2.9**	**1.9**	**1.7**	**1.5**	**1.4**	**1.9**	**1.7**	**1.6**	**1.1**	**1.2**	**1.1**	**1.1**	**1.3**
	SE	0.3	0.4	0.3	0.2	0.2	0.2	0.3	0.2	0.2	0.1	0.1	0.1	0.1	0.1
		P=0.076									*P*=0.486			*P*=0.409	

As only significant differences were found for bee visits and time spent these factors were used in the model (Table 36). Southstoke, Hellenge Hill, Woolacombe Warren and sand treatment soil all had significantly less bee visits and time spent, compared to the model. Additionally Folly Farm and Dawlish Warren had significantly less visits.

Table 36. Generalized Linear Mixed Effects Regression (GLMM) model of bee visits and time spent per visit. Cockey Down in calcareous loam + grazed treatment is used as the standard, 'significant higher' and 'significant lower' columns refer to significant differences in relation to this standard. See Appendix X for P values and Table 21 for ecotype and treatment key.

	Ecotype		Soil Treatment		Management Treatment	
	Significant Lower	**Significant Higher**	**Significant Lower**	**Significant Higher**	**Significant Lower**	**Significant Higher**
Bee Visits	ss, ff, hh, ww, dw		S			
Time Spent	ss, hh, ww		N,S			

Home-site condition models were completed for visits and time spent per visit. No significant differences were found for time spent. However, ecotypes from sand and neutral loam soils received significantly fewer bee visits (Figure 75) than calcareous loam.

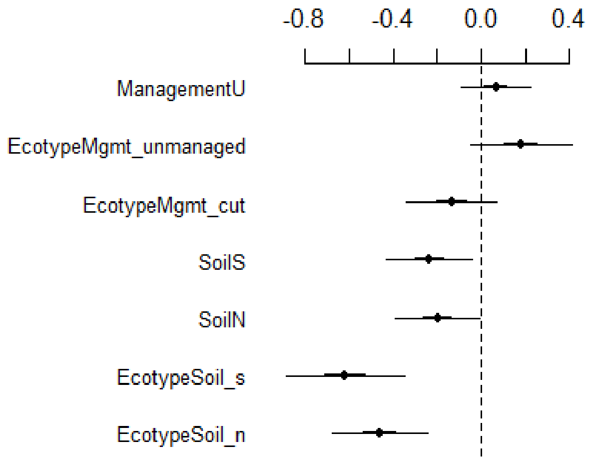


Figure 75. GLMM of bee visits between ecotype and treatment. Factors with capital letter (S, N, U) are treatments, those with lower case letters (s, n, unmanaged, cut) are ecotype factors. Cockey Down in calcareous loam + grazed treatment is used as the standard.

Data derived for plant growth and leaf-content were chosen to include in the model to identify any effect from these on the bee activity (Table 37). Bee visits were shown to be positively influenced with higher leaf-nitrogen (P=0.006), and lower leaf-HCN (P=0.016) indicating the amounts of these chemical properties may be reflected in the nectar. Bee use of plants was also positively related to higher vegetation biomass [perhaps making the plant, and potentially flowers elevated and more conspicuous] (bee visits P=0.001, time spent P=0.005), dry flower biomass [more flowers] (bee visits P<0.001, time spent P<0.001) and relative moisture content [potentially more nectar] (bee visits P<0.001, time spent P<0.001). Surprisingly the bee use of plants was negatively influenced by stronger pre-harvest flower scent (bee visits P<0.001, time spent P<0.001), usually thought to be an attractive quality to pollinators (Junker & Parachnowitsch, 2015), more so than flower colour (Murrell *et al.*, 1982). There were no significant differences found for hirsuteness or flower number.

Table 37. Generalized Linear Mixed Effects Regression (GLMM) model of effect from vegetation and flower factors in relation to bee visits and time spent per visit. Greyed out * indicates this factor was important but not significant (P=0.059). The significant lower or higher categories relate to significant differences of bee use, in relation to the numeric continuous predictor variable. See Appendix X for P values and Table 21 for ecotype and treatment key.

	Vegetation		**Flowers**	
	Significant Lower	**Significant Higher**	**Significant Lower**	**Significant Higher**
Bee Visits	HCN (quantitative)	Leaf-nitrogen, Dry biomass	Pre-harvest flower scent	Dry flower biomass (harvest), Relative moisture content
Time Spent		Dry biomass, Leaf-nitrogen *	Pre-harvest flower scent	Dry flower biomass (harvest), Relative moisture content

Seven bee species were found to use the plants during the experiment (Table 38), total time spent on flowers ranged from 60 seconds divided between 12 plants by *B. vestalis*, to 205 plants visited by *B. lapidarius* making 806 seconds, this species also spent the shortest mean time per visit. *M. centuncularis* spent the longest mean time per plant at 7.4 seconds.

Table 38. Bee species recorded in bee preference study surveys. Total bee visits: number of plant visits per study. Total surveys: Number of surveys the bee occurred in study. Total time spent: total number of time (seconds) on all plants in study. Mean time spent: Mean time spent per plant visit (*n*= Total bee visits).

	Total Bee visits	**Total Surveys**	**Total Time spent (seconds)**	**Mean Time spent (seconds)**	**Standard Error**
Apis mellifera	40	2	203	5.1	1.0
Bombus lapidarius	205	4	806	3.9	0.4
Bombus pascuorum	97	5	460	4.7	0.4
Bombus terrestris	55	2	292	5.3	0.5
Bombus vestalis	12	2	60	5.0	1.0
Megachile centuncularis	112	7	834	7.4	0.8
Megachile willughbiella	64	4	323	5.0	1.0

14.4 **Bee Preference Study - Discussion**

Most variation was seen between ecotypes for ‘bee visits’ and ‘time spent’ per visit (Table 35). The model (Table 36) showed Southstoke, Hellenge Hill and Woolacombe Warren had significantly fewer ($P<0.001$) and shorter ($P=0.054$) bee visits than the standard (Cockey Down). The two ecotypes Folly Farm and Dawlish Warren had significantly fewer bee visits ($P<0.001$) than the standard but no difference in time spent, indicating these flowers may have shown lower initial attractive qualities [such as aroma (Junker & Parachnowitsch, 2015) and high floral density (Waddington, 1980)] to the bees but contained high enough rewards to make it worthwhile for bees to stay for longer (Galen & Plowright, 1985). Four out of the five ecotypes with significantly lower visit numbers were either from sand (Woolacombe Warren and Dawlish Warren) or cut home-sites (Folly Farm and Southstoke), both of which were previously found to contain significantly greater amounts of leaf-HCN ($P<0.001$) (Figure 65), which may have been a contributing factor. However, previous research has shown bee choice isn’t influenced by toxic alkaloid levels in nectar; instead time spent visiting the flower would be the response which would be reduced (Alder & Irwin, 2005; Manson *et al*., 2012). In addition, three out of the five ecotypes with significantly lower visit numbers were from the three closest (geographically) sites to the glasshouse (Southstoke, Folly Farm and Hellenge Hill), indicating no preference of the bees to ecotypes of closer geographical distance to the test foraging area.

In the first model (Table 36), the calcareous loam treatment soil was significantly higher for both time spent ($P=0.015$) and bee visits ($P=0.037$). When the model was split between home-site conditions (Figure 75) it was also identified that the calcareous loam ecotype received significantly more bee visits ($P<0.001$) than the other two soil ecotypes [though there was no differences for time spent]. As the surrounding local area of the glasshouse location (Newton Park, Newton St. Loe) is clayey soils over white Lias limestone (Dallimore, 2001), the results may indicate that bees were more attracted to ecotypes derived from a similar soil type and also grown in similar treatment soil type to their home-site soil. Alternatively, these findings for bee visits may reflect plant vigour and fitness presented earlier (Table 19, Table 22, Table 30). Plants grown in calcareous loam treatment soil showed significantly greater main stem length, stems per plant, leaflets and branching per main stem, seed pods and dry biomass than the plants grown in alternative treatment soils. Shykoff and Bucheli (1995) found that pollinators preferred healthy *Silene alba* (Mill.) plants to diseased ones. Although in this case there are no diseased plants, the healthier plants may have been favoured by the pollinators due to the possibility of higher nutritional quality of the nectar. In contrast the plants grown in sand treatment soil had significantly lower leaf-nitrogen ($P<0.001$) (Table 32) which was suspected to be caused by increased nutrient leaching of the soil (Aerts, 1996; Cornelissen *et al*., 2003; IPNI, 2013). This too may have been an aspect mirrored in the flower properties whereby reduced soil nutrients in turn reduced the nutritional properties of nectar, as found in previous research whereby fertiliser nutrients increased alkaloids and amino acids of nectar (Gardener & Gillman, 2001; Alder *et al*., 2006).

The second model (Table 37) which included the already-studied variables (leaf-nitrogen, leaf-HCN, hirsuteness, dry vegetation biomass, dry flower biomass, flower relative moisture content, pre-harvest flower scent, harvest flower number) [now used as predictor variables] supported the above suggestions. More bee visits were made by bees to plants with significantly lower leaf-HCN ($P=0.016$) and significantly higher leaf-nitrogen ($P=0.007$), and more time was also spent on plants with higher leaf-nitrogen (important but not significant $P=0.059$). These chemical parameters may therefore be reflected in flower nectar quality as found for *Asclepias sp.*, where nectar cardenolide concentrations were found to positively correlate with that of leaves (Manson *et al.,* 2012). However, it may only be higher leaf-nitrogen levels which encourage higher visit numbers [rather than low leaf-HCN as well] as work by Alder and Irwin (2005) and by Manson *et al*. (2012) indicates that defence chemicals are not detected by bees in initial flower choice.

More plants were visited and used for longer which had significantly higher dry vegetation biomass (bee visits $P=0.001$; time spent $P=0.005$) [perhaps the larger the plant, the more prolific the flowers seem to passing bees], dry flower biomass at harvest [high floral density (Waddington, 1980)] (bee visits $P<0.001$; time spent $P<0.001$), and higher relative moisture content of flowers ($P<0.001$). This latter element may reflect water content of the nectar, nectar water is important for bees, with different species requiring different dietary amounts (Wilmer, 1988). Surprisingly, more flowers were used which had significantly weaker flower scent (pre-harvest flower scent). This finding contrasts with previous research indicating a bee's olfactory sense is important in flower choice, even for generalist pollinators and those using small-flowered plants (Ashman *et al*., 2004), with weak flower volatiles generally indicating low nectar (Murrel *et al*., 1982) and reward levels adjusting bee behaviour to flower choice (Cartar, 2004; Junker & Parachnowitsch, 2015). Yet Waddington, 1980 found unrewarded visits didn't alter bee choice of *L. corniculatus* varieties, only flight pattern after the unrewarded visit.

Seven species of bee were recorded to use the plants during the bee surveys (Table 38), the majority of which were bumblebees, which are known to be the main pollinators of *L.cornicultaus* (Ollerton, 1993). The most prolific species was *B. lapidarius* (visited 205 plants in 4 surveys), *B. pascorum* (visited 97 plants in 5 surveys), and *Megachile centuncularis* (visited 112 plants in 7 surveys). The species assemblage was a mixture of bumble bees, honey bees and solitary bees with a range of short and long tongues (Goulson *et al*., 2005; Heard *et al*., 2007) and pollen transfer efficiencies (Woodcock *et al.*, 2013). However, there was little variation between them within plant choice, though *Apis melifera* used Folly Farm, one of the closer sites 8.77km (Table 34) to the glasshouse for significantly more time than it did the other ecotypes. The lower use of the plants by honeybees may have been related to the surrounding landscape quality and distance to managed hives, found to influence honeybee visitation rates in previous research on oilseed rape, as apposed to bumblebees and solitary bees which were not found to respond to these (Woodcock *et al.*, 2013).

15 CONCLUDING DISCUSSION

Please note that within this chapter, calcareous sand is referred to as ‘sand’ and cut with aftermath grazing is referred to as ‘cut’. ‘Grazing treatment’ refers to simulated grazing from cutting by hand.

Most variation throughout the experiment was seen to be from treatments. Plants of unmanaged treatment exhibited significantly more leaflets and branches per main stem, more seed pods, greater biomass (dry weight), shorter time to flowering, shorter time to seed pod formation, and stronger flower scent. However, they also had significantly lower numbers of stems per plant, leaf-nitrogen and relative moisture content. Most ecotypes grown in calcareous loam soil appeared the easiest to establish and had greatest vigour and fitness as reflected by larger main stem length, greater number of stems (per plant) and leaflets (per main stem), greater degree of branching (per main stem), more seed pods, greater dry biomass, and shorter time to first flowering. Neutral loam soil treatment was shown to be the most difficult for *Lotus corniculatus* to successfully establish in with significantly more mortality. Sand soil treatment also showed limiting factors presumably due to nutrient leaching, reducing the plants’ nutritional value to herbivores with significantly lower leaf-nitrogen and, in the second year, shortest main stem length.

Ecotypic and geographic variation have been considered against the objectives and aim of this research (Chapter 2) as detailed below.

Objective A: Plant Fitness

Ecotypic variation was found to persist in the reciprocal planting. Ecotypes from sand home-sites produced significantly more stems per plant (mean 44), and leaflet number per main stem (mean 118), the model additionally highlighted delayed seed pod formation compared to the standard. Ecotypes from cut sites showed a number of significant differences with fewer stems per plant (mean 33), fewer leaflets per main stem (mean 65), and more seeds per pod (mean 7.3). The model also showed cut ecotypes had fewer seed pods [in the unmanaged treatment].

Neutral ecotypes grown within matching soil type produced earlier pod formation than the standard. There were no further significant fitness benefits found for ecotypes grown within matching soil type/management treatments.

Spatial distance between ecotype sites did not show any significant effect on the models of plant fitness parameters.

Objective B: Herbivore Requirement

Sand ecotypes contained significantly lower leaf-HCN content (mean 16.6 degrees of colour) than the other ecotypes, though the model identified sand ecotypes as having significantly higher leaf-HCN as well as greater hirsuteness than the standard. Ecotypes from cut sites also displayed significant differences with more leaf-HCN (mean 32.9 degrees of colour), shorter time to first flower and in having two clear flowering peaks in both years. Other ecotypic traits were shown in calcareous loam ecotypes having two clear flowering peaks (as with cut ecotypes) in both years of the experiment and highest leaf-HCN (mean 57.03 degrees of colour), and unmanaged ecotypes having lower leaf-HCN (mean 9.5 degrees of colour). Although sand home-sites showed significantly greater stem number (per plant), and leaflet number (per main stem), which were primarily categorised in the plant fitness objective, differences in plant part ratios can also have an effect on herbivory as nitrogen content of different plant tissues has been found to vary (Mattsson, 1980).

Sand ecotypes grown in matching soil type, and unmanaged ecotypes receiving matching management treatment produced significantly more flowers than all other ecotypes or combinations. There were no further significant herbivore benefits found for ecotypes grown within matching soil/management treatments.

Spatial distance between ecotype sites did not show any significant effect on the models.

Objective C: Bee Preference Study

Results from the bee preference study showed significant differences in terms of ecotype preference of bees, with avoidance of plants containing highest leaf-HCN. Plants grown in calcareous loam soil treatment were preferred which suggests nectar of plants are of most value to bees when grown in optimum [for *L. corniculatus* growth] conditions (Grime *et al*., 1992). It could also be that plants grown in soil type similar to the presumed homerange of the bees are of highest quality to them.

There was not found to be any preference by bees of ecotypes growing within matching soil type, or receiving matching management to the ecotype donor site.

There was no preference found by bees for ecotypes geographically close to the test foraging area.

Research Aim: To assess the importance of using ecologically-similar seed rather than geographically-local seed in grassland restoration projects, with particular reference to herbivorous invertebrates, including pollinators.

In meeting the objectives, the aim of this research has been answered as follows:

When considering importance of the results for large herbivores (livestock) the ecotypic differences would make little nutritional difference as the biomass and nitrogen [which would be of most importance in large quantities to these animals] were affected by treatments more so than ecotype, which is a positive outcome for seed used for species enrichment of agricultural grasslands.

There were however, ecotypic differences (induced by both home-site soil and management), which could cause concerns for invertebrate herbivores and pollinators. For example, the secondary plant chemical leaf-HCN, which was present in significantly greater amounts in ecotypes of cut home sites could cause toxicity, mortality or avoidance if introduced to an unmanaged site where local invertebrates are not adapted to high levels (Howe & Westley, 1999; Pentzold *et al*., 2014), thus reducing the value of the restoration scheme for local biota, as shown in a study using cyanogenic and acyanogenic cultivars of *Trifolium repens* (Mowat & Shakeel, 1989). The ecotypes from cut home-sites comprised less hirsute plants (a physical plant defence) than the standard and so herbivores at Cockey Down may experience reduced fitness from more densely hirsute ecotypes being introduced (Hanley *et al*., 2007). In addition, pollinators of calcareous loam cut sites could miss peak flowering times of ecotypes translocated from unmanaged sand sites due to flowering asynchrony. It could be argued that flowering asynchrony may also act as a benefit to the invertebrates whereby different timed flowers extend the pollinator season (Rathcke & Lacey, 1985), and also for plant populations in extending reproductive potential and compensating risk of seed pod failure or seed predation (Tarayre, 2007).

Both home-site soil type and management also showed potential influence on the viability of the plant population itself in reducing fecundity. Delayed seed pod formation in sand ecotypes (compared to the standard) suggests this may be an adaptation to summer temperatures and desiccation of the sand (Maun, 1998; 2009; Zhu *et al*., 2014), which calcareous loam ecotypes lack when planted to these sites and therefore genotypes not adapted could fail due to poorly timed (seasonal) germination or deep seed burial from shifting sand dunes (Maun, 1998; 2009; Liu *et al*., 2011). It may also be an adaptation to low soil nutrients at the home-site with more resource initially allocated to plant growth rather than reproduction or herbivore defence (Herms & Mattson, 1992; Aerts, 1996; Cornelissen *et al*., 2003). Cut ecotypes also produced significantly fewer seed pods (than the standard) with significantly greater seed numbers per pod. Again, this could be an adaptation to cutting regime, as found to be a genetic influence on flowering by Reisch & Poschlod (2009), the timing of which could be critical for seed return. It may be, in such cases, that natural selection of genotypes would occur, perhaps enabling the population to survive and establish slowly. However, should such a founder population survive, there could be further issues such as genetic bottlenecks and reduced genetic diversity (Ollerton & Lack, 1998).

There were only two significant interactions, indicating a typically additive character of factor effects. Neutral loam ecotypes grown in neutral loam soil treatment had earlier pod formation than the standard, and sand ecotypes grown in sand treatment soil had greater numbers of flowers per plant (over both years) than all other ecotypes, treatments and combinations. Both of these results show factors which are potentially influenced by soil resources (Stephenson, 1984; Nagy & Proctor, 1997), therefore matching soils in these instances may have promoted higher reproduction.

It should be noted that there was also some genetic variability noticed within ecotypes, as found with leaf-HCN where both acyanogenic and cyanogenic plants were present in all ecotypes. This variable in particular has been shown to be polymorphic (Ellis *et al*., 1977). As this species shows plasticity to a range of habitats (Rodwell ed., 1991; Grime *et al.*, 1992; Rodwell ed., 1992; 2000), some degree of genetic variability would be expected. Billington *et al.* (1988) stated that differences in genetic variation between adjacent populations could be the case even for species with large population sizes.

It was noted that most of the significant ecotypic differences recorded (both for plant fitness as well as herbivore/pollinator importance) were from ecotypes of sand sites or those from cut management regimes. These were considered to be the more stressed environments of the study. Such sand soils are prone to stresses such as drought, desiccation and nutrient leaching from lack of organic matter and clay (Aerts, 1996; Cornelissen *et al*., 2003; Rosen *et al.,* 2008; IPNI, 2013) and extreme pH, often elevated from the high calcium of shell particles (Horne, 2006). Cut fields also experience stress in the form of periodic machine-cutting followed by trampling and tearing action of the vegetation from grazing. *L. corniculatus* as well as other plant species introduced to either of these habitats, would require rapid adaptation and to a greater extent to these harsh soil and management conditions than ecotypes introduced to a calcareous loam site receiving extensive grazing. However, it could be argued that these [less stressed] ecotypes would need to adapt to other environmental factors such as inter-specific and intra-specific competition. As shown to be the case in control treatments which illustrated more pronounced competitive strategies than their droughted counterparts in a calcareous grassland study (Ravenscroft *et al*., 2014).

Results from this study agree with previous concerns stressing geographical proximity is not always better than ecological similarity (Bischoff *et al.*, 2006; Warren, 2012), and that ecotypic variation can manifest in physiological and morphological differences that could cause difficulties for use by herbivores (Hufford & Mazer, 2003; Walker *et al*., 2004). This shows better decision-making is needed when selecting seed for restoration projects.

Unless seed is harvested from a particular site with known history, establishing the seed provenance can be difficult. As discussed in Chapter 1, seed company information can often be misleading with 'native origin' actually meaning the location the seed were collected and 'local provenance' having being collected geographically close to where it will be planted (Flora Locale, 2012). Often seed companies advise whether certain grassland mixes can be sown in neutral or calcareous soil (Cotswold Grass Seed Direct, 2015), though this indicates management regime adaptation hasn't been considered. Some companies are now offering seed mixes for particular habitats (Emorsgate Seed, 2015; The Grass Seed Store Ltd, 2015), these are collected from the habitat that the seed is expected to be planted in (i.e. meadows) with this information listed in the seed description (Emorsgate Seed, 2015). However, this description still doesn't specify the soil type or the management regime/history to which the ecotype has become adapted. It also excludes sowing of seed at dry sand sites which would seemingly require highly adapted ecotypes. Although some specialist species such as *Filipendula ulmaria* L. [shaded mire and river banks] and *Molinia caerulea* L. [acidic pastures, unshaded mire/river banks] (Grime *et al*., 1992) would only have been grown in soils they would then be planted in, more plastic species like *L. corniculatus,* such as *Leucanthemum vulgare* L. [limestone quarries, neutral meadows, drought tolerant] and *Agrostis capillaris* L. [limestone and acidic pastures, wasteland] (Grime *et al*., 1992), may show wider ecotypic adaptations. These species should have the adapted soil type and management regime specified on the seed container, with such information easily available in the online/catalogue seed description. Emorsgate Seed (2015) do offer further services to use their various local stocks or matching stock from similar habitats, but seed should be more widely and easily available with greater description to make this more popular with land managers.

Findings from this study are of national relevance, and Natural England should adopt new recommendations on seed provenance in agri-environment schemes. These recommendations would also be relevant for use by additional bodies such as Highways Agency (design manual for roads and bridges (Highways England, 2015) and local authorities (landscaping within infrastructure and school projects) as well as consultants. Instead of recommending strictly geographically local seed, the management regime (particularly details of intensity and timing of management operations) should ideally be similar between the donor and receptor sites. Soil types, especially pH and clay/organic matter content should also be matched as far as possible as these were the greatest limiting factors within this study. Suitable donor sites may be local sites of similar habitat. If no such sites are available then recommendations from this study should be followed in seeking suitable sowing material. If seed suppliers are used, then they should provide greater detail on donor site conditions to aid land managers.

16 REFINEMENTS & FURTHER WORK

There have been some adjustments to the methodology during the course of the study:

Originally ecotypes were to have been collected from a larger range of soil types and from more sites, they were also planned to be planted within their own and each-others soil types rather than three ‘treatment’ soils. This design however became too large an experiment resulting in an experimental design of over 1800 plants for just the soil type study. It also would have incurred a collection of approximately 1350L of soil from each site which would have been impractical and not permitted from SSSI sites.

As soils were originally to be collected for planting in, from each of the ecotype sites, it was first thought that the management treatments could be conducted on plants growing in home-site soils. However, the minimisation of the soil type study to 216 plants allowed the addition of a reciprocal experiment using all plants in all soil types to be conducted, allowing differences from management to be distinguished from that of treatment soil.

More than the needed amount of ecotype seed was sown as back-up for large germination failures. Certain ecotypes were selected beforehand as most appropriate for the studies, the only one of these not used was Valley of Stones due to the poorest germination levels of all, however Hellenge Hill was used successfully in its place. This site was geographically closer to the rest of the sites than Valley of Stones but had the same management regime and soil type so was an acceptable replacement.

The three management regimes of ecotypes collected were the original proposals for treatments’ simulations. It was found that the variation between simulated grazing and cutting treatments was so minor that difference between plant treatments would have been negligible, therefore simulated grazing and unmanaged treatments were the regimes implemented.

It was hoped that suitable treatment soils could be found outside a distance of at least 16km radius to ecotype sites to easier clarify differences/similarities between plants as effects of geographical differences or edaphic differences, which is why Fontmell was removed from the sowing. However, the difficulty in finding a suitable sand site where permission could be granted for soil extraction was difficult, hence Woolacombe Warren was used.

Goblin Combe was initially collected when more than three soil types were to be sampled. Due to it being calcareous heath and having a unique particle size range compared to the others (Figure 8), Goblin Combe was removed from further study.

At the beginning of the experimental design, it was decided [with use of the Agricolae package version 1.0-9 (De Mendiburu, 2010) in R statistical software version 3.1.0 (R Core Team, 2013), and in statistical discussion with Smith (2011)] that nine replicates would be ideal to decrease the chance of a Type II error, and also create easier analysis by having a Latin Square Block design. However, due to lower than anticipated germination, only eight replicates were viable. It was still thought that replication of eight would be suitable for the analysis and rule out pseudoreplication. This turned out to be a positive compromise logistically as the experiment was at the uppermost limit with regard to manageability for one part-time researcher and the glasshouse space available, the original design would have added a further 54 plants to this.

Initial thoughts on the bee preference study included keeping domestic honey bees under mosquito nets, over each replicate of plants to monitor time spent using the plants. However, advice was sought from Lunt (2012) of the Bath Bee Keepers' Teaching Apiary who warned of problems with absence of adequate nectar sources and hives. When it was instead decided to monitor the bees naturally occuring in the glasshouse, camera source monitoring was tried. Plants were rearranged into replicates under the frames of nine motion-activated video cameras. These cameras were left in place for three days and set at the highest sensitivity level, to record video for 5 minutes when activated. However, problems were encountered with this method from sensitivity of the cameras. Bees were often not large enough to trigger filming unless they were very close to the camera, yet slugs at night [the cameras had night vision] and slight breezes which moved leaves were enough to set the cameras off, and did so most of the time. Therefore battery and memory of the cameras only lasted around four hours and footage was of little value. Therefore the method of monitoring the bees at one hour intervals was used instead.

The following further research is needed on this subject.

Similar experimental designs need to be undertaken with a range of herb and grass species often used in grassland restoration.

In-situ investigations including reciprocal translocations would be useful to eliminate any glasshouse effects and detect any effect from other site variables not measured in a controlled environment. This could include plant traits that influence the species ability to establish and persist in a range of environments (Pywell *et al.*, 2003).

Further variables need investigation such as inter-specific and intra-specific competition in relation to management regime, and climatic conditions of home-sites. Water holding capacity of soil would also be of interest as soil moisture has been found to cause genetic variation in biomass and fecundity of *Lotus corniculatus* (Carter *et al.*, 1997). Also the symbiotic relationship between *L. corniculatus* and *Rhizobium* sp., previously found to alter aspects of fitness (Gwynne *&* Beckett, 1980), and the possibility of mycorrhizal associations would be worth further study. The latter of which has been shown to be a major factor in European calcareous grassland composition (Van der Heijden *et al.*, 1998) and is found to differ between legumes and non-legumes (Scheublin *et al.*, 2004).

REFERENCES

Aerts, R. (1996) ‘Nutrient resorption from senescing leaves of perennials: Are there general patterns?’ *Journal of Ecology,* 84 (4) pp.597-608.

Aerts, R.J., Barry, T.N. & McNabb, W.C. (1999) ‘Polyphenols and agriculture: beneficial effects of proanthocyanidins in forages.’ *Agriculture, Ecosystems and Environment,* 75, pp.1-12.

Ahmad, Z. (2011) ‘Impact of Alluvial Deposits on Soil Fertility during the Floods of 2010 in Punjab, Pakistan’. *International Potash Institute* [online] available from: http://www.ipipotash.org/udocs/eifc_no26-rf3.pdf.

Alder, L.S., Wink, M., Distl, M., Lentz, A.J. (2006) ‘Leaf herbivory and nutrients increase nectar alkaloids’, *Ecology Letters,* 9 pp.960-967.

Arteca, R.N. (2015) *Introduction to horticultural science*. 2nd ed. Stamford, USA: Cengage Learning [online] available from: Google Books http://www.bwars.com/index.php?q=species_gallery/Bee [accessed: 13/08/2014].

Ash, D., Toynton, P. & Davis, S. (2005) *Salisbury Plain Life Project - Management of chalk grassland on Salisbury Plain military training area. Defence Estates & English Nature.* [online] available from: http://ec.europa.eu/environment/life/project/Projects/index.cfm?fuseaction=home.showFile&rep=file&fil=ChalkGrasslandsFactsheet.pdf.

Ashman, T., Bradburn, M., Cole, D.H., Blaney, B.H., Rauso, R.A (2004) 'The scent of a male: The role of floral volatiles in pollination of a gender dimorphic plant'*. Ecological Society of America*, 86 pp.2099-2105.

Avon Wildlife Trust. (1999) *Non-SSSI Grassland/Barn owl Corridors Folly Farm Nature Reserve Management Plan 1999-2004*. Bristol: Avon Wildlife Trust.

Avon Wildlife Trust. (2001) *Hellenge Hill Nature Reserve Management Plan 2001-2006.* Bristol: Avon Wildlife Trust.

Avon Wildlife Trust. (2011) *Hellenge Hill* [online] available from: http://www.avonwildlifetrust.org.uk/reserves/documents/HHNatureReserveInformation.pdf [accessed: 20/11/13].

Avon Wildlife Trust. (2012[a]) *Folly Farm – A Historic Landscape* [online] available from: http://www.avonwildlifetrust.org.uk/reserves/folly_farm/history.htm [accessed: 20/11/13].

Avon Wildlife Trust. (2012[b]) *Folly Farm – Welcome to the reserve* [online] available from:

http://www.avonwildlifetrust.org.uk/reserves/folly_farm/welcome.htm [accessed: 20/11/13].

BBC News. (2012) ‘Weather spells hard times for UK dairy farmers’ 25th December 2012.

BBC News UK (2013) ‘Bee decline: Government announced ‘urgent’ review’ 28 June [online] available from: http://www.bbc.co.uk/news/uk-23090339 [accessed: 16/03/15].

Baer, S.G., Blair, J.M., Collins, S.L. & Knapp, A.K. (2003) ‘Soil resources regulate productivity and diversity in newly established tall grass prairie’. *Ecological Society of America*, 84 (3), pp.724-735.

Baker, W,H. & Thompson, T.L. (1992) ‘Determination of Total Nitrogen In Plant Samples by Kjeldahl’. *In:* Plank, O. *Plant analysis reference procedures for the southern region of the United States.* Georgia: The University of Georgia, pp.13-16.

Banks, K. (2009) kbanks@dorsetwildlifetrust.org.uk *Brooklands Farm, Haydon Hills, Upton Heath information*. Email to Erica Mortimer, 02/09/2009. eriberi.mortimer@gmail.com.

Banks, K. (2013) kbanks@dorsetwildlifetrust.org.uk *Brooklands information.* Email to Erica Mortimer, 04/09/2013. eriberi.mortimer@gmail.com.

Bardgett, R.R., Wardle, D.A., & Yeates, G.W. (1998) ‘Linking above-ground and below-ground interactions: how plant responses to foliar herbivory influence soil organisms’ *Soil Biology and Biochemistry,* 30 (14) pp.1867-1878.

Basra, A.S. *ed*. (1994) *Mechanisms of Plant Growth and Improved Productivity - Modern Approaches.* New York: Marcel Dekker Inc. [online] available from:http://books.google.co.uk/books?hl=en&lr=&id=snuffPh9RqwkC&oi=fnd&pg=PA137&dq=lateral+shoot+stem+tiller+growth+sand+nutrient+deficiency&ots=WGBwwHhEtn&sig=M8ewpBizfuozz6FFBQcr67hw5uo#v=onepage&q=tillers&f=false. [accessed: 01/03/15].

Batáry, P., Báldi, A., Sárospataki, M., Kohler, F., Verhulst, J., Knop, E., Herzog, F., Kleijn, D. (2010) ‘Effect of conversation management on bees and insect-pollinated grassland plant communities in three European countries’. Agriculture, Ecosystems and Environment, 136, pp.35-39.

Bazin, A., Goverde, M., Erdhardt, A., Shykoff, J.A. (2002) ‘Influence of atmospheric carbon dioxide enrichment on induced response and growth compensation after herbivore damage in *Lotus corniculatus’. Ecological Entomology,* 27 (3), pp271-278.

Bazzaz, F.A., & Harper, J.L. (1977) ‘Demographic analysis of the growth of Linum usitatissimum’ *New Phytology,* 78 pp.193-208.

Bees, Wasps and Ants Recording Society (BWARS) (2014) Bees [online] available from: http://www.bwars.com/index.php?q=species_gallery/Bee [accessed: 13/09/2014].

Begon, M., Harper, J.L. & Townsend, C.R. (1990) *Ecology: Individuals, populations and communities.* 2nd ed. London: Blackwell Scientific Publications.

Bignal, E., Jones G., & McCracken D. (2001) 'Comment: Future directions in agricultural policy and nature conservation'. *British Wildlife*, 13 (1), PP.16-20.

Billington, H, L., Mortimer, A.M., & McNeilly. (1988) 'Divergence and Genetic Structure in Adjacent Grass Populations. I. Quantitative Genetics'. *Evolution*, 42 (6) pp.1267-1277.

Bischoff, A., Cremieux, L., Smilauerova, M., Lawson, C.S., Mortimer, S.R., Dolezal, J., Lanta, V., Edwards, A.R., Brook, A.J., Macel, M., Leps, J., Steinger, T., Műller-Schärer, H. (2006) 'Detecting local adaptation in widespread grassland species: the importance of scale and local plant community'. *Journal of Ecology*, 94 (6), pp.1130-1142.

Black, R. (2010) 'Bee decline linked to falling biodiversity' BBC news [online] available from: http://news.bbc.co.uk/1/hi/sci/tech/8467746.stm [accessed: 16/03/15].

Blake, R.J., Westbury, D.D., Woodcock, B.A., Sutton, P., & Potts, S.G. (2012) 'Enhancement of buffer stripscan improve provision of multiple ecosystem services'. Outlooks on Pest Management, 23 (6) pp.258-263.

Bloom, A.J., Chapin, S. & Mooney, H.A. (1985) 'Resource limitation in plants: An economic anology'. *Annual Review of Ecology and Systematics*, 16, pp.363-392.

Borowicz, V.A. (1993) 'Effects of benomyl, clipping, and competition on growth of pre-reproductive *Lotus corniculatus'*. *Canadian Journal of Botany.* 71 (9) pp.1169-1175.

Bragg, N.C., & Chambers, B.J. (1988) 'Interpretation and advisory applications of compost air-filled porosity (AFP) measurements' [online] *International Society of Horticultural Science.* Available from: http://www.actahort.org/books/221/221_3.htm accessed: 10/02/12.

Bunce, R.G.H., Barr, C.J., Gillespie, M.K., Howard, D.C., Scott, W.A., Smart, S.M., Van de Poll, H.M. & Watkins, J.W. (1999) *ECOFACT 1: Vegetation of the British countryside - the Countryside Vegetation System* [online] available from: http://www.ceh.ac.uk/products/software/CEHSoftware-CVS.htm [accessed: 01/11/09].

Brain, B.G. (1994) *Folly Farm Reminiscences over more than three score years and ten*. [online] available from: http://www.bwcw.org/folfarm.htm [accessed:02/04/13].

Bristol Regional Environmental Records Centre. (2009) *Lotus corniculatus records enq1354 spreadsheet*, 29th May. Bristol.

Brewer, H.E. (1947) 'Response of certain legumes to variations in soil and microclimate on eroded areas in areas in Southeastern Iowa'. *Ecological Monographs* 17 (4), pp.471-500.

Briggs, M.A. (1991) 'Influence of herbivory and nutrient availability on biomass, reproductions and chemical defences of *Lotus corniculatus* L.'. *Functional Ecology* 5 (6), pp.780-786.

British Society of Soil Science (2013) *For the advancement of soil science: Soils of Britain – An interesting pattern of sand, clay and peat packed into a small island* [online] http://www.soils.org.uk/media/uploads/education/BSSS-SoilsOfBritain-A4-DL-Leaflet-WEB.pdf [accessed: 05/03/14].

Bryant, J.P. & Kuropat, P.J. (1980) 'Selection of winter forage by subarctic browsing vertebrates: The role of plant chemistry'. *Annual Review of Ecology and Systematics*, 11, pp.261-285.

Buglife. (2012) *B-Lines Factsheet 2 – Wildflower rich grassland restoration*, [online] available from http://www.buglife.org.uk/sites/default/files/B-Lines%20fact%20sheet%202-%20Wildflower-rich%20grassland%20restoration.pdf [accessed: 02/10/2013].

Bullock, J.M., Aronson, J., Newton, A.C., Pywell, R.F., Rey-Benayas, J.M. (2011) 'Restoration of ecosystem services and biodiversity: conflicts and opportunities'. *Trends in Ecology & Evolution*, 26 (10) pp.541-549.

Bumblebee Conservation. (2012) *Grassland restoration and creation for bumblebees. Factsheet 3 Land Management Series* [online] available from: http://bumblebeeconservation.org/images/uploads/Resources/BBCT_Land_Factsheet_4_Grassland_restoration.pdf [accessed:21/04/14].

Bunt, A.C. (1983) 'Physical properties of mixtures of peats and minerals of different particle size and bulk density for potting substrates'. *International Society of Horticultural Science.* [online] available from: http://www.actahort.org/books/150/150_15.htm [accessed: 10/02/12].

Burton, G.W., Jackson, J,E., & Hart, R,H. (1963) 'Effects of cutting frequency and nitrogen on yield, in vitro digestibility, and protein, fibre and carotene content of costal Bermudagrass' *Agronomy Journal,* 55 (5) pp.500-502.

Canals, R. & Sebastia, R. (2009) *Soil nutrient fluxes and vegetation changes on molehills*. Journal of Vegetation Science, 11 (1) pp.23-30 [accessed: 04/02/12].

Capewell, M. & Agriculture Solutions LLC. (2013) *The why and how to testing the electrical conductivity of soils* [online] available from: http://www.agriculturesolutions.com/resources/92-the-why-and-how-to-testing-the-electrical-conductivity-of-soils [accessed: 20/01/2014].

Carrington, D. (2012) ‘Pesticides linked to honeybee decline’. The Guardian. Thursday 29 March [online] available from: *http://www.theguardian.com/ environment/2012/ mar/29/crop-pesticides-honeybee-decline* [accessed: 06/03/15].

Carson, T. (2009) *Telephone conversation with Erica Mortimer*. 4 September 2009.

Cartar, R.V. (2004) 'Resource tracking by bumblebees: responses to plant-level differences in quality'. *Ecological Society of America*, 85 pp2764-2771.

Carter, E.B., Theodorou, M.K. & Morris, P. (1997) ‘Responses of *Lotus corniculatus* to environmental change: I. Effects of elevated CO_2, temperature and drought on growth and plant development’. *New Phytologist*, 136 (2), pp.245-253.

Castillo, S., Popma, J. & Moreno-Casasola, P. (1991) ‘Coastal sand dune vegetation of Tabasco and Campeche, Mexio’. *Journal of Vegetation Science,* 2, pp.73-88.

Chambers, P. (2009) phil.chambers@teignbridge.gov.uk *Study site Dawlish Warren*. Email to Erica Mortimer, 29/09/2009. eriberi.mortimer@gmail.com.

Chinery, M. *ed.* (1982) *The Natural History of Britain and Europe*. London: Kingfisher Books Ltd.

Chinery, M. (2004) *Butterflies*. Glasgow: Harper Collins Publishers.

Chinery, M. (2005) *Complete guide to British insects*. London: Collins.

Christie Estates (undated) *Visiting Braunton Burrows – Management* [online] http://www.brauntonburrows.org/#/management/4558965258 [accessed:20/10/13].

Clarke-Smith, E. (1969) *Drumbeat – Salisbury Plain – An Historical Introduction* [online] available from http://www.drumbeat.org.uk/salisburyplain_history.htm [accessed: 02/12/2013].

Clua, A., Conti, M. & Beltrano, J. (2012) ‘The effect of glyphosphate on growth of bird’s foot trefoil *(Lotus corniculatus)* and its interaction with different phosphorus contents in soil’, *Journal of Agricultural Science*, 4 (7), pp.208-218.

Collins, R.P., Fothergill, M., Macduff, J.H., & Puzio, S. (2003) 'Morphological compatability of White clover and Perrennial ryegrass cultivars grown under two nitrate levels in flowing solution culture'. *Annals of Botany*, 92 (2), pp.247-258.

Cooper, A., & Etherington, J.R. (1974) 'The Vegetation of Carboniferous Limestone Soils in South Wales: I. Dolomitization, Soil Magnesium Status and Plant Growth'. *Journal of Ecology,* 62 (1), pp.179-190.

Cornelissen, J.H.C., Sibma, F., Van Logtestijn, R S P., Broekman, R,A., Thompson,K. (2011) 'Leaf pH as a plant trait: species-driven rather than soil-driven variation'. *Functional Ecology* 25 pp.449-455.

Cornelissen, J.H.C., Lavorel, S., Garnier, E., Diaz, S., Buchmann., N., Gurvich, D.E., Reich, P.B., ter Steege, H., Morgan, H.D., van der Heijden, M.G.A., Pausas, J.G. & Poorter, H. (2003) 'A Handbook of Protocols for standardised and easy measurement of plant functional traits worldwide'. *Australian Journal of Botany*, 51, pp.335-380.

Corner, L. (2009) Lucy_Corner@bathnes.gov.uk *Twerton Round Hill information*. Email to Erica Mortimer, 12/08/2009. eriberi.mortimer@gmail.com.

Cotswold Grass Seeds Direct (2015) *Wildflower seeds*. [online] available from: https://www.cotswoldseeds.com/seedmix/wild-flowers [accessed: 07/05/2015].

Courtney, A.D. (1968) 'Seed dormancy and field emergence in *Polygonum aviculare'*. Journal of Applied Ecology, 5 (3), pp.675-684.

Cox, J. (2009) jonathan.cox@naturalengland.org.uk *Study site Valley of Stones*. Email to Erica Mortimer, 05/08/2009. eriberi.mortimer@gmail.com.

Crawford, R.M.M. (1990) *Studies in plant survival*. Oxford: Blackwell Scientific Publications.

Crawley, M.J. (1983) *Herbivory: The dynamics of animal-plant interactions*. Oxford: Blackwell Scientific publications.

Crawley, M.J. (1986) *Plant Ecology*. Oxford: Blackwell Scientific publications.

Critchley, C.N.R., Burke, M.J.W., & Stevens, D.P. (2003). 'Conservation of lowland semi-natural grasslands in the UK: a review of botanical monitoring results from agri environment schemes' *Biological* Conservation115, pp.263-278.

DTE SP. (2008) *Salisbury Plain Training Area management map*. DTE SP.

Dallimore, J. (2001) *Newton St Loe, N.E.Somerset*. Bath: Bath & North East Somerset Council [online] availabled from: http://www.bathnes.gov.uk/sites/

default/files/newton_st_loe_-_study_of_the_vernacular_building_survey.pdf [accessed: 17/03/15].

Detzel, A., & Wink, M. (1993) ‘Attraction, deterrence or intoxication of bees (*Apis melifera*) by plant alleochemicals’. *Chemoecology,* 4 (1) pp.8-18.

De Mendiburu, F. (2010) *R: Agricolae version 1.0-9.* La Molina National Agrarian University.

Dean, J. *ed*. (undated) *Peat alternatives for commercial plant production in the UK*.
Horticultural Development Council.

Defra. (2011) *Biodiversity 2020 – A strategy for England's wildlife and ecosystem services* [online] available from: https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/69446/pb13583-biodiversity-strategy-2020-111111.pdf [accessed: 18/09/15].

Defra. (2012) *The Rural Development Programme for England* [online] available from: http://www.Defra.gov.uk/rural/rdpe/what-is-rdpe/ [accessed: 26/02/2012].

Defra. (2014) *The national pollinator strategy: for bees and other pollinators in England.* [online] available from: https://www.gov.uk/government/publications/national-pollinator-strategy-for-bees-and-other-pollinators-in-england [accessed: 12/03/15].

Department of Environment and Primary Industries – Victoria Australia, (1998) *Agriculture: What is Soil? Updated 2009 AG0058* [online] available from: http://www.dpi.vic.gov.au/agriculture/farming-management/soil-water/soil /what-is-soil [accessed: 31/12/13].

Devoto, M., Memmot, J., & Bailey, S. (2014) ‘Ecological meta-networks integrate spatial and temporal dynamics of plant-bumble bee interactions’. *Oikos,* 123 pp.714-720.

Dorrough, J., Ash, J. & McIntyre, S. (2004) ‘Plant responses to livestock grazing frequency in an Australian temperate grassland’, *Ecography*, 27, pp.798-810.

Duffy, L.K. (2001) *Tannin detection in Alaskan plants* [online] Available from: http://www.uaf.edu/csem/asra/archive/2002_site/2001_asra/program_descriptions/tannin_chemistry.html [accessed: 20/11/2009].

Dunmore and District Vintage Club. (undated) *History of Silage* [online] available from: http://dunmorevintage.com/historyofsilage/ [accessed: 10/11/13].

Eaton, F.M., & Ergle, D.R. (1954) ‘Effects of shade and partial defoliation on carbohydrate levels and the growth, fruiting and fibre properties of cotton plants’. *Plant Physiology.* 29 (1), pp.39-49.

Edgington, M. (2009) *Study site permissions Crook Peak and The Perch*. Letter to Erica Mortimer, 12/08/2009.

Egan, S.V., Yeoh, H.H. & Bradbury, J.H. (1998) ‘Simple picrate paper kit for determination of cyanogenic potential of Cassava flour’. *Journal of Science of Food and Agriculture*. 76, pp.36-48.

Ellis, W.M., Heymer, R.J., & Jones, D.A. (1977) ‘On the polymorphism of cyanogenesis in *Lotus corniculatus* L.’ *Heredity* 39, pp.45–65.

Emorsgate Seeds. (2013) *Lotus corniculatus – Birds foot trefoil* [online] available from: http://wildseed.co.uk/species/view/79 [accessed:20/01/14].

Emorsgate Seeds. (2015) *Wildflower seed origin options*. [online] available from: http://wildseed.co.uk/page/wild-flower-seed-origin-options [accessed: 07/05/2015].

Engels, F.M., & Jungs H.G. (1998) ‘Alfalfa stem tissues: cell wall development and lignification’. *Annals of Botany*, 82 pp.561-568.

English Nature. (2005) *The importance of livestock grazing for wildlife conservation* [online] available from: http://www.google.co.uk/url?sa =t&rct=j&q=&esrc=s&frm= 1&source=web&cd=1&ved=0CEEQFjAA&url =http%3A%2F%2Fpublications.naturalengland.org.uk%2Ffile%2F612038&ei= 0J3CUuqSObL07AbPpIHYAg&usg=AFQjCNFy7zbkmKqAMFatlpbBvFugf0tyI Q&sig2=pbXGKbor-dguN5tQ2uJbRg&bvm=bv. 58187178,d.ZGU [accessed: 26/02/2011].

Ehrlich, P.R. & Raven, P.H. (1964) ‘Butterflies and plants: A study in coevolution’. *Evolution*, 18 (4), pp.586-608.

Escaray, F.J., Menendez, A.B., Garriz, A., Pieckenstain, F.L., Estrella M.J., Castago, L.N., Carasco, P., Sanjuan, J., Ruiz, O.A. (2012) ‘Economical and agronomic importance of the plant genus *Lotus.* Its application in grassland sustainability and the amelioration of constrained and contaminated soils’. *Plant Science,* 182, pp.121-133.

Etherington, J.R. (1984) ‘Relationship between morphological adaptation to grazing, carbon balance and waterlogging tolerance in clones of *Dactylis glomerata* L.’. *New Phytologist,* 98 (4) pp.647-658.

European Commission. (2008) *LIFE and Europe’s grassland. Restoring a forgotten habitat* [online]. Available from: http://ec.europa.eu/environment/ life/publications/lifepublications/lifefocus/documents/grassland.pdf [accessed: 26/02/2012].

European Environment Agency. (2010) *Distribution and targeting of the CAP budget from a biodiversity perspective* [online] European Environment Agency. Available from: http://www.eea.europa.eu/publications/distribution-and-targeting-of-the-cap-budget-from-a-biodiversity-perspective [accessed: 26/02/2012].

European Commission. (2012[a]*) The Common Agricultural Policy – A Partnership Between Europe and Farmers*. Luxembourg: Publications Office of the European Union.

European Commission (2012[b]) *Natura 2000 Network* [online]. Available from http://ec.europa.eu/environment/nature/natura2000/index_en.htm [accessed: 26.02.2013].

European Commission (2012[c]) *Joint Research Centre, European Soil Portal – Soil Data and Information Systems* [online] available from: http://eusoils.jrc.ec.europa.eu/ [accessed: 20/11/13]

European Commission. (2013) *Assessing the risk of farmland abandonment in the EU - Final report. Terres, J,M., Nisini, L., Anguiano,E. Italy.* [online] available from: http://ec.europa.eu/agriculture/external-studies/2013/farmland-abandonment/fulltext_en.pdf [accessed: 20/11/2013].

Felderer, B., Boldt-Burisch, K.M., Schneider, B.U., Huttl, J. & Schulin, R. (2013) 'Root growth of *Lotus corniculatus* interacts with P distribution in young sandy soil' *Biogeosciences*, 10, pp.1737–1749.

Feneley,. M (2009) Margaret.Feneley@naturalengland.org.uk *Study Sites Heath Hill Farm*. Email to Erica Mortimer,04/08/2009. eriberi.mortimer@gmail.com.

Fenner, M. *ed.* (2000) *Seeds: The Ecology of Regeneration in Plant Communities*, 2nd ed. CABI Publishing: Oxon.

Fern, K. (2007) *Plants for a future: Lotus corniculatus* [online] available from: http://www.pfaf.org/user/Plant.aspx?LatinName=Lotus+corniculatus [accessed: 23.03.2011].

Ferris, R. & Taylor, G. (1993) 'Contrasting effects of elevated CO_2 on the root and shoot growth of four native herbs commonly found in chalk grassland'. *New Phytology*, 125 pp. 855-866 [accessed: 02.02.12].

Field, A., Miles, J., Field, Z. (2012) *Discovering Statistics Using R*. London: SAGE Publications Ltd.

Fitter, A.H. & Hay, R.K.M. (2002) *Environmental physiology of plants*. 3rd ed. London: Academic Press.

Flora Locale. (2005[a]) *Go native! Enhancing the floral diversity of semi-natural grassland.* Berkshire: Flora locale.

Flora Locale. (2005[b]) *Go native! Sowing wild flora seed.* Berkshire: Flora locale.

Flora Locale. (2005[c]) *Go native! Managing recently created grasslands*. Berkshire: Flora locale.

Flora Locale. (2008) *Go native! Planting for Biodiversity: Guidelines for Planting Projects in the Countryside: Marlborough.* Berkshire: Flora Locale.

Flora Locale. (2012) *Code of Practice for collectors, growers and suppliers of native flora: Planting with wildlife in mind*. Berkshire: Flora Locale.

Flower, C. (2008) *Where have all the flowers gone? Restoring Wild flowers to the garden and countryside*. Berkshire: Papadakis.

Forrest, J.R.K. (2014) 'Plant size, sexual selection and the evolution of protandry in dioecious plants'. *American Naturalist,* 184 (3) pp.338-351.

Foulds, W. (1978) 'Response to soil moisture supply in three leguminous species: II. Rate of N_2 (C_2H_2)-Fixation', *New Phytologist*, 80, pp.547-555.

Fox, J., & Bouchet-Valat, M. (2014) R Commander (RCmdr).

Fredrickson, E.L., Estell, R.E., Havstad, K.M., Ksiksi, T, Van Tol, J., & Remmenga, M.D. (1997) 'Effects of ruminant digestion on germination of Lehmann love-grass seed' *Journal of Range Management,* 50 (1), pp.20-26.

Freeman, B.C., & Beattie, G.A. (2008) 'An overview of plant defences against pathogens and herbivores' *The Plant Health Instructor* [online] Available from: https://www.apsnet.org/edcenter/intropp/topics/Pages/OverviewOfPlantDiseases.aspx [accessed: 29/11/13].

Frei, E.R., Ghazoul, J., Pluess, A.R. (2014) 'Plastic responses to elevated temperature in low and high elevation populations of three grassland species'. *PLoS ONE*, 9 (6) pp.1-13.

Fridley, J.D. (2010) *Lecture notes from Bio793: Plant Ecology / Niche modeling / Modeling in R. Generalized Linear Models.* New York: Syracruse University [online] available from http://plantecology.syr.edu/fridley/bio793/glm.html. [accessed: 21/01/2014].

Fu, B., Qi, Yanbing., Chang, Q.(2015) 'Impacts of revegetation management modes on soil properties and vegetation ecological restoration in degraded sandy grassland in farming-pastoral ecotone'*. International Journal of Agricultural & Biological Engineering,* 8 (1) pp.26-34.

Fuller, R.M. (1987) 'The Changing extent and conservation interest of lowland grasslands in England and Wales: A review of grassland surveys 1930'. *Biological Conservation,* 40, pp.281-300.

Fussell, M., & Corbet, S.A. (1992) ‘Flower Usage by Bumble-Bees: A Basis for Forage Plant Management’. *Journal of Applied Ecology*, 29 (2) pp.451-465.

Galen, C., & Plowright, R.C. (1985) 'The effects of nectar level and flower development on pollen carry-over in inflorescences of fireweed *(Epilobium augustifolium*) (Onagraceae*)'. Canadian Journal of Botany*, 63 (3) pp.488-491.

Gardener, M.C., & Gillman, M.P. (2001) ‘The effects of soil fertilizer on amino acids in the floral nectar of corncockle, Agrostemma githago (Caryophyllaceae)’. *OIKOS*, 92 pp.101-106.

Gathmann, A., & Tscharntke, T. (2002) ‘Foraging ranges of solitary bees’. *Journal of Animal Ecology*, 71, pp.757-764.

Gebrehiwot, L. & Beuselinck, R. (2001) ‘Seasonal variations in hydrogen cyanide concentration of three *Lotus* species’. *Agronomy Journal*, 93, pp.603-608.

Geddes, I. (2003) *Hidden Depths: Wiltshire’s Geology and Landscapes.* Ex-Libris Press: Bradford on Avon.

Gibson, D.J., Connolly, J., Hartnett, D.C. & Weidenhamer, J.D. (1999) ‘Designs for Greenhouse studies of interactions between plants’. *Journal of Ecology,* 87 pp.1-17.

Gilbert, M.E., & Ripley, B.S. (2008) ‘Biomass reallocation and the mobilization of leaf resources support dune plant growth after sand burial’. *Physiologia Plantarum*, 134 (3) pp.464-472.

Glazebrook, P. (2009[a]) polyglazebrook@avonwildlifetrust.org.uk *Avon Wildlife Trust study site information Burledge Hill, Folly Farm, Goblin Combe, Hellenge Hill, Lawrence Weston Moor*. Email to Erica Mortimer, 03/09/2009. eriberi.mortimer@gmail.com

Glazebrook, P. (2009[b]) *Telephone conversation with Erica Mortimer*. 07/09/2009.

Glazebrook, P. (2013) polyglazebrook@avonwildlifetrust.org.uk *Lawrence Weston Moor information*. Email to Erica Mortimer, 03/09/2013. eriberi.mortimer@gmail.com

Gleadow, R.M., & Møller, B.L. (2014) ‘Cyanogenic glycosides: synthesis, physiology, and phenotypic plasticity’. *Annual review of plant biology’*, 65 pp.155-185.

Goldenberg, S. (2014) ‘White House task force charged with saving bees from mysterious decline’. The Guardian. Friday 20 June. [online] available from: http://www.theguardian.com/environment/2014/jun/20/bees-die-off-mystery-white-house-plan-save [accessed: 16/03/15].

Goulson, D. (2009) *Conservation of bumblebees (Bombus spp.) in the UK.* Food and Agriculture of the United Nations [online] available from: http://www.internationalpollinatorsinitiative.org/uploads /6-018.pdf [accessed:16/03/14].

Goulson, D., & Hanley, M.E. (2004) 'Distribution and forage use of exotic bumblebees in South Island'. *New Zealand Journal of Ecology*, 28 (2) pp.225-232.

Goulson, D., Hanley, M.E., Darvill, J,S., & Knight, M.E. (2005) 'Causes of rarity in bumblebees' *Biological Conservation* 122, pp.1-8.

Gough, M.W. & Marrs, R.H. (1990) 'A comparison of soil fertility between semi-natural and agricultural plant communities: Implications for the creations of species-rich grassland on abandoned agricultural land'. *Biological Conservation*, 51 (2) pp.83-96.

Gough, L., Shaver, G.R., Carroll, J., Royer, D.L. & Laundre, J,A. (2000) 'Vascular plant species richness in Alaskan arctic tundra: the importance of soil pH'. *Journal of Ecology*, 88 (1) pp. 54 – 66.

Graham, D.J. & Hutchings, M.J. (1988) 'Estimation of the seed bank of a chalk grassland ley established on former arable land'. *Journal of Applied Ecology,* 25 pp.241-252.

Graham, P.H & Vance, C.P. (2003) 'Legumes Importance and constraints to greater use'. *Plant Physiology***,** 131 (3), pp**.** 872-877.

Gray, L. (2012) 'Dairy farmers are being skimmed alive'. *Telegraph.* 18th July [online] available from: http://www.telegraph.co.uk/earth/agriculture/ farming/9408891/Dairy-farmers-are-being-skimmed-alive.html [accessed: 29/10/2013].

Grazing Animals Project. (2009) *Map of UK Conservation Grazing Schemes Dawlish Warren NNR* [online] available from: http://www.grazinganimals project.org.uk/gap_site/dawlish_warren_nnr.html [accessed: 20/10/13].

Green, S. & Renault, S. (2007) 'Influence of papermill sludge on growth of *Medicago sativa*, *Festuca rubra* and *Agropyron trachycaulum* in gold mine tailings: A greenhouse study'. *Environmental Pollution*, 151, pp.524-531.

Greenwood, K.L. & McKenzie, B.M. (2001) 'Grazing effects on soil physical properties and the consequences for pastures: a review', *Australian Journal of Experimental Agriculture*, 41 (8), pp.1231-1250.

Grime, J.P., Hodgson, J.G. & Hunt, R. (1992) *The Abridged Comparative Plant Ecology*. London: Chapman & Hall.

Grogan, A.I. (2010) *Maintenance of soil organic matter. Department of Agriculture, Food and Marine for Ireland* [online] Available from: http://www.agriculture.gov.ie/media/migration/farmingschemesandpayments/crosscompliance/soilorganicmatter/Soil%20organic%20matter%20guidelines.pdf [accessed 07/10/12].

Gudin, C., & Syratt, W.J. (1970) 'Biological aspects of land rehabilitation following hydrocarbon contamination' *Environmental Pollution*, 8 (2), pp.107-112.

Guisan, A, Weiss, S. & Weiss A. (1999) 'GLM versus CCA spatial modeling of plant species distribution'. *Plant Ecology,* 143 (1), pp.107-122.

Gwynne, D.C. & Beckett, R.E. (1980) 'The response of *Lotus uliginosus* L. grown on hill soils to inoculation with Rhizobium'. *Grass and Forage Science*, 35 (3), pp. 213-217.

Hagler, J.R., Mueller, S., Teuber, L.R., Machtley, S.A., & Van Deynze, A. (2011) 'Foraging range of honey bees, *Apis mellifera*, in Alfalfa seed production fields'. *Journal of Insect Science*, 11 pp.144.

Hancock, C. (2009) *Telephone conversation with Erica Mortimer about Chancellors Farm*. 01/09/2009.

Hanley, M.E., Lamont, B.B., Fairbanks, M.M., & Raffertyb C.M. (2007) 'Plant structural traits and their role in anti-herbivore defence'. *Perspectives in Plant Ecology, Evolution and Systematics.* 8 pp.157–178.

Haplin, A. (2013) annehalpin@avonwildlifetrust.org.uk *Folly Farm*. Email to Erica Mortimer, 11/12/2013. eriberi.mortimer@gmail.com.

Hare, M.D. (1983) 'Seed development and the influence of severity and date of defoliation and plant population upon seed yields in 'Grasslands Maku' lotus (*Lotus pedunculatus* Cav.)'. Lincoln College University of Canterbury (Thesis) [online] available from; https://researcharchive.lincoln.ac.nz/handle/10182/2488 [accessed: 31st May 2015].

Havill, D.C., Lee, J.A. & Stewart, G.R. (1974) 'Nitrate utilization by species from acidic and calcareous soils'. *New Phytologist,* 73 (6) pp.1221-1231.

Hawke, M.A., & Maun, M., A. (1988) 'Some aspects of nitrogen, phosphorus, and potassium nutrition of three colonizing beach species'. *Canadian Journal of Botany,* 66 (8) pp.1490-1496.

Heard, M.S., Carvell, C., Carreck, N.L., Rothery, P., Osbourne, J.L., Bourke, A.F.G. (2007) 'Landscape context not patch size determines bumble-bee density on flower mixtures sown for agri-environment schemes'. Biology Letters, 3.

Henry, M., Beguin, M., Requier, F., Rollin, O., Odoux, J,F. (2012) ‘A common pesticide decreases foraging success and survival in honey bees’. *Science*, 336 (6079) pp.348-350.

Herms, D.A., & Mattson, W.J. (1992) ‘The Dilemma of Plants: To Grow or Defend’. *The Quarterly Review of Biology.* 67 (3) pp. 283-335.

Herrera, C.M. (1989) ‘Pollinator abundance, morphology, and flower visitation rate:analysis of the “quantity” component in a plant-poffinator system’. *Oecologia*, 80 pp.241-248.

Highways England. (2015). *Design manual for roads and bridges – Volume 10 Environmental design.* [online] available from http://www.standardsforhighways.co.uk /ha/standards/dmrb/vol10/index.htm [accessed: 19/09/15].

Hill, M.O., Mountford, J.O, Roy, D.B. & Bunce, R.G.H. (1999) *ECOFACT 2a Technical Annex - Ellenberg’s indicator values for British Plants*. DETR.

Himanen, K., Boccardi, T.M., Rycke, R.D., Odeny, O.P. & Van Lijsebettens, M. (2012) ‘Is HUB1 a hub for plant fitness?’ *Plant Signal Behaviour.* 7 (12) pp.1537-1540.

Hole, G. (2011) *Research Skills Mann-Whitney test handout version 1.0* [online] available from:http://www.sussex.ac.uk/Users/grahamh/RM1web /MannWhitney Handout%202011.pdf [accessed: 15/02/2013].

Holland, J.S., (2013) ‘The Plight of the Honeybee’. National Geographic. 10 May. [online] available from:http://news.nationalgeographic.com/news/2013/ 13/130510-honeybee-bee-science-european-union-pesticides-colony-collapse-epa-science/ [accessed: 16/03/15].

Hook, P.B. & Burke, I.C. (2000) ‘Biochemistry in a shortgrass landscape: control by topography, soil texture, and microclimate’. *Ecology,* 81 pp.2686-2703.

Hopkins, A. & Clements, R.O. (2004) *Re-creating flower-rich grassland* [online] available from: http://www.iger.bbsrc.ac.uk/Practice/Publications_ &_Leaflets/informationsheets/Re-creatingflower-richgrassland.htm [accessed: 01/02/2007].

Hopkins, A. & Murray, P.J. (1998) ‘Acceptability of *Lotus corniculatus* and *Lotus uliginosus* as a food source for non-pest butterfly larvae’. [online] available from: http://www.plantsci.missouri.edu/lnl/v29/Hopkins.htm [accessed: 01/02/2007].

Horne, F. (2006) American Scientist.com: How are seashells created? Or any other shell, such as a snail's or a turtle's?’ [online] Available from: http://sciam. com/print_version.cfm?articleID=000BBB1D-EE1A-1537-AE1A83414B7F000 [accessed on: 20.12.2006].

Houle, G. (1997) ‘No evidence for interspecific interactions between plants in the first stage of succession on coastal dunes in subarctic Quebec, Canada’. *Canadian Journal of Botany,* 75 (6) pp.902-915.

Howe, H.F. & Westley, L.C. (1988) *Ecological relationships of plants and animals*. Oxford: Oxford University Press.

Hughes, M.A. (1991) ‘The cyanogenic polymorphism in *Trifolium repens* L. (white clover)’. *Heredity*, 66 pp.105–115.

Hurben, T. & Huber-Sannwold. (2002) ‘Effect of Management on species-richness of grasslands: sward-scale processes lead to large scale patterns’. *Grassland Science in Europe,* 7, pp.634-643.

Hufford, K.M. & Mazer, S.J. (2003) ‘Plant ecotypes:Genetic differentiation in the age of ecological restoration’. *Trends in Ecology & Evolution*, 18 (3) pp.147-155.

International Plant Nutrition Institute (IPNI), (2013) *Nitrogen Notes – number 3.* [online] available from http://www.ipni.net/publication/nitrogen-en.nsf/book/FDEE48CFF7600CE585257C13004C7BB0/$FILE/NitrogenNotes-EN-03.pdf [accessed: 27/02/15].

JNCC. (2012) *The UK Biodiversity Action Plan (UK BAP) 1992-2012*. [online] available from: http://jncc.defra.gov.uk/page-5155 [accessed: 05/10/2013].

JNCC., & Defra (2012) UK Post-2010 Biodiversity Framework July 2012 - on behalf of the Four Countries Biodiversity Group. [online] available from: http://jncc.defra.gov.uk/page-6189 [accessed:06/11/13].

Jackson, D.K. (1973) ‘Some characteristics of perlite as an experimental growth medium’. *Plant and Soil*, 40 (1) pp.161-167.

Jacquemyn, H., Brys, R. & Hermy, M. (2003) ‘Short term effects of different management regimes on the response of calcareous grassland vegetation to increased nitrogen’. *Biological Conservation*, 111 (2) pp.137-147.

Janssens, F., Peeters, A., Tallowin, J.R.B., Bakker, J.P., Bekker, R.M., Fillat, F. & Oomes, M.J.M. (1998) ‘Relationship between soil chemical factors and grassland diversity’. Plant and Soil, 202 pp.69-78.

Jiménez-Ambriz, G., Petit, C., Bourrié, I., Dubois, S., Olivieri, I. & Ronce, O. (2007) ‘Life history variation in the heavy metal tolerant plant *Thlaspi caerulescens* growing in a network of contaminated and noncontaminated sites in southern France: role of gene flow, selection and phenotypic plasticity’. *New Phytologist,* 173 (1), pp.199-215.

Jing, J., Raaijmakers, C., Kostenko, O., Kos, M., Mulder, P.P.J., Bezemer, T.M (2015) ‘Interactive effects of above-and belowground herbivory and plant competition on plant growth and defence'. *Basic and Applied Ecology*. [online] available from: Bath Spa University Library Academic Search Premier [accessed: 30/06/15].

Jones, N (undated) BWARS: *Bombus* images. [online] available from: http://www.bwars.com/index.php?q=taxonomy/term/88/all&page=2 [accessed: 15/03/15].

Jones, D.A. & Turkington, R. (1986) 'Biological flora of the Bristish Isles: *Lotus corniculatus* L.'. *Journal of Ecology* 74 pp.1185-1212.

Jones, M.L.M., Norman, K., Rhind, P.M. (2010) 'Topsoil inversion as a restoration measure in sand dunes, early results from a UK field-trial'. *Journal of Coastal Conservation,* 14 (2) pp.139-151.

Jones, D.A., Parsons, J. & Rothschild, M. (1962) 'Release of hydrocyanic acid from crushed tissues of all stages in the life-cycle of species of the Zygaeninae (Lepidoptera)'. *Nature,* 193 (4810), pp.52-53.

Junker, R.R., & Parachnowitsch, A.L. (2015). 'Working towards a holistic view on flower traits - how floral scents mediate plant-animal interactions in concert with other floral characters'. *Journal of the Indian Institute of Science*, 95 (1) pp.43-67.

Kakes, P. (1991) 'A rapid and sensitive method to detect cyanogenesis using microtiterplates'. *Biochem. Syst. Ecol*. 19 (6) pp.519–522.

Keesing, V. & Wratten, S.D. (1998) 'Indigenous invertebrate components in ecological restoration in agricultural landscapes'. *New Zealand Journal of Ecology*, 22 (1) pp.99-104.

Kelman, W.M., Blumenthal, M.J. & Harris, C.A. (1997) 'Genetic variation for seasonal herbage yield, growth habit and condensed tannins in *Lotus pendunculatus* Cav. and *Lotus corniculatus* L.'. *Australian Journal of Agricultural Research,* 48 (7) pp.959-968.

Ketterings, Q., Reid, S. & Rao, R. (2007) *Agronomy Factsheet Series. FactSheet 22. Cation Exchange Capacity (CEC)*. Cornell University Cooperative Extension [online] available from http://nmsp.cals.cornell.edu/publications/factsheets/factsheet22.pdf [accessed: 05/02/13].

Killpack, S.C & Buchholz, D. (1993) *Nitrogen in the Environment, Soils.* Department of Agronomy: Mississipi State University [online] available from http://msucares.com/crops/soils/nitrogen.html) [accessed: 03/11/13].

King, T.J. (1977) 'The Plant Ecology of Ant-Hills in Calcareous Grasslands: I. Patterns of Species in Relation to Ant-Hills in Southern England'. *Journal of Ecology*, 65 (1), pp. 235-256.

Kleijn, D., Baquero, R.A., Clough, Y., Dı´az, M., DeEsteban, J., Ferna´ ndez, F., Gabriel, D., Herzog, F., Holzschuh, A., Jo¨ hl, R., Knop, E., Krues, A., Marshall, E.J.P., Steffan-Dewenter, I., Tscharntke, T., Verhulst, J., West, T.M., Yela, J.L. (2006) 'Mixed biodiversity benefits of agri-environment schemes in five European countries'. *Ecology Letters*, 9, pp. 243–254.

Kleijn, D., Berendse, F., Smit, R., Gilissen, N. (2001) 'Agri-environment Schemes do not Effectively Protect Biodiversity in Dutch Agricultural Landscapes'. *Nature,* 413, pp.723-725.

Kovach, W. (2006) *Multi-Variate Statistical Package – Version 3.13n*. Kovach Computing Services.

Kozuharova, E.K. (2000) 'Entomophilous plant species inhabiting the southern limestone slopes of Mt. VitoS'a (SW Bulgaria) and their pollinators' *Flora Mediterranea* 10, pp.227-234.

Krauss, S.L. & Koch, J.M. (2004) 'Methodological insights: Rapid genetic delineation of provenance for plant community restoration'. *Journal of Applied Ecology*, 41 (6) pp.1162-1173.

Kydd, D.D. (1964) 'The effect of different systems of cattle grazing on the botanical composition of permanent downland pasture'. *Journal of Ecology*, 52 pp.139-149.

Land Use Consultants. (2005) *Wiltshire Landscape Character Assessment: 10. The Landscape Character of Wiltshire.* Wiltshire Council.

Langford Evangelical Church. (2013) Hellenge Hill Photograph [online] available from: http://langfordevangelicalchurch.org/news [accessed: 22/10/2013].

Lawrence, K. (2009) *Telephone conversation with Erica Mortimer*. 29/08/2009.

Lesica, P. & Allendorf, F.W. (1999) 'Ecological genetics and the restoration of plant communities: Mix or match?'. *Restoration Ecology*, 7 (1) pp. 42-50.

Liu, H.L., Shi, X., Wang, J.C., Yin, L.K., Huang, Z.Y., & Zhang, D.Y. (2011) 'Effects of sand burial, soil water content and distribution pattern of seeds in sand on seed germination and seedling survival of *Eremosparton songoricum* (Fabaceae), a rare species inhabiting the moving sand dunes of the Gurbantunggut Desert of China'. *Plant & Soil*, 345 (1/2) pp.69-87.

Living Countryside. (2013) *UK Agriculture: the Importance of agriculture to the UK – Civilisation and society* [online] available from: http://www.ukagriculture.com/the_importance_of_agriculture.cfm [accessed: 25/10/13].

Lowry, A.A. (1996) *Influence of Ruminant Digestive Processes on Germination of Ingested Seeds.* MSc thesis. Oregon State University. [online] available from: http://www.google.co.uk/url?sa=t&rct=j&q=&esrc=s&frm=1&source=web&cd=4&ved=0CEcQFjAD&url=http%3A%2F%2Fir.library.oregonstate.edu%2Fxmlui%2Fbitstream%2Fhandle%2F1957%2F33924%2FLowryAmayaA1997.pdf%3Fsequence%3D1&ei=0CCqUrrYIs2rhQfN_IGwBw&usg=AFQjCNFKCW2Jx_DRyVWd1J5Ik2ifbUaCwQ&sig2=Qcfg3-kK0BvAGoP3wVMD6A&bvm=bv.57967247,d.ZG4 [accessed: 01/10/2013].

Lunt, G. (2012) lynchetts@hotmail.com *Honey bee research enquiry reply.* Email to Erica Mortimer, 22/06/2012. eriberi.mortimer@gmail.com.

Lynn, S., Adler, R., & Irwin, E. (2005) 'Ecological costs and benefits of defences in nectar'. Ecology, 86 (11) pp.2968-2978.

MAFF. (1986) *Analysis of Agricultural Materials*. London: HMSO.

MAGIC. (2013) *Magic Interactive Mapping* [online] available from: http://www.magic.gov.uk/MagicMap.aspx [accessed: 04/10/2013].

Majak, W., Hall, J.W. & McCaughley, W.P. (1995) 'Pasture management strategies for reducing the risk of legume bloat in cattle'. *Journal of Animal Science*, 73, pp.1493–1498.

Manchester, S.J., Bullock, J.M. (2001) 'The impacts of non-native species on UK biodiversity and the effectiveness of control'. *Journal of Applied Ecology*, 37 (5) pp.845-864.

Manchester, S. J., McNally, S., Treweek, J. R., Sparks, T.H. (1999) 'The cost and practicality of techniques for the reversion of arable land to lowland wet grassland—an experimental study and review'. *Journal of Environmental Management*, 55 pp. 91–109.

Manson, J.S., Rasmann, S., Halitschke, R., Thomson, J.D., & Agrawal, A.A. (2012) 'Cardenolides in nectar may be more than a consequence of allocation to other plant parts: a phylogenetic study of *Asclepias*', *Functional Ecology*, 26 pp.1100-1110.

Marley, C.L., Cook, R., Keatinge, R., Barrett, J. & Lampkin N.H. (2003) 'The effect of birdsfoot trefoil (*Lotus corniculatus*) and chicory (Cichorium intybus) on parasite intensities and performance of lambs naturally infected with helminth parasites'. *Veterinary Parasitology,* 112 (1-2) pp.147-155.

Marshall, S. (2009) SarahM2@wiltshirewildlife.org *Wiltshire Wildlife Trust Study Sites.* Email to Erica Mortimer,08/05/2009. eriberi.mortimer@gmail.com.

Martin, J. (2009[a]) John.p.martin@naturalengland.org.uk *Study site permissions St. Catherines Valley*. Email to Erica Mortimer, 10/08/2009. eriberi.mortimer@gmail.com.

Martin, J. (2009[b]) John.p.martin@naturalengland.org.uk *Study site permissions Goblin Combe and Hellenge Hill*. Email to Erica Mortimer, 02/09/2009. eriberi.mortimer@gmail.com.

Martin, J. (2009[c]) *Telephone conversation with Erica Mortimer*. 02/09/2009.

Martin, L.M. & Wilsey, B.J. (2006) 'Assessing grassland restoration success: relative roles of seed additions and native ungulate activities'. *Journal of Applied Ecology*, 43 (6), pp. 1098–1109.

Maun, M,A. (1998) 'Adaptations of plants to burial in coastal sand dunes' *Canadian Journal of Botany* 76 (5) pp.713-738.

Maun, A.M. (2009) *The Biology of Coastal Sand Dunes*. Oxford: The Oxford University Press. [online] available from https://books.google.co.uk/books?id=Z9XPa7- Je8YC&pg=PA80&lpg=PA80&dq=higher+desication+in+sand+soils&source=bl&ots=9LBfOBiz7t&sig=8StMxHsHrupvXHRql63i-cDmruE&hl=en&sa =X&ei= 0Y7xVKDwI-Sp7Aa8mIHoDw&ved=0CEAQ6AEwAw#v=onepage&q= higher%20desication%20in%20sand%20soils&f=false [accessed: 27.02.2015].

McBride, J.R. & Stone, E.C. (1976) 'Plant succession on the sand dunes of the Montery Peninsula, California'. *American Midland Journalist*, 96 (1) pp.118-132.

McCracken, D. (2011) *Farmland Biodiversity and the Common Agricultural Policy (CAP).* SAC Rural Policy Centre.

McNaughton, S.J., Wallace, L.L., & Coughenour, M,B. (1983) 'Plant adaptation in an ecosystem context: Effects of defoliation, nitrogen and water on growth of an African C4 sedge' *Ecology* 64 (2) pp.307-318.

McNeilly, T. (1980) 'Ecotypic differentiation in *Poa annua*: Interpopulation differences in response to competition and cutting'. *New Phytologist*, 88, pp.593-547.

Meineri, E., Skarpaas, O., Spindelböck, J., Bargmann, T., & Vandvik, V. (2014) 'Direct and size-dependant effects of climate on flowering performance in alpine and lowland herbaceous species' *Journal of vegetation science* 25 (1) pp.275-286.

Met Office (2013) *Historic Station Data: Yeovilton.* [online] available from http://www.metoffice.gov.uk/climate/uk/stationdata/yeoviltondata.txt [accessed: 12/12/2013].

Microsoft. (2003) version 15.0.4551.1005.

Millien, V., Lyons, S.K., Olson, L., Smith, F.A., Wilson, A.B. & Yom-Tov, Y. (2006) *Ecology Letters*, 9 pp.853-869.

Molan, A.L., Waghorn, G.C., Min, B.R. & McNabb, W.C. (2000*)* 'Remove from marked Records The effect of condensed tannins from seven herbages on Trichostrongylus colubriformis larval migration in vitro'*. Folia Parasitologica,* 47 (1), pp.39-44.

Montalvo, A.M., Williams Rice, S.L., Buchmann, S.L., Cory, C., Handel, S.N., Nabhan, G.P., Primack, R. & Robichaux, R.H. (1997) 'Restoration biology: A population biology perspective'. *Restoration Ecology*, 5 (4) pp.277-290.

Moore, P.D., & Chapman, S.B. (1986) *Methods in Plant Ecology*. 2nd ed. Oxford: Blackwell Scientific Publications.

Morant, A.V., Jørgensen, K., Jørgensen, C., Paquette, S.M., Sánchez-Pérez, R., Møller, B.L., & Bak, S. (2008) 'ß-Glucosidases as detonators of plant chemical defence'. Phytochemistry, 69 pp.1795-1813.

Morrison, H. (2009) *Telephone conversation with Erica Mortimer*. 04/09/2009.

Mortimer, E.J. (2010). Farm Environment Plan survey of Avis Meadow for Environmental Stewardship. Wiltshire Wildlife Trust.

Mountford, J.O., & Cook, A.I. (editors) Amy, S.R., Baker, A., Carey, P.D., Dean, H.J., Kirby, V.G., Nisbet, A., Peyton, J.M., Pywel, R.F., Redhead, J.W., Smart, S.M. (2013) *Monitoring the outcomes of Higher Level Stewardship: Results of a 3-year agreement monitoring programme.* Natural England Commissioned Reports, Number 114.

Mowat, D.J., & Shakeel, M.A. (1989) 'The effect of different cultivars of clover on numbers of and leaf damage by, some invertebrate species'. *Grass and Forage Science*, 44 (1) pp.11-18.

Mulhollond, B. & Fullen, M.A. (2007) 'Cattle trampling and soil compaction on loamy sands'. *Soil Use and Management*, 7 (4) pp.189-193.

Müller, A., Diener, S., Schnyder, S., Stutz, K., Sedivy C., Dorn, S. (2006) 'Quantitative pollen requirements of solitary bees: Implications for bee conservation and the evolution of bee-flower relationships'. Biological Conservation, 130. pp.604-615.

Munns, R. (2002) 'Comparative physiology of salt and water stress'. *Plant, Cell and Environment,* 25 pp.239-250.

Murrell, D.C., Shuelt, R.W. & Tomes, D.T. (1982) 'Nectar production and floral characteristics in bird's foot trefoil (*Lotus corniculatus* L.)'. *Canadian Journal of Plant Science*, 62 pp.361-371.

Nagy, L., & Proctor, J. (1997) 'Plant growth and reproduction on a toxic alpine ultramafic soil: adaptation to nutrient limitation'. *New Phytologist*, 137 (2) pp.267-274.

National Biodiversity Network. (2009) *Lotus corniculatus records* [online] available from: https://data.nbn.org.uk/Taxa/NBNSYS0000003258 [accessed: 04/04/2009].

Natural England. (1975[a]) *Special Site of Scientific Interest Citation – Cockey Down* [online] available from: http://www.sssi.naturalengland.org.uk/ citation/citation_photo/1003265.pdf [accessed: 20/10/13].

Natural England. (1975[b]) *Special Site of Scientific Interest Citation – Salisbury Plain* [online] available from: http://www.english-nature.org.uk/citation/citation_ photo/1006531.pdf [accessed: 20/10/13].

Natural England. (1999) *Soil sampling for habitat recreation and restoration; Technical Information Note TIN035.* Natural England.

Natural England. (2008[a]) *Seed Sources for Grassland Restoration and Re-creation in Environmental Stewardship*; *Technical Information Note TIN038.* Natural England.

Natural England. (2008[b]) *Soil and agri-environment schemes: interpretation of soil analysis*; *Technical Information Note TIN036.* Natural England.

Natural England. (2010) *Arable Reversion to species-rich grassland: site selection and choice of methods*; *Technical Information Note TIN066.* Natural England.

Natural England. (2012[a]) *Evolution of Agri-environment Schemes in England*. [online] available from: http://publications.naturalengland.org.uk/ publication/3567470?category=35001 [accessed: 28/11/13].

Natural England. (2012[b]) *National Character Area Profile: 108. Upper Thames Vale*. [online] available from: http://publications.naturalengland.org.uk /category/587130 [accessed: 20/10/2013].

Natural England. (2012[c]) *National Character Area Profile: 117. Avon Vale*. [online] available from: http://publications.naturalengland.org.uk /category/587130 [accessed: 20/10/2013].

Natural England. (2012[d]) *National Character Area Profile: 135. Dorset Heaths*. [online] available from: http://publications.naturalengland.org.uk/category/587130 [accessed: 20/10/2013].

Natural England. (2012[e]) *National Character Area Profile: 145. Exmoor*. [online] available from: http://publications.naturalengland.org.uk /category/587130 [accessed: 20/10/2013].

Natural England. (2012[f]) *National Character Area Profile: 147. Blackdowns*. [online] available from: http://publications.naturalengland.org.uk /category/587130 [accessed: 21/10/2013].

Natural England. (2012[g]) *Sites of Special Scientific Interest - SSSI units for Cockey Down* [online] available from: http://www.sssi.naturalengland.org.uk /special/sssi/unitlist.cfm?sssi_id=1003265 [accessed: 21/10/2013].

Natural England. (2013[a]) *Funding for land management: Closed Schemes*. [online] available from http://www.naturalengland.org.uk /ourwork/farming/funding/closedsch emes/default.aspx [accessed 14/11/2013].

Natural England. (2013[b]) *National Character Area Profile: 107. Cotswolds*. [online] available from: http://publications.naturalengland.org.uk /category/587130 [accessed: 20/10/2013].

Natural England. (2013[c]) *National Character Area Profile: 116. Berkshire and Marlborough Downs*. [online] available from: http://publications.naturalengland .org.uk/category/587130 [accessed: 20/10/2013].

Natural England. (2013[d]) *National Character Area Profile: 118. Bristol Avon Valleys and Ridges*. [online] available from: http://publications.naturalengland .org.uk/category/587130 [accessed: 20/10/2013].

Natural England. (2013[e]) *National Character Area Profile: 132. Salisbury Plain and West Wiltshire Downs*. [online] available from: http://publications. naturalengland.org.uk/category/587130 [accessed: 20/10/2013].

Natural England. (2013[f]) *National Character Area Profile: 113. Blackmoor Down and Vale of Wardour.* [online] available from: http://publications. naturalengland.org.uk/category/587130 [accessed: 20/10/2013].

Natural England. (2013[g]) *National Character Area Profile: 134. Dorset Downs and Cranborne Chase*. [online] available from: http://publications. naturalengland.org.uk/category/587130 [accessed: 20/10/2013].

Natural England. (2013[h]) *National Character Area Profile: 141. Mendip Hills.* [online] available from: http://publications.naturalengland.org.uk /category/587130 [accessed: 20/10/2013].

Natural England. (2013[i]) *National Character Area Profile: 142. Somerset Levels and Moors.* [online] available from: http://publications.naturalengland.org.uk/category/587130 [accessed: 20/10/2013].

Natural England. (2013[j]) *National Character Area Profile: 143. Devon Redlands*. [online] available from: http://publications.naturalengland.org.uk /category/587130 [accessed: 20/10/2013].

Natural England. (2013k) *Our Work: Soils.* [online] http://www.naturalengland.org.uk/ourwork/conservation/geodiversity/soils/default.aspx [accessed: 07/10/2013].

Natural England. (undated) *National Character Areas – Defining England's Natural Boundaries* [online] available from: http://www.naturalengland.org.uk/publications/nca/ [accessed: 20/04/2009].

NERC. (2013) *British Geological Survey. Geology of Britain Viewer* [online] available from: http://mapapps.bgs.ac.uk/geologyofbritain/home.html [accessed: 02/10/2013].

Neal, P.R. (1998) 'Pollinator restoration'. *Trends in Ecology & Evolution*, 13 (4) pp.132-133.

Neuffer, B. (1990) 'Ecotype Differentiation in Capsella'. *Plant Ecology,* 89 (2) pp.165-171.

North Devon Biosphere. (2011) *Sand Dunes* [online] available from: http://www.northdevon.gov.uk/lct_4f_dunes.pdf [accessed: 01/10/2013].

North Devon Coast AONB. (2013) *Explore the North Devon Coast – Woolacombe and Mortehoe* [online] available from: http://explorethecoast.org/waypoint/109 [accessed: 01/10/2013].

O'Hare, G. (1990) *Soils, vegetation and ecosystems*. London: Oliver & Boyd.

O'Leary, M., Rehm, G. & Schmitt, M. (2002) *Understanding Nitrogen in Soils. University of Minnesota* [online] available from http://www.extension.umn.edu/distribution/cropsystems /dc3770.html [accessed 07/10/12].

Ödman, A.M., Mårtensson, L.M., Sjöholm, C., Olsson, P.A. (2011) 'Immediate responses in soil chemistry, vegetation and ground beetles to soil perturbation when implemented as a restoration measure in decalcified sandy grassland'. *Biodiversity and Conservation*, 20 (13) pp.3039-3058.

Ollerton, J. (1993) *Ecology of fruiting and flowering in Lotus corniculatus*. PhD thesis. Oxford Brooks University. [online] available from: http://ethos.bl.uk/OrderDetails.do?did=1&uin=uk.bl.ethos.359911 [accessed: 01/10/2013].

Ollerton, J. & Lack, A. (1998) 'Relationships between flowering phenology, plant size and reproductive success in *Lotus corniculatus*'. *Plant Ecology,* 139, pp.35-47.

Olsson, P.A. & Tyler, G. (2004) 'Occurance of non-mycorrhizal plant species in south Swedish rocky habitats related to exchangeable soil phosphate'. *Journal of Ecology*, 92 (5) pp.808-815.

Ormerod, S.J. (2003) 'Restoration in applied ecology: editor's introduction'. *Journal of Applied Ecology*, 40 pp.44-50.

Osborne, J.L., Martin, A.P., Carreck, N.L., Swain, J.L., Knight, M.E., Goulson, D., Hale, R.J., Sanderson, R.A. (2008) 'Bumblebee flight distances in relation to the forage landscape'. *Journal of Animal Ecology*, 77 (2) pp.406-15.

Ouhadi, V.R. & Goodarzi, A.R. (2007) 'Factors impacting the electro conductivity variations of clayey soils'. *Iranian Journal of Science and Technology,* 31 (B2) pp.109-121.

Ovenden, G.N., Swash, A.R.H. & Smallshire, D. (1998) 'Agri-environment schemes and their contribution to the conservation of biodiversity in England' *Journal of Applied Ecology* 35, pp.955-960.

PDA. (2011) *The Potash Development Association Leaflet 24. Soil Analysis Key to Nutrient Management Planning* [online] available from http://www.pda.org.uk/leaflets/pdf/PDA-lf24.pdf [accessed: 01/10/2013].

Pady, J. (2009) gorenfarm@hotmail.com *Goren Farm grassland information*. Email to Erica Mortimer, 06/09/2009. eriberi.mortimer@gmail.com.

Parker, D.M. (1995) *Habitat Creation: A critical guide (ENS21).* English Nature Science Reports [online] available from: http://publications.naturalengland .org.uk/publication/2294780 [accessed: 18/09/15].

Palmer, M.W. (undated) *Ordination Methods – an overview.* Oklahoma State University [online] available from: http://ordination.okstate.edu/overview.htm [accessed: 02/08/2009].

Panagiota, G., Eleni, M.A., Zoi, M.P., & Apostolos, P.K. (2014) 'Phenotypic variation of *Lotus corniculatus* L. natural populations in relation to habitat type and management regimes'. *Archives of Biological Sciences*. [online] available from: http://www.doiserbia.nb.rs/img/doi/0354-4664/2015%20OnLine-First/0354-46641500043G.pdf [accessed: 30/06/2015].

Parsons, A.J., Harvey, A., & Woledge, J. (1991) 'Plant-Animal Interactions in a Continuously Grazed Mixture. I. Differences in the Physiology of Leaf Expansion and the Fate of Leaves of Grass and Clover'. *Journal of Applied Ecology*, 28 (2) pp. 619-634

Patien, K.D., Shanks, C.H., Mayer, D.F. (1993) 'Evaluation of herbaceous plants for attractiveness to bumble bees for use near cranberry farms', *Journal of Apicultural Research,* 32 (2) pp.73-79.

Pavlů, V., Hejcman, M., Pavlů, L. & Gaisler, J. (2007) 'Restoration of grazing management and its effect on vegetation in an upland grassland', *Applied Vegetation Science* 10, pp.375-382.

Pegtell, D.M., Bakker, J.P., Verweij, G.L. & Fresco, L.F.M. (1996) 'N,K and P deficiency in chronosequential cut summer-dry grasslands on grey podzol after the cessation of fertilizer application'. *Plant and Soil*, 178 pp.121-131.

Pérez-Barrales, R., Arroyo, J. & Scott Armbruster, W. (2007) ‘Differences in pollinator faunas may generate geographic differences in floral morphology and integration in *Narcissus papyraceus* (Armaryllidaceae)’. *Oikos,* 116 (11) pp.1904-1918.

Perlite.info. (2004) *Indoor gardening with perlite* [online] available from http://www.perlite.info/hbk/pg07.htm [accessed: 10.03.2007].

Pettis, J.S., VanEngelsdorn, D., Johnson, J., Dively, G. (2012) ‘Pesticide exposure in honeybees results in increased levels of the gut pathogen *Nosema*’. *Naturwissenscaften*, 99 (2) pp.153-158.

Poschlod, P., Hoffmann, J. & Bernhardt-Römermann, M. (2011) ‘Effect of grassland management on the age and reproduction structure of *Helianthemum nummularium* and *Lotus corniculatus* populations’. *Preslia*, 83 pp.421-435.

Price, P.W., Denno, R.F., Eubanks, M.D., Finke, D.L., Kaplan, I. (2011) ‘Insect Ecology: Behavior, Populations and Communities’. *Cambridge University Press*, pp.123. [online] available from Google Books [accessed: 04.08.2014].

Prine, G.M., & Burton, G.W. (1956) ‘The effect of nitrogen rate and clippingfrequency upon the yield, protein content and certain morphological characteristics of coastal Bermudagrass (*Cynodon dactylon,(L) Pers.) Agronomy Journal*, 48 (7), pp.296-301.

Pywell, R.F., Bullock, J.M., Roy, D.B., Warman, L., Walker, K.J., & Rothery, P. (2003) ‘Plant Traits as Predictors of Performance in Ecological Restoration’. *Journal of Applied Ecology,* 40, (1) pp. 65-77

Pywell, R.F., Bullock, J.M., Hopkins, A., Walker, K.J., Sparks, T.H., Burke, M.J.W., & Peel, S. (2002). ‘Restoration of species-rich grassland on arable land: assessing the limiting processes using a multi-site experiment’. *Journal of Applied Ecology,* 39 pp.294-309.

Pywell, R.F., Heard, M.S., Bradbury, R.B., Hinsley, S., Nowakowski, M., Walker, K.J., Bullock, J.M. (2012) ‘Wildlife-friendly farming benefits rare birds, bees and plants’. *Biology Letters,* 8, 772-775.

Pywell, R.F., Warman, E.A., Hulmes, L., Hulmes, S., Nuttall, P., Sparks, T.H., Critchley, C.N R. & Sherwood, A. (2006) ‘Effectiveness of agri-environment schemes in providing foraging resources for bumblebees in intensively farmed landscapes’. *Biological Conservation* 129 (2) pp.192-206.

Pywell, R.F., Warman, E.A., Carvella, C., Sparksa, T.H., Dicksa, L.V., Bennetta, D., Wrighta, A., Critchley, C.N.R., & Sherwood, A. (2004) ‘Providing foraging resources for bumblebees in intensively farmed landscapes’. *Biological Conservation,* 121 (4) pp.479-494.

R Core Team (2013) *R: A language and environment for statistical computing.* Vienna, Austria: R Foundation for Statistical computing.

Rackham, O. (2003) *The illustrated history of the countryside.* London: Weidenfield & Nicolson Ltd.

Rathcke, B., & Lacey, E.P. (1985) 'Phenological Patterns of Terrestrial Plants'. *Annual Review of Ecology and Systematics*. 16 pp. 179-214

Ravenscroft, C., Fridley, J.D., & Grime, J.P. (2014). 'Intraspecific functional differentiation suggests local adaptation to long-term climate change in a calcareous grassland'. *Journal of Ecology*, 102 (1) pp.65-73.

Rayner, J. (2013) 'Britain's farming crisis: 'People don't realise how tough it is'. *The Guardian.* Saturday 16th March [online] available from: http://www.theguardian.com/environment/2013/mar/16/britain-farming-crisis-how-tough [accessed: 01/10/2013].

Real, D., Sandral, G.A., Rebuffo, M., Hughes, S.J., Kelman, W.M., Mieres, J.M., Dods, K. & Crossa, J. (2012) 'Breeding of an early-flowering and drought-tolerant *Lotus corniculatus* L. variety for the high-rainfall zone of southern Australia', *Crop and Pasture Science,* 63, pp.848-857.

Redmon, L.A., Caddel, J.L. & Enis, J.D. (undated) *Forages legumes for Oklahoma* [online] available from http://courses.missouristate.edu/WestonWalker/AGA375_Forages/Forage%20Mgmt/References/2Forages/3Legume/OSUF2585ForageLegumesOklahoma.pdf [accessed: 30.03.2007].

Reisch, C., & Poschlod, P. (2009) 'Land use affects flowering time: seasonal and genetic differentiation in the grassland plant *Scabiosa columbaria'. Evolutionary Ecology*. 23 (5), pp.753-764.

Rhykerd, C.L., Hankins, B.J., Johnson, K.D., Bauman, T.T., & Williams, J.L. (2007) 'Birdsfoot trefoil production and utilization in Indiana'. [online] available from: http://www.agry.purdue.edu/ext/forages/publications/id-139.htm [accessed: 03/05/2015].

Richardson, M. (1976) *Translocation in Plants: Studies in Biology no.10* (2nd ed.). London: Edward Arnold (Publishers) Ltd.

RICS (2015) *Agricultural land prices* [online] available from: http://www.rics.org/uk/knowledge/glossary/agricultural-land-prices/ [accessed: 19/09/15].

Rodwell, J.S. *ed*. (1992) *British Plant Communities. Volume 3. Grassland and montane communities.* Cambridge University Press.

Rodwell, J.S. *ed.* (1991) *British Plant Communities. Volume 2. Mires and heath*. Cambridge University Press.

Rodwell, J.S. *ed.* (2000) *British plant communities. Volume 5. Maritime communities and vegetation of open habitats*. Cambridge University Press.

Roulston, S. (2003) *Grey Dunes* [online] available from: http://www.geographyinaction.co.uk/Text_based_site/Magilligan/Mag_grey.html [accessed: 20.12.2006].

Rorison, I.H. & Robinson, D. (1986) 'Mineral Nutrition'. *In*: Moore, P.D. & Chapman, S.B. *eds. Methods in plant ecology*. Oxford: Blackwell Scientific Publications.

Rose, F. (2006) *The Wild Flower Key.* London: Penguin Group.

Rosen, C.J., Bierman, P.M., & Eliason, R.D. (2008) *Overview of essential nutrients* [online] University of Minnesota. available from: http://www.extension.umn.edu/distribution/horticulture/components/1731-7-essentialnutrients.pdf [accessed: 04.02.12].

Rowell, D.L. (1994) *Soil science; methods and applications*. Harlow: Longman Scientific.

Royal Botanic Gardens, Kew. (2002) *A field manual for seed collectors; Seed collecting for the millennium seed bank project* [online] available from: http://www.kew.org/msbp/scitech/publications/fieldmanual.pdf [accessed: 02.09.09].

Royal Botanic Gardens, Kew (2008) *Post harvest handling of seed; Millenium seed bank project* [online] *a*vailable from: http://www.kew.org/msbp/scitech/publications/04-Post%20harvest%20handling.pdf [accessed: 02.09.09].

RStudio. (2013) RStudio: Integrated development environment for R (version 0.98.994). Boston, MA [accessed: 01/10/2014].

Ruiz-Jaen, M.C. & Mitchell Aide, T. (2005) 'Restoration Success: How is it being measured?'. *Restoration Ecology*, 13 (3) pp.569-577.

Sackville Hamilton, N.R. (2001) 'Forum: Is local provenance important in habitat creation? A reply'. *Journal of appyplied ecology*, 38, pp. 1374-1376.

Sareen, S. & Dev, I. (2003) *A preliminary study to explore potential of Lotus corniculatus L. (Birdsfoot trefoil) in Palampur (Himachal Pradesh, India)* [online] available from: http://www.inia.org.uy/sitios/lnl/vol33/sareen.pdf [accessed: 01.02.2007].

Scheublin, T.R., Ridgway, K.P., Young, J.P.W., Van der Heijden, M.G.A. (1998) 'Nonlegumes, Legumes, and Root Nodules Harbor Different Arbuscular Mycorrhizal Fungal Communities'. *Applied and Environmental Biology,* 70 (10) pp.6240-6246.

Schmitt, J., & Wulff, R,D. (1993) ‘Light spectral quality, phytochrome and plant competition’ *Tree*, 8 (2) pp.47-50.

Schnelle, M.A. & Henderson, J.C. (2007) *Containers and Media for the Nursery - Oklahoma Cooperative Extension Service, Oklahoma State University* [online] available from http://osufacts.okstate.edu [accessed: 10.02.12].

Schroeder, L,A. (1986) ‘Changes in leaf quality and growth performance of Lepidopteran larvae’. *Ecology*, 67 (6) pp.1628-1636.

Schröder, R. & Prasse, R. (2013) ‘From nursery into nature: A study on performance of cultivated varieties of native plants used in re-vegetation, their wild relatives and evolving wild × cultivar hybrids’. *Ecological Engineering* 60 pp.428-437.

Schulte, E.E. & Kelling, K.A. (1996) *Understanding Plant Nutrients. Soil and Applied Phosphorus. University of Wisconsin* [online] available from: http://www.soils.wisc.edu/extension/pubs/A2520.pdf [accessed: 09/12/13].

Sedgemoor District Council. (undated) *Berrow Dunes Local Nature Reserve - The dune slacks, fixed dunes, foredunes and ponds.* [online] available from: http://www.sedgemoor.gov.uk/index.aspx?articleid=6220 [accessed: 01/10/2013].

Seedaholic.com. (undated) *Lotus corniculatus Birds foot trefoil* [online] available from: http://www.seedaholic.com/lotus-corniculatus-bird-s-foot-trefoil.html [accessed: 02/02/2011].

Self, J.R. & Waskon, R.M. (2011) *Nitrates in drinking water - Colorado State University* [online] available from http://www.ext.colostate.edu/pubs/crops/00517.html [accessed: 30/09/13]

Shi, L., Zhang, Z,J., Zhang, C,Y., & Zhang, J,Z. (2004) ‘Effects of sand burial on survival, growth, gas exchange and biomass allocation of *Ulmus pumila* seedlings in the Hunshandak Sandland, China’ *Annals of Botany*, 94 pp.553-560.

Shrubb, M. (2003) *Birds, Scyths and Combines: A history of birds and agricultural change*. Cambridge University Press: Cambridge.

Shykoff, J.A., & Bucheli, E. (1995) ‘Pollinator visitation patterns, floral rewards and the probability of transmission of *Microbotryum violaceum*, a veneral disease of plants’. *Journal of Ecology*, 83 (2) pp.189-198.

Siddique, K.H.M., & Sedgley, R.H. (1986) ‘Chickpea (*Cicer arietinum* L.), a potential grain legume for South-Western Australia: Seasonal growth and yield’. *Australian Journal of Agricultural Research.* 37(3) pp.245 – 261.

Silcock, P., Allen, B. & Hart, K. (2012) *Land Stewardship in England Post-2013: CAP Greening and Agri-environment*. Prepared for National Trust and The Co-operative Forms.

Smart, S. (2009) *Telephone conversation with Erica Mortimer*. 30/08/2009.

Smith, G. (2011) *Interview with Erica Mortimer on Experimental Design.* 07.07.2011.

Smith, G. (2014) *Interview with Erica Mortimer on Statistics.* 07.08.2014.

Smith, B.M., Diaz, A., Winder, L. & Daniels, R. (2005) 'The effect of provenance on the establishment and performance of *Lotus corniculatus* L. in a re-creation environment'. *Biological Conservation*, 125 (1) pp.37-46.

Smith, R.S., Shiel, R.S., Millward, D. & Corkhill, P. (2000) 'The Interactive Effects of Management on the Productivity and Plant Community Structure of an Upland Meadow: An 8-Year Field Trial'. *Journal of Applied Ecology*, 37, (6) pp. 1029-1043.

Smith, B.M., Diaz, A., Daniels, R., Winder, L. & Holland, J.M. (2009) 'Regional and ecotype traits in *Lotus corniculatus* L., with reference to restoration ecology'. *Restoration Ecology,* 17 (1) pp.12-23.

Stephenson, A.G. (1980) 'Fruit Set, Herbivory, Fruit Reduction, and the Fruiting Strategy of *Catalpa Speciosa* (Bignoniaceae)'. *Ecology*. 61 (1) pp. 57-64.

Stephenson, A.G. (1984) 'The regulation of maternal investment in an indeterminate flowering plant *(Lotus corniculatus)*'. *Ecology*, 65 (1) pp.113-121.

Stephenson, A.G. & Winsor, J.A. (1986) '*Lotus corniculatus* regulates offspring quality through sensitive fruit abortion'. *Evolution*, 40 (3), pp.453-458.

Spectrum Analytic. (undated) *Agronomic Library: Calcium in the soil* [online] available from: http://www.spectrumanalytic.com/support/library/ff/Ca_Basics.htm [accessed: 30/09/12].

Springate, D.A., & Kover, P. (2014) 'Plant responses to elevated temperatires: a field study on phonological sensitivity and fitness responses to simulated climate warming'. *Global change biology*, 20 (2) pp.456-465.

Stace, C (1997) *New Flora of the British Isles.* 2nd ed. Cambridge University Press: Cambridge.

Suter, L., Ruegg, M., Zemp, N., Hennig, L., Widmer, A. (2014) 'Gene regulatory variation mediates flowering responses to vernalization along an altitudal gradient in Arabidopsis thaliana' *Plant Physiology*, pp.114.

Sykes, M.T., & Bastow Wilson, J. (1990) 'Dark tolerance in plants of dunes'. *Functional Ecology,* 4 pp.799-805.

Taiz, L. & Zeiger, E. (1991) *Plant Physiology*. California: The Benjamin/ Cummings Publishing Company, Inc.

Tarayre, M., Bowman, G., Schermann-Legionnet, A., Barat, M., & Atlan, A. (2007) 'Flowering phenology of Ulex europaeus : ecological consequences of variation within and among populations'. *Evolutionary Ecology,* 21 (3) pp.395-409

Teignbridge District Council. (undated) *Dawlish Warren: Wildlife* [online] available from: http://www.teignbridge.gov.uk/index.aspx?ArticleID=2668 [accessed: 01/10/2013].

The Grass Seed Store Ltd. (2015) *Acidic soil wildflower seeds*. [online] available from: http://www.thegrassseedstore.co.uk/environmental/wildflower-only-mixtures/reclamation-wild-flower-mix/250g-acidic-soil-wildflower-seeds.html [accessed: 07/05/2015].

The Laboratory of Apiculture and Social Insects (LASI), (2015) University of Sussex. [online] available from: http://www.sussex.ac.uk/lasi/ [accessed: 16/03/15].

Thompson, K. & Grime, J.P. (1979) 'Seasonal variation in the seed banks of herbaceous species in ten contrasting habitats'. *Journal of Ecology*, 67 pp.893-921.

Thompson, K., Band, S.R. & Hodgson, J.G. (1993) 'Seed Size and Shape Predict Persistence in Soil'. *Functional Ecology*, 7 (2) pp. 236-241.

Thompstone, S. (2009) stephen.thompstone@sky.com *Study sites Southstoke and Iford*. Email to Erica Mortimer, 06/09/2009. eriberi.mortimer@gmail.com.

Till-Bottraud, I. & Gouyon, P.H. (1992) 'Intra-versus interplant batesian mimicry? A model on cyanogenesis and herbivory in clonal plants'. *The American Naturalist,* 139 (3), pp.509-520.

Tintometer Ltd. (undated). *Colorimetric chemical analytical methods; determination of phosphate (7).* Salisbury: The Tintometer Limited.

Tirado, R., Gergely, S., Johnston,P. (2013) Bees in decline. A Review of factors that put pollinators and agriculture in Europe at risk. University if Exeter; Greenpeace International [online] available from:

http://www.greenpeace.org/switzerland/Global/international/publications /agriculture/2013/BeesInDecline.pdf [accessed: 16/03/15].

Tivy, J. (1993) *Biogeography: A study of plants in the ecosphere.* Essex: Longman Group UK Ltd.

Trlica, M. J., Cook, C. W (1971) 'Defoliation effects on the carbohydrate reserves of desert range species'. *Journal of Range Management*. 24 pp.418-25.

Underwood, A.J. (1997) *Experiments in Ecology: Their Logical Design and Interpretation Using Analysis of Variance.* Cambridge: Cambridge University Press.

United States Department of Agriculture. (1999) *Agricultural Research Service: Perlite and hydroponics: possible substitute for MeBr?* [online] available from http://www.ars.usda.gov/is/np/mba/apr99/perlite.htm [accessed: 28.03.2007].

United States Department of Agriculture (USDA). (2015) Research programs and projects at this location. [online] available from: http://www.ars.usda.gov/ research/projects_programs.htm?modecode=20-22-05-00 [accessed: 16/03/15].

Van Assche, J.A., Debucquoy, K.L.A. & Rommens, W.A.F. (2003) 'Seasonal cycles in the germination capacity of buried seeds of some leguminosae'. *New Phytologist* 158 pp. 315-323.

Van der Heijden, M.G.A., Klironomos, J.N., Ursic, Margot., Moutoglis, P., Streitwolf-Engel, R., Boller, T., Wiemken, A., & Sanders, I.R. (1998) 'Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity'. *Nature*, 396 pp.69-72.

Vickery, P.J., Wheeler, J.L., & Mulcahy, C. (1987). 'Factors affecting the hydrogen cyanide potential of white clover (*Trifolium repens* L.)'. *Australian Journal of Agricultural Research,* 38 (6) pp.1053-1059.

Vignolio, A.B., Cambareri, A., & Maceira, A. (2010) 'Seed production of *Lotus tenuis* (Fabaceae), a forage legume: effects of row spacing, seeding date, and plant defoliation'. *Crop and Pasture Science.* 61(12) pp.1027-1035.

Virteiu, A-M., Grozea, I., Stef, R., Carabet, A., Damianov, S., & Florian, T. (2014) 'Insect community structures of bird's-foot trefoil (*Lotus corniculatus* L.) inflorescences along the seed dispersal'. *Bulletin of the University of Agricultural Sciences & Veterinary Medicine Cluj-Napoca. Agriculture.* 71 (2) pp.354-359.

Vuckovic, S., Stojanovic, I., Prodanovic, S., Branko, C., Toma, Z., Svetko, V, & Slavica, J. (2006) 'Morphological and nutritional properties of birdsfoot trefoil

(*Lotus corniculatus* L.) autochthonous populations in Serbia and Bosnia and Herzegovina’. *Genetic Resources and Crop Evolution*, 54 (2), pp.421-428.

Waddington, K.D. (1980) 'Flight patterns of foraging bees relative to density of artificial flowers and distribution of nectar'. *Oecologia*, 44 (2) pp.199-204

Walker, K.J., Hodder, K.H., Bullock, J.B., Pywell, R.F. (2004[a]) *A review of the potential effects of seed sowing for habitat re-creation on the conservation of intraspecific biodiversity*. Defra Contract BD1447. Centre for Ecology and Hydrology, Monks Wood.

Walker, K.J., Pywell R.F., Warman, E.A., Fowbert, J.A., Bhogal.A., Chambers B.J. (2004[b]) ‘The importance of former land use in determining successful re-creation of lowland heath in southern England’ *Biological Conservation* 116 pp.289-303.

Walker, K.J., Stevens, P.A., Stevens, D.P., Mountford, J.O., Manchester, S.J., & Pywell, R.F. (2004[c]) ‘The restoration and re-creation of species-rich lowland grassland on land formerly managed for intensive agriculture in the UK’. *Biological Conservation*, 119 pp.1–18.

Warda, M. (2002) *Persistency and productivity of legumes in the pasture sward on black soil and peat-muck soil* [online] available from: http://www.fao.org/DOCREP/006/AD236E/ad236e0n.htm [accessed: 23.03.2007].

Wardle, D.A., Barker, G.M., Bonner, K.I., Nicholson, K.S. (1998) ‘Can comparative approaches based on plant ecophysical traits predict the nature of biotic interactions and individual plant species effects in ecosystems?’. *Journal of Ecology* 86 pp.405-420.

Warren, J. (2012) *Genetic variation and local provenance.* Scottish Agricultural College. Flora Locale.

Warren, J., & Billington, T. (2005) ‘Flower colour phenology in British mesotrophic grassland communities’. *Grass and Forage Science*, 60 pp.332-336.

Weiner, J. (1985) ‘Size Hierarchies in Experimental Populations of Annual Plants’. *Ecology.* 66 pp.743–752.

Wells, T.C.E. (1969) ‘Botanical aspects of conservation management of chalk grasslands’ *Biological Conservation* 2 (1) pp.36-44.

Whitbourn, C. (2009) *Interview with Erica Mortimer about Fontmell Downs*. 26/05/2009.

Whitbourn, C. (2011) *Interview with Erica Mortimer about Wingreen*. 04/11/2011.

Whittle, J. (2009) *Telephone conversation with Erica Mortimer*. 10/09/2009.

Who killed the honeybee? (2014) London: BBC Four. Tuesday 15 April. [Documentary].

Wilkins, R.J. *ed*. (2001) *IGER: Legume silages for animal production* [online] available from http://www.fao.org/ag/agp/agpc/doc/silage/legume/english.pdf [accessed: 10.02.2012].

Willmer, P.G. (1988) 'The role of insect water balance in pollination ecology: Xylocopa and Calotropis'. *Oecologia*, 76 (3) pp.430-438.

Wilson, D. & Jones, J.G. (1981) 'Effect of selection for dark respiration rate of mature leaves on crop yields of *Lolium perenne* cv. S23'. *Annals of Botany* 49 pp.313-320.

Wiltshire Council. (2011) *Wiltshire Community History – Donhead St. Mary* [online] available from: http://history.wiltshire.gov.uk/community/getcom.php?id=81 [accessed: 09/12/13].

Wiltshire Geology Group. (undated) *Geology – Geological Map of Wiltshire* [online] available from: http://www.wiltshiregeologygroup.org.uk/geology/ [accessed: 01/10/14].

Wiltshire Wildlife Trust. (2003[a]) *Reserve Management Plan – Avis Meadows 1st October2003 – 30th September2008.* Devizes: Wiltshire Wildlife Trust.

Wiltshire Wildlife Trust. (2003[b]) *Cockey Down Reserve Management Plan 1st Oct 03 – 30 Sept 08* (3rd ed). Devizes: Wiltshire Wildlife Trust.

Wiltshire Wildlife Trust. (2013) *Cockey Down photograph* [online] available from: http://www.wildlifetrusts.org/reserves/cockey-down accessed: 01/10/2013.

Winter, B. (2013) *A very basic tutorial for performing linear mixed effects analyses (Tutorial 2).* Univeristy of California [online] available from : http://www.bodowinter. com/tutorial/bw_LME_tutorial2.pdf [accessed: 02/04/14].

Woodcock, B.A., Savage, J., Bullock, J.M., Nowakowski, R., Orr, J., Tallowin, J.R.B. (2014) 'Enhancing floral resources for pollinators in productive agricultural grasslands'. *Biological Conservation*, 171 pp.44-51.

Woodcock, B.A., Edwards, M., Redhead, J., Meek, W.R., Nuttall, P., Falk, S., Nowakowski, M., Pywell, R.F. (2013) 'Crop flower visitation by honeybees, bumblebees and solitary bees: behavioural differences and diversity responses to landscape'. *Agriculture, Ecosystems & Environment*, 171 pp.1-8.

Woodward, S,L., Laboyrie, P, J., & Jansen, E, B, L. (2000) '*Lotus corniculatus* and condensed tannins – Effects on milk production by dairy cows'. *Asian-Australiasian Journal of Animal Sciences*, 13 pp. 521-525.

Xu, L., Huber, H., During, H.J., Dong, M., & Anten, N.P.R. (2013) 'Intraspecific variation of a desert shrub species in phenotypic plasticity in response to sand burial'. *New Phytologist,* 199 (4) pp.991-1000.

Yasseen, M., Barringer, S.A., Splittstoesser, W.E. & Costanza, S. (1994) 'The role of seed coats in seed viability'. *The Botanical Review* 60 (4) pp.426-439.

Young, T.P. (2000) 'Restoration Ecology and Conservation Biology'. *Biological Conservation,* 92 p.73-83.

Zagrobelny M, Møller B.L. (2011) 'Cyanogenic glucosides in the biological warfare between plants and insects: the Burnet moth-Birdsfoot trefoil model system'. *Phytochemistry.* 72 (13) pp.1585-92.

Zhang, J., & Maun, M,A. (1990) 'Effects of sand burial on seed germination, seedling emergence, survival, and growth of *Agropyron psammophilum*' *Canadian Journal of Botany,* 68 (2) pp.304-310.

Zhang, T., Guo, R., Gao, S., Guo, J., & Sun, Wei. (2015) 'Responses of plant community composition and biomass production to warming and nitrogen deposition in a temporate meadow ecosystem'. *PLoS ONE*, 10 (4) pp.1-16.

Zhu, Y., Yang, Y., Baskin, C., Baskin, J., Dong, M., & Huang, Z. (2014) 'Effects of amount and frequency of precipitation and sand burial on seed germination, seedling emergence and survival of the dune grass *Leymus secalinus* in semiarid China'. *Plant & Soil*, 374 (1/2) pp.399-409.

Zuur, A.F., Ieno, E,N., Walker, N,J., Saveliev, A, A., Smith, G, M. (2009) *Mixed Effects Models and Extensions in Ecology with R.* New York: Springer Science & Business Media.

APPENDICES

TABLE OF APPENDICES

APPENDIX I – National Character Area Summaries

NCA No.	NCA Name	NCA Description
107	**Cotswolds**	Running from the Dorset coast to Lincolnshire, the Cotswolds NCA character is influenced by the oolithic limestone geology, creating steep scarp usually capped by open wold rising above lowland valleys. Beech and mixed oak woods are a feature, with mixed farmland and large parkland estates. Soils are thin and brashy and therefore prone to erosion. Unimproved limestone grassland and wet meadows are some of the important habitats found here (Natural England, 2013[b]).
108	**Upper Thames & Clay Vales**	This undulating clay vale NCA is a strip of low-lying land running through south central England from Somerset to Lincolnshire. Quaternary river deposition covers large areas with clay, silt, sands and gravels present over a large proportion of the NCA. Extensive surface water, river catchment systems are prominent. Oxford clay and Kimmeridge clay from the Upper and Middle Jurassic years dominate the area. Land use is mainly mixed farming impeded heavy soils are usually pasture with the arable restricted to areas of better drainage. Low lying areas often have gleyed soils (Natural England, 2012[2]).
116	**Berkshire & Marlborough Downs**	A largely arable landscape with immense fields stretching across rolling Chalk hills of the NCA. White horse figures cut out in the chalk hills, sarson stones and ancient monuments are seen often here. The chalk escarpments enclose the Vale of Pewsey which lies on Upper Greensand. Beech clumps, small remnants of species-rich chalk grassland, dry valley slopes and free-draining arable are important habitats here with farmland birds and rare arable plants benefitting from them (Natural England, 2013[c]).
117	**Avon Vale**	A generally flat, low-lying landscape dominated by Middle and Upper Jurassic clays which include both Kimmeridge and Oxford clay. Nine main soil types are found in this NCA, ranging from 'slowly permeable seasonally wet slightly acid but base-rich loamy and clayey soils' which covers the majority (46%), to 'shallow lime-rich soils over chalk or limestone', covering (15%) (Natural England, 2012[c]. pp.6-7). Large landscaped woodlands, historic parkland, calcareous grassland, floodplain grazing marsh and lowland meadow are some of the important habitats here (Natural England, 2012[c]).

Continued…

NCA No.	NCA Name	NCA Description
118	**Bristol Avon & Valleys**	The city of Bristol is included in this NCA with its surrounding landscape, including the areas of Chew and Yeo valleys, and parts of the Cotswolds and Mendips. Ridges and valleys dominate the characteristic of the area as influenced by the complex geology. This includes geomorphological features such as the Avon Gorge, carboniferous limestones and sandstones, silts, muds and clays. Soil types are widely varied with poor draining gleys on clay and brown earths on limestone. Important habitats include broadleaved wood, often on slopes, large reservoirs and wet valley and dry slope grasslands. (Natural England, 2013[d]).
132	**Salisbury Plain & West Wiltshire Downs**	Salisbury Plain is characterised by the underlying cretaceous chalk and forms the dominant expanse of gently rolling unimproved chalk downland, one of the largest remnants of this habitat In north-west Europe. Predominantly agricultural, and used for military training, there are few hedgerows. In-between are sheltered valleys with chalk streams and often woodland on valley slopes. Vast arable fields predominate with many scheduled monuments and earthworks dotted throughout, including Stonehenge, numerous burial mounds and cut out figures on chalk scarps (Natural England, 2013[e]).
133	**Blackmoor Down & Vale of Wardour**	The core of this NCA is the fertile clay vales, fringed by Upper Greensand hills and scarps. Occasional large forms and low rounded limestone hills are present. Fields are small and hedged with oak standards common and many small broad leaved woods scattered. Streams cut through the landscape of the vales supporting the predominantly lush grassland. The area is predominantly mixed farmland (Natural England, 2013[f]).
134	**Dorset Downs & Cranborne Chase**	This NCA lies across the counties of Dorset, Wiltshire and Hampshire over cretaceous chalk geology. It is characterised by large, open fields of arable and pasture with dotted clumps of woodland, dramatic scarps and plateaux's of chalk grassland with gentle, sheltered valleys. Archaeological monuments here are numerous, Cranborne Chase chalk plateau features predominantly in the landscape. Important habitats include species-rich calcareous grassland, water meadows and ancient woodland in cut with species-rich chalk streams and rivers (Natural England, 2013[g]).
135	**Dorset Heaths**	Surrounded by chalk downland, this NCA lies in the Poole Basin where the chalk bedrock is geology overlain by clays, gravels and sands of the Reading Beds and London Cley series, creating more acidic soils, encompassing approximately 34 ha of the New Forest National Park. Major watercourses exist together with Floodplain grazing marsh BAP habitat, also supported by the NCA are important arable habitats supporting nationally important farmland birds, lowland heath and mixed/and broadleaved woodland. Livestock farming is the dominant land use here (Natural England, 2012[d]).

Continued…

NCA No.	NCA Name	NCA Description
141	**Mendip Hills**	Here is a karst landscape with a sequence of carboniferous limestone hills with cores of Silurian and Devonian rock and sandstone outcrops. The limestone which can be eroded by weathering then creates underground caves and gorges such as those seen in this NCA. There is a mosaic of habitats here with valleys of woodlands and improved pasture grassland bordered with drystone walls and smaller fields bounded by hedgerows further east. Habitats of the centre and west are influenced by varying pH with unimproved neutral grassland below the calcareous grassland of the plateau and then heathland on sandstone hilltops (Natural England, 2013[h]).
142	**Somerset Levels and Moors**	This is a predominantly flat, open landscape which contains vast amounts of wetland and waterways, historically drained and agriculturally-improved to create productive farmland. Wet pasture and wetland are common, separated by wet ditches. Important habitats include lowland grazing marsh, water meadow, reed bed, fens and mires. Scattered hills are found throughout such as Polden and Mendip hills and Glastonbury Tor. Trees are mainly those along ditches such as pollard willows, with individual moorland areas often treeless (Natural England, 2013[i]).
145	**Exmoor**	The predominantly upland landscape of Exmoor NCA is formed on a plateaux of Devonian sandstones, slates and shale. Here the most common habitat is high treeless moorland, grazed by sheep and ponies, where acid soils contribute to heath, blanket bog and bracken. Along the Devon coast line of this NCA Woolacombe and Braunton Burrows contain geological features creating sandy beaches and rocky shores and cliffs, whereas the coast further into Somerset such as Porlock Bay is instead shingle (Natural England, 2012[e]).
147	**Blackdowns**	This NCA has a large amount of rivers and valleys with a varied underlying geology, therefore giving a range of elevations to the landscape with plateaus, narrow ridges, cliffs, and valley sides. Cliffs are often formed from a geology of red Triassic Mudstone and sandstone, where the chalk cliffs (at Beer) are located these bedrocks are overlain with Upper Greensand and clay flint capped chalk. The valleys are cut into the Upper Greensand and Triassic Mudstone, sometimes with Lias Mudstones which create fertile brown earth and clay soils. Contrary to this on the higher ground are poor soils, often acidic from the clay and Upper Greensand and usually covered in heath or forestry. Pasture land is mainly confined to the valleys, with arable more represented on the chalk plateau. On the steeper slopes rough grassland, scrub and mixed woodland predominate. There are also salt marshes where the rivers reach the sea (Natural England, 2012[f]).

Continued…

NCA No.	NCA Name	NCA Description
148	**Devon Redlands**	A character red soil, coloured by the underlying red sandstone Rolling hills with fertile farmland, enclosed into small hedged fields used for mixed agriculture dominate the landscape. Important habitats include the sand dunes and salt and grazing marshes (Natural England, 2013[j]).

APPENDIX II – Soil Element Descriptions

Table II 1. Descriptions of soil elements measured in soil analysis of experiment

pH

Soil pH is the measure of hydrogen (H^+) and hydroxyl (OH^-) concentrations. With a low pH (acid) soil resulting from higher H^+ than OH^- and vice-versa for a high pH (alkaline) soil (Bunt, 1983). Very acidic soils can in themselves be toxic to plants from such high H+ levels, but pH also plays an important role in its interactions with other soil nutrients, with amounts available to plants ultimately determined by pH related reactions (Fitter & Hay, 2002). Certain species are specialised to cope with these deficiencies and toxicities; calcicoles such as *Asperula cynanchica* L. (Cooper & Ethington, 1974) and *Sanquisorba minor* Scop. (Bunce *et al*., 1999) are able to flourish in high pH soil. These specialist species are more tolerant to deficiencies such as low aluminium, therefore survive well in this calcium rich soil (Gough *et al.*, 2000). Calcifuges such as *Calluna vulgaris* L.(Etherington, 1984) and *Deschampsia flexuosa* L. (Havill *et al*., 1974), can survive in highly acid soils where the calcicoles cannot due to their low tolerance to toxicities (i.e. aluminium) associated with acidic soil (Gough *et al.,* 2000). These herbs show narrow ecotypic adaptation and populations exist within the communities largely because of their tolerance to soil pH extremes. Soil pH is therefore important in grassland restoration as this will determine which species have a better chance of survival in the sward.

Phosphorus (P)

An essential macro-nutrient for plants, however high levels of phosphorus are especially associated with increased growth of grasses (Buglife, 2012) and as one of the soil macro-nutrients which are slower to leach from soil, some projects, especially arable reversion can be blighted by this due to high levels of past phosphorus application. A P index of 0 is recommended as 'ideal' for grassland creation, 1 is 'satisfactory', 2 'marginal' and higher than this is deemed 'usually unsuitable' by the Natural England Technical Advice Note TIN066 (Natural England, 2010). However, other considerations should be considered, and additional soil factors taken into account, for example a high pH can reduce the availability of phosphorus to a plant (Bunt, 1983). It has been found that phosphorus applications of 100ppm and 200ppm to *L. corniculatus* significantly increased aerial dry weight and main stem length compared to no phosphorus application (Clua *et al.,* 2012), in addition another study also found low soil P limited *L. corniculatus* growth (Felderer *et al*., 2013).

Nitrate (NO_3^-)

An essential macro-nutrient which is mainly assimilated into organic nitrogen compounds by the plant roots before use (Taiz & Zeiger 1998). Major functions of plants aided by nitrogen translocation include synthesis of proteins and nucleic acid (Richardson, 1976). Plants need nitrogen in large amounts compared to other minerals (Taiz & Zeiger, 1998), however excess nitrate in the soil formed from fertilizer application can change species composition; diversity and richness in a restoration study was shown to decrease under annual nitrogen application whereas richness increased and diversity stabilised over time in soil with reduced soil nitrogen availability (Baer *et al.,* 2003). Nitrate does leach from soils easily (Killpack & Buchholz, 1993) and therefore levels decrease fairly quickly when appropriate management is implemented (Jacquemyn *et al*., 2003).

Potassium (K)
An essential macro-nutrient in plant nutrition, especially important for plant cell osmotic functions (Richardson, 1976), and as an activator of respiration and photosynthesis enzymes (Taiz & Zeiger, 1998). This is added regularly to improved agricultural land with nitrogen and phosphorus in the form of fertiliser.

Calcium (Ca)
An essential macro-nutrient required for cell-wall formation and translocation of amino acids and carbohydrates (Bunt, 1983). An easily leached element with which deficiencies can be shown by stunted growth and pale leaf margins (Bunt, 1983).

Organic Matter
Organic matter is formed by decomposition of plant and animal material, the major component is organic carbon which is highly important for all soil processes. Many factors influence the formation and loss rate of organic matter including human influenced activities such as land management and natural factors including climate and topography (European Commission, 2012[c]).

Sodium (Na)
Often more commonly high in soils of arid or coastal areas where rainfall isn't sufficient enough to leach sodium out of the soils (Taiz & Zeiger, 1998). Too much sodium can cause problems for plants and may be limited to mainly halophyte species such as *Armenia maritime* (Hill *et al.,* 1999).

Electrical Conductivity/Salinity (EC)
The ability of a soil solution to conduct electricity will indicate the relative amount of ions (dissolved salts) present (Bunt, 1983), such salts include but are not limited to sodium, calcium, magnesium, chloride, sulphate (European Commission, 2012[c]). A very low EC can indicate low nutrients and possibly limited microbial activity, whereas a very high EC can highlight faults such as excess fertiliser application or waterlogging (Capewell, 2013).

Soil Texture
The inorganic segment of a soil is the broken down fragments of rocks, weathered from the original bedrock such as granite or sandstone, the further broken down these become creates their horizon classifications such as gravel, sand, silt and clay. During this process soluble materials are also released (Department of Environment & Primary Industries - Victoria Australia, 1998). Soil texture has been found to be a primary control of vegetation cover and biogeochemical processes of the landscape (Hook & Burke, 2000).

APPENDIX III – Ecotype Soil Analysis Raw Data

Table III 1. pH and Cond.mS results of ecotype soils. Numbered ecotypes are those used in the main experiment.

	Replicates								
pH	**1**	**2**	**3**	**4**	**5**	**6**	**Median**	**Min**	**Max**
1. Cockey Down	7.51	7.39	7.60	7.47	7.46	7.44	7.47	7.39	7.60
2. Southstoke CA	7.35	7.47	7.33	7.39	7.47	7.49	7.43	7.33	7.49
3. Woodborough	7.37	7.41	7.48	7.47	7.40	7.37	7.41	7.37	7.48
4. Folly Farm	6.00	6.40	5.50	7.18	6.61	6.08	6.24	5.50	7.18
5. Hellenge Hill	5.96	6.43	6.17	6.67	6.57	6.87	6.50	5.96	6.87
6. Salisbury Plain	6.51	6.73	7.38	6.96	6.74	6.34	6.74	6.34	7.38
7. Berrow Dunes	7.40	7.41	7.43	7.44	7.29	7.49	7.42	7.29	7.49
8. Woolacombe	8.05	7.98	8.08	8.03	7.66	7.61	8.01	7.61	8.08
9. Dawlish Warren	7.65	7.75	7.96	7.54	7.42	7.72	7.69	7.42	7.96
Southstoke G	7.38	7.39	7.35	7.49	7.48	7.43	7.41	7.35	7.49
Fontmell	7.43	7.38	7.61	7.58	7.56	7.52	7.54	7.38	7.61
Goblin Combe	7.60	7.52	7.52	8.08	7.57	7.40	7.55	7.40	8.08
St.Catherines	5.31	5.55	5.59	5.86	5.88	5.34	5.57	5.31	5.88
Burledge Hill	5.45	6.10	6.65	5.57	5.55	5.50	5.56	5.45	6.65
Valley of Stones	4.96	5.26	5.41	5.27	5.29	5.12	5.27	4.96	5.41

Cond. mS	**1**	**2**	**3**	**4**	**5**	**6**	**Mean**	**St.Error**
1. Cockey Down	0.78	0.82	0.64	0.80	0.76	0.75	0.76	0.02
2. Southstoke CA	0.52	0.51	0.53	0.54	0.51	0.56	0.53	0.01
3. Woodborough	0.74	0.68	0.67	0.64	0.75	0.65	0.69	0.02
4. Folly Farm	0.17	0.30	0.15	0.33	0.28	0.24	0.24	0.03
5. Hellenge Hill	0.55	0.45	0.56	0.70	0.78	0.77	0.64	0.05
6. Salisbury Plain	0.36	0.44	0.47	0.47	0.41	0.32	0.41	0.02
7. Berrow Dunes	0.54	0.41	0.34	0.47	0.38	0.39	0.42	0.03
8. Woolacombe	0.33	0.30	0.25	0.55	0.59	0.40	0.40	0.05
9. Dawlish Warren	0.16	0.18	0.10	0.11	0.11	0.09	0.13	0.01
Southstoke G	0.75	0.64	0.69	0.68	0.67	0.61	0.67	0.02
Fontmell	0.62	0.57	0.57	0.56	0.56	0.56	0.57	0.01
Goblin Combe	0.61	0.68	0.72	0.60	0.67	0.74	0.67	0.02
St.Catherines	0.24	0.29	0.27	0.41	0.29	0.28	0.29	0.02
Burledge Hill	0.48	0.48	0.68	0.39	0.41	0.40	0.47	0.04
Valley of Stones	0.14	0.16	0.19	0.12	0.14	0.12	0.14	0.01

Table III 2. Nitrate, phosphate and organic matter results of ecotype soils. Numbered ecotypes are those used in the main experiment.

	Replicates							
N (PPM)	**1**	**2**	**3**	**4**	**5**	**6**	**Mean**	**St.Error**
1. Cockey Down	19.00	15.73	15.58	33.05	12.26	12.65	18.05	2.89
2. Southstoke C	26.60	36.58	21.11	13.33	12.74	9.41	19.96	3.83
3. Woodborough	10.16	32.08	12.41	14.60	22.79	16.33	18.06	3.02
4. Folly Farm	16.79	30.29	9.20	14.58	31.35	17.73	19.99	3.32
5. Hellenge Hill	14.53	16.90	18.47	24.89	15.89	15.75	17.74	1.39
6. Salisbury Plain	8.55	20.19	22.60	11.73	16.80	9.50	14.89	2.18
7. Berrow Dunes	16.87	8.78	6.64	14.44	9.36	8.71	10.80	1.47
8. Woolacombe	20.48	38.79	16.59	28.37	40.82	29.40	29.08	3.58
9. Dawlish Warren	13.81	9.28	1.77	4.36	3.39	4.10	6.12	1.69
Southstoke G	6.20	8.68	7.93	5.64	5.93	13.76	8.02	1.14
Fontmell	50.92	11.31	28.50	45.63	7.03	7.03	25.07	7.35
Goblin Combe	27.12	15.77	13.39	17.41	18.88	16.63	18.20	1.77
St.Catherines	6.10	6.76	8.81	28.53	27.36	8.30	14.31	3.96
Burledge Hill	10.52	27.77	27.08	14.60	12.17	15.33	17.91	2.82
Valley of Stones	5.95	6.55	8.24	6.54	29.19	7.97	7.05	0.36
P (PPM)	**1**	**2**	**3**	**4**	**5**	**6**	**Mean**	**St.Error**
1. Cockey Down	11.19	25.33	7.61	40.72	24.61	21.48	21.82	4.39
2. Southstoke CA	6.32	11.00	6.00	11.03	7.66	88.52	21.75	12.22
3. Woodborough	43.15	42.83	21.03	14.77	32.28	32.72	31.13	4.27
4. Folly Farm	2.03	3.43	3.64	6.21	6.00	4.07	4.23	0.60
5. Hellenge Hill	8.80	12.93	10.54	15.87	10.98	11.74	11.81	0.90
6. Salisbury Plain	3.10	3.53	3.96	3.75	4.60	3.53	3.75	0.19
7. Berrow Dunes	36.20	40.33	25.43	17.90	40.65	24.61	30.85	3.54
8. Woolacombe	0.00	9.09	2.87	42.11	2.39	8.35	10.80	5.87
9. Dawlish Warren	4.91	5.66	2.64	2.64	2.26	0.00	3.02	0.75
Southstoke G	33.04	45.11	35.54	17.45	13.87	23.71	28.12	4.42
Fontmell	11.96	16.17	25.36	25.36	12.92	8.13	16.65	2.69
Goblin Combe	13.42	27.74	35.35	29.53	10.74	17.83	22.43	3.67
St. Catherines	4.60	5.14	7.71	6.32	3.75	4.39	5.32	0.54
Burledge Hill	9.24	9.35	11.09	12.39	10.33	18.04	11.74	1.23
Valley of Stones	3.00	3.32	2.03	3.43	2.57	2.78	2.86	0.19
OM %	**1**	**2**	**3**	**4**	**5**	**6**	**Mean**	**St.Error**
1. Cockey Down	20.06	21.00	15.91	18.63	22.34	24.88	20.47	1.15
2. Southstoke CA	23.98	23.74	24.80	22.09	19.88	20.83	22.55	0.72
3. Woodborough	17.72	19.11	18.51	15.45	18.71	24.03	18.92	1.05
4. Folly Farm	10.14	14.19	14.37	13.25	15.79	15.18	13.82	0.75
5. Hellenge Hill	25.33	19.90	21.83	23.28	25.12	26.64	23.68	0.93
6. Salisbury Plain	18.20	19.03	19.35	21.97	25.82	22.83	21.20	1.08
7. Berrow Dunes	9.37	7.50	5.98	7.77	5.80	4.09	6.75	0.69
8. Woolacombe	1.80	1.55	1.67	1.37	3.48	6.83	2.78	0.79
9. Dawlish Warren	5.71	4.81	2.56	11.22	5.00	0.97	5.04	1.31
Southstoke G	25.52	24.03	25.78	22.85	23.18	24.31	24.28	0.44
Fontmell	24.10	26.71	25.02	20.40	24.73	17.66	23.10	1.26
Goblin Combe	18.16	20.65	20.52	16.82	18.06	21.17	19.23	0.66
St.Catherines	31.88	32.83	38.22	33.60	18.71	34.16	31.57	2.48
Burledge Hill	23.77	25.82	27.69	24.41	23.79	25.71	25.20	0.56
Valley of Stones	13.37	12.78	12.28	12.77	11.01	12.04	12.38	0.30

Table III 3. Potassium and sodium results of ecotype soils. Numbered ecotypes are those used in the main experiment.

	Replicates							
K (ppm)	**1**	**2**	**3**	**4**	**5**	**6**	**Mean**	**St.Error**
1. Cockey Down	43.40	53.45	35.80	49.70	52.25	61.35	49.33	3.28
2. Southstoke CA	298.57	220.10	178.45	131.31	204.49	117.67	191.77	24.55
3. Woodborough	100.90	82.40	67.05	53.60	64.20	72.35	73.42	6.14
4. Folly Farm	110.98	112.33	90.59	154.44	173.91	128.07	128.39	11.48
5. Hellenge Hill	214.80	223.20	156.20	342.45	171.75	202.40	218.47	24.57
6. Salisbury Plain	190.27	100.54	79.57	59.60	90.25	74.59	99.14	17.43
7. Berrow Dunes	115.70	34.50	53.65	33.20	43.45	48.55	54.84	11.49
8. Woolacombe	30.03	25.11	45.69	32.86	37.88	52.61	37.36	3.82
9. Dawlish Warren	44.90	97.25	41.10	64.95	38.40	29.75	52.73	9.22
Southstoke G	180.00	134.75	129.85	134.40	86.90	120.25	131.03	11.17
Fontmell	50.31	60.38	48.38	39.45	49.36	26.80	45.78	4.26
Goblin Combe	115.90	66.50	201.75	97.25	83.65	144.90	118.33	18.26
St.Catherines	136.50	116.37	116.58	148.90	85.27	127.95	121.93	8.13
Burledge Hill	373.00	317.20	230.05	319.85	156.65	114.85	251.93	37.96
Valley of Stones	81.61	69.00	65.24	32.31	52.83	48.96	58.33	6.45
Na (ppm)	**1**	**2**	**3**	**4**	**5**	**6**	**Mean**	**St.Error**
1. Cockey Down	10.40	11.24	23.70	24.57	24.07	28.21	20.37	2.82
2. Southstoke CA	21.90	23.32	22.43	21.90	17.05	30.06	22.78	1.56
3. Woodborough	14.74	15.06	10.95	10.87	15.25	15.75	13.77	0.83
4. Folly Farm	25.36	31.11	24.12	25.00	24.75	32.09	27.07	1.32
5. Hellenge Hill	22.46	20.34	23.63	20.31	23.34	18.88	21.50	0.72
6. Salisbury Plain	34.94	37.82	26.68	31.32	22.56	33.32	31.11	2.09
7. Berrow Dunes	17.61	21.70	15.74	17.49	18.80	14.91	17.71	0.90
8. Woolacombe	26.06	21.48	29.06	23.12	29.23	40.19	28.19	2.48
9. Dawlish Warren	35.75	55.87	19.11	45.47	44.81	31.51	38.75	4.78
Southstoke G	23.92	19.43	25.71	28.33	23.30	37.21	26.31	2.27
Fontmell	35.10	45.06	68.90	40.21	58.30	19.44	44.50	6.50
Goblin Combe	21.74	22.98	24.89	21.46	18.28	16.09	20.91	1.19
St.Catherines	45.47	44.31	50.16	62.65	27.19	41.88	45.28	4.30
Burledge Hill	37.99	35.94	33.81	40.52	47.46	41.77	39.58	1.80
Valley of Stones	35.02	40.36	48.68	38.07	30.97	32.59	37.61	2.39

Table III 4. Calcium results of ecotype soils. Numbered ecotypes are those used in the main experiment.

	Replicates							
Ca (ppm)	**1**	**2**	**3**	**4**	**5**	**6**	**Mean**	**St.Error**
1. Cockey Down	170211.50	208671.80	129766.40	151739.80	92712.22	54163.87	134544.27	20613.16
2. Southstoke CA	20304.61	27808.47	40236.61	61462.80	66903.59	50546.97	44543.84	6888.82
3. Woodborough	131804.60	130757.10	129271.60	187998.10	201656.90	171836.60	158887.48	12071.81
4. Folly Farm	992.77	2137.72	2586.03	4049.22	1385.99	12640.99	3965.45	1633.04
5. Hellenge Hill	4524.97	3119.65	1337.79	4084.74	7876.34	1931.37	3812.48	869.86
6. Salisbury Plain	3665.42	3366.63	4288.05	23652.94	3184.99	6698.18	7476.04	2992.03
7. Berrow Dunes	23910.71	27971.34	31587.51	22570.07	28624.34	24302.48	26494.41	1286.02
8. Woolacombe	38688.49	43331.48	37277.81	34386.38	36297.24	29236.42	36536.30	1742.63
9. Dawlish Warren	46379.90	127462.44	38619.11	2621.98	425.06	176060.52	65261.50	26530.48
S.Stoke g	93680.60	63486.67	64407.93	77467.27	71420.19	79880.89	75057.26	4204.56
Fontmell	7868.06	101444.40	98178.23	119591.70	94078.08	93971.07	85855.26	14670.94
Goblin combe	79413.94	49096.50	35929.64	42819.00	41912.32	207798.40	76161.63	24706.44
St.Catherines	5267.98	4796.61	2798.72	2777.90	1701.04	2498.78	3306.84	522.68
Burledge Hill	4170.21	2831.05	3054.66	3058.51	2912.16	10415.55	4407.02	1112.11
Valley of Stones	1004.14	461.65	185.14	1362.92	274.32	521.57	634.96	170.09

Table III 5. Particle analysis of ecotype soils

Site	Sample	% A	% B	% C	% <63µm & OM	From previous OM results: Potential % OM	From previous OM results: Potential % <63µm
1. Cockey Down	1	0.98	8.56	22.00	68.46	*20.06*	*48.40*
	2	2.26	8.89	17.70	71.15	*21.00*	*50.15*
	3	2.70	15.35	13.08	68.87	*15.91*	*52.97*
	4	5.87	9.77	23.08	61.28	*18.63*	*42.64*
	5	6.05	21.47	34.27	38.21	*22.34*	*15.87*
	6	4.54	17.89	33.12	44.45	*24.88*	*19.57*
2. Southstoke CA	1	1.41	4.16	22.83	71.60	*23.98*	*47.62*
	2	13.72	10.44	22.43	53.41	*23.74*	*29.67*
	3	1.67	9.32	18.23	70.78	*24.80*	*45.98*
	4	3.63	7.69	11.88	76.79	*22.09*	*54.70*
	5	5.03	7.89	29.93	57.14	*19.88*	*37.26*
	6	1.91	6.25	20.20	71.64	*20.83*	*50.81*
3. Woodborough	1	2.21	3.44	14.75	79.60	*17.72*	*61.88*
	2	2.96	5.87	16.05	75.12	*19.11*	*56.01*
	3	1.02	3.91	17.08	77.99	*18.51*	*59.48*
	4	1.38	1.92	17.96	78.75	*15.45*	*63.30*
	5	1.29	2.81	15.43	80.47	*18.71*	*61.76*
	6	5.79	9.76	20.54	63.91	*24.03*	*39.89*
4. Folly Farm	1	0.94	0.90	6.61	91.55	*10.14*	*81.41*
	2	0.61	1.70	8.72	88.97	*14.19*	*74.78*
	3	0.80	2.55	9.82	86.83	*14.37*	*72.46*
	4	0.58	2.21	11.69	85.52	*13.25*	*72.27*
	5	7.14	3.85	16.78	72.23	*15.79*	*56.43*
	6	0.40	10.07	16.10	73.42	*15.18*	*58.23*
5. Hellenge Hill	1	1.24	16.00	29.28	53.49	*25.33*	*28.15*
	2	1.33	9.71	21.98	66.97	*19.90*	*47.07*
	3	0.83	5.24	19.49	74.44	*21.83*	*52.61*
	4	0.32	11.11	17.88	70.69	*23.28*	*47.41*
	5	-0.10	10.35	28.04	61.71	*25.12*	*36.58*
	6	0.47	5.63	27.85	66.05	*26.64*	*39.41*
6. Salisbury Plain	1	10.94	5.43	21.25	62.38	*18.20*	*44.18*
	2	7.56	4.20	15.50	72.73	*19.03*	*53.70*
	3	7.11	6.60	21.28	65.01	*19.35*	*45.66*
	4	1.46	4.03	27.09	67.42	*21.97*	*45.45*
	5	4.13	3.10	11.55	81.22	*25.82*	*55.40*
	6	1.90	4.46	25.14	68.50	*22.83*	*45.67*
7. Berrow Dunes	1	0.07	4.66	88.06	7.22	*9.37*	*-2.16*
	2	0.03	1.65	95.95	2.37	*7.50*	*-5.12*
	3	0.18	1.51	86.85	11.46	*5.98*	*5.48*
	4	0.07	1.32	90.14	8.46	*7.77*	*0.69*
	5	0.22	2.92	91.74	5.12	*5.80*	*-0.68*
	6	0.02	1.49	93.98	4.51	*4.09*	*0.42*
8. Woolacombe	1	0.35	27.29	64.51	7.86	*1.80*	*6.06*
	2	1.05	50.13	43.84	4.98	*1.55*	*3.42*
	3	0.09	53.21	41.63	5.07	*1.67*	*3.40*

Site	Sample	% A	% B	% C	% <63µm & OM	From previous OM results: Potential % OM	From previous OM results: Potential % <63µm
	4	-0.01	59.48	38.42	2.11	*1.37*	*0.74*
	5	0.18	45.59	49.56	4.67	*3.48*	*1.19*
	6	-0.03	46.63	49.75	3.64	*6.83*	*-3.19*
9. Dawlish Warren	1	0.40	6.7129	89.78	8.82	5.71	3.11
	2	1.10	2.2672	94.72	6.73	4.81	1.92
	3	7.18	4.0993	88.18	3.10	2.56	0.54
	4	3.52	4.6292	90.07	13.00	11.22	1.78
	5	0.50	2.4064	93.61	8.48	5.00	3.48
	6	0.30	5.7154	90.58	4.38	0.97	3.41
Southstoke G	1	5.55	14.56	28.38	51.51	*25.52*	*25.98*
	2	3.80	11.84	21.76	62.61	*24.03*	*38.58*
	3	3.08	10.12	26.13	60.66	*25.78*	*34.89*
	4	5.39	16.00	26.32	52.28	*22.85*	*29.43*
	5	5.10	13.85	19.84	61.21	*23.18*	*38.02*
	6	3.10	7.42	23.65	65.83	*24.31*	*41.52*
Fontmell	1	11.74	16.18	34.61	37.47	*24.10*	*13.37*
	2	5.43	10.86	41.47	42.25	*26.71*	*15.54*
	3	4.26	14.95	31.59	49.20	*25.02*	*24.17*
	4	7.74	6.10	27.01	59.16	*20.40*	*38.76*
	5	5.12	9.98	38.43	46.46	*24.73*	*21.73*
	6	1.50	2.54	10.53	85.43	*17.66*	*67.77*
Goblin Combe	1	0.80	4.48	38.84	55.88	*18.16*	*37.72*
	2	0.00	3.57	29.02	67.42	*20.65*	*46.77*
	3	12.66	8.04	21.91	57.38	*20.52*	*36.87*
	4	2.98	3.87	19.35	73.80	*16.82*	*56.99*
	5	1.06	3.96	22.90	72.08	*18.06*	*54.02*
	6	0.19	3.52	18.25	78.05	*21.17*	*56.88*
St.Catherines	1	0.45	4.41	13.98	81.16	*31.88*	*49.28*
	2	-0.13	8.06	16.86	75.21	*32.83*	*42.37*
	3	0.36	0.19	9.55	89.90	*38.22*	*51.68*
	4	8.04	6.70	10.70	74.56	*33.60*	*40.96*
	5	0.36	0.19	32.32	67.14	*18.71*	*48.42*
	6	-0.26	6.67	23.82	69.77	*34.16*	*35.61*
Burledge Hill	1	0.72	2.22	11.01	86.05	*23.77*	*62.27*
	2	1.12	4.46	9.21	85.21	*25.82*	*59.38*
	3	-0.11	7.11	7.39	85.61	*27.69*	*57.93*
	4	0.19	3.50	11.75	84.56	*24.41*	*60.15*
	5	-0.19	4.00	12.61	83.59	*23.79*	*59.79*
	6	0.26	5.36	16.81	77.57	*25.71*	*51.86*
Valley of Stones	1	8.73	14.19	29.79	47.28	*13.37*	*33.91*
	2	10.07	19.57	28.34	42.03	*12.78*	*29.24*
	3	11.55	16.31	26.69	45.45	*12.28*	*33.17*
	4	15.28	19.36	27.85	37.51	*12.77*	*24.74*
	5	14.01	21.11	28.54	36.34	*11.01*	*25.33*
	6	13.96	22.38	32.48	31.18	*12.04*	*19.14*

APPENDIX IV – Seed Weights and Germination

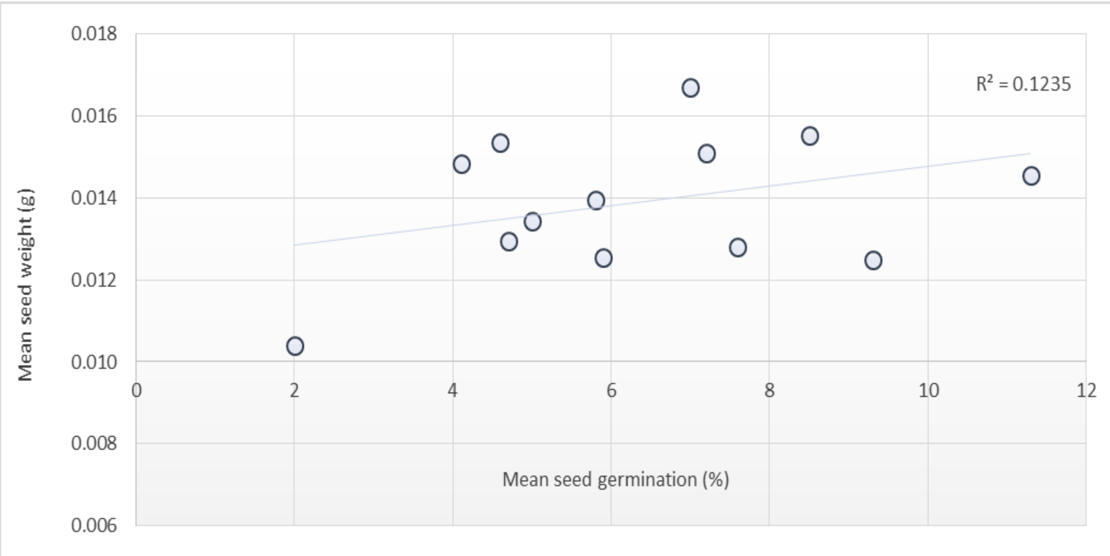
Table IV 1. Seed weights (n=10 seeds per replicate). Soil Key: C=calcareous loam, N=neutral loam, S=Calcareous sand. Management key: G=grazed, C=cut with aftermath grazing, U=unmanaged.

	Soil	Mgmt	Seed Weights (g)											
			1	2	3	4	5	6	7	8	9	10	Mean	SE
Cockey Down	**C**	**G**	0.017	0.014	0.012	0.015	0.016	0.013	0.014	0.019	0.021	0.015	**0.016**	0.001
Southstoke (cut)	**C**	**C**	0.010	0.012	0.009	0.014	0.013	0.014	0.013	0.017	0.014	0.014	**0.013**	0.001
Woodborough	**C**	**C**	0.013	0.012	0.015	0.016	0.016	0.012	0.015	0.010	0.010	0.016	**0.013**	0.001
Southstoke (grazed)	**C**	**G**	0.014	0.015	0.012	0.018	0.016	0.017	0.015	0.016	0.014	0.015	**0.015**	0.001
Folly Farm	**N**	**C**	0.006	0.009	0.017	0.013	0.013	0.013	0.012	0.015	0.017	0.013	**0.013**	0.001
Hellenge Hill	**N**	**G**	0.014	0.021	0.010	0.011	0.015	0.016	0.012	0.012	0.016	0.018	**0.015**	0.001
Salisbury Plain	**N**	**U**	0.013	0.014	0.009	0.013	0.015	0.014	0.015	0.015	0.017	0.014	**0.014**	0.001
Burledge Hill	**N**	**G**	0.015	0.014	0.021	0.017	0.018	0.015	0.017	0.015	0.018	0.018	**0.017**	0.001
St. Catherine's	**N**	**G**	0.014	0.013	0.016	0.012	0.014	0.011	0.016	0.011	0.011	0.006	**0.012**	0.001
Valley of Stones	**N**	**G**	0.010	0.013	0.017	0.010	0.005	0.009	0.013	0.015	0.005	0.007	**0.010**	0.001
Berrow Dunes	**S**	**U**	0.009	0.012	0.015	0.016	0.019	0.018	0.010	0.012	0.016	0.017	**0.015**	0.001
Woolacombe Warren	**S**	**U**	0.015	0.015	0.016	0.017	0.012	0.013	0.014	0.013	0.017	0.017	**0.015**	0.001
Dawlish Warren	**S**	**G**	0.015	0.009	0.013	0.010	0.011	0.013	0.013	0.014	0.014	0.013	**0.013**	0.001

Table IV 2. Seeds germinated per replicate. (At least 18 seeds were sown per replicate). Soil Key: C=calcareous loam, N=neutral loam, S=Calcareous sand. Management key: G=grazed, C=cut with aftermath grazing, U=unmanaged.

			Seeds Germinated per replicate												
Ecotype	**Soil**	**Mgmt**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**Total**	**Mean**	**SE**
Cockey Down	**C**	**G**	13	7	9	12	8	4	7	8	13	4	**85**	**9**	1
Southstoke (cut)	**C**	**C**	9	0	5	5	10	6	6	1	5	0	**47**	**5**	1
Woodborough	**C**	**C**	7	3	6	9	10	9	3	1	2	0	**50**	**5**	1
Southstoke (grazed)	**C**	**G**	8	7	10	7	12	8	1	10	4	5	**72**	**7**	1
Folly Farm	**N**	**C**	6	2	7	5	4	10	8	12	15	7	**76**	**8**	1
Hellenge Hill	**N**	**G**	3	15	11	7	13	16	14	14	16	4	**113**	**11**	2
Salisbury Plain	**N**	**U**	3	7	7	7	6	5	10	10	1	2	**58**	**6**	1
Burledge Hill	**N**	**G**	12	9	9	10	2	7	6	10	4	1	**70**	**7**	1
St. Catherine's	**N**	**G**	14	6	18	11	11	6	16	10	0	1	**93**	**9**	2
Valley of Stones	**N**	**G**	1	5	5	2	1	0	0	0	5	1	**20**	**2**	1
Berrow Dunes	**S**	**U**	0	0	7	2	7	3	10	4	11	2	**46**	**5**	1
Woolacombe Warren	**S**	**U**	9	6	5	6	1	3	7	3	0	1	**41**	**4**	1
Dawlish Warren	**S**	**G**	3	3	2	1	5	1	15	19	6	4	**59**	**6**	2

Figure IV 1. Scatter graph to compare mean dry seed weights (g) (taken from 10 seeds per ecotype replicate) with germination mean (taken from 18 seeds per ecotype replicate).



APPENDIX V – Detailed Descriptions of Chosen Ecotype Sites.

Cockey Down

Overview and History

Cockey Down is a chalk downland escarpment overlooking the city of Salisbury (3km NE of Salisbury) in Wiltshire. Part of this site has had SSSI status since 1971 (Natural England, 1975[a]), with the south section privately owned (Magic, 2013) and the north, a Wiltshire Wildlife Trust (WWT) reserve since it was acquired by them in the 1970's (WWT, 2003[b]). The site is an unimproved escarpment of herb-rich chalk grassland including calcicoles such as *Sanguisorba minor* Scop. and *Anacamptis pyramidalis* L., other herbs present during the sampling included *Galium verum* L., *Knautia arvensis* L., *Leontodon hispidus* L. and *Campanula rotundifolia* L. With *Bromus erectus* Huds. becoming a dominant grass in places. Arable land surrounds all sides of this area of chalk grassland.

Figure V 1. Cockey Down. Looking south-west across the reserve (Source: Wiltshire Wildlife Trust, 2013)

Site Characteristics

This high chalk plain (Land Use Consultants, 2005) consists of rendzina soils of the Andover 2 series (MSC team, undated *in* WWT, 2003[b]), identified as Soilscape 3; shallow lime-rich soils with free drainage (Magic, 2013), over-lying chalk sedimentary bedrock with flints, formed in the cretaceous period (NERC, 2013). The soils over the strip lynchets are more nutrient-rich in places where they are deeper (WWT, 2003[b]), the steep gradient (Magic, 2013) would have limited historical improvement. Soil sample results (Chapter 6) confirmed site soils as being a calcareous loam with a median pH of 7.45 and one of the highest mean calcium values (134544ppm) reflecting the chalk bedrock. The National Character Area (NCA) is 132. Salisbury Plain and West Wiltshire Downs is shared with the neutral loam site 'Salisbury Plain' which also contains this chalk bedrock (Natural England, 2013[e]).

Grassland Management

Cockey Down is grazed extensively, principally by cattle. The management prior to 1971 is unclear, but it is thought likely that cattle grazing would have been predominant as the previous owner was a dairy farmer (WWT, 2003[b]; Natural England, 2012[g]). In the Natural England SSSI condition assessment (Natural England, 2012[g]) the site is classified as 'unfavourable recovering', due to encroaching scrub which is currently being tackled. Although the grassland has a long history of grazing, the scrub encroachment may have occurred due to grazing inconsistencies in the past at the north (WWT) end due to access restrictions. However, it appears there has been some form of grazing at least annually here. Prior to 1988 the land was primarily cattle-grazed, winter grazing by sheep was introduced in 1988 and lasted until 1994, then cattle grazing was resumed until 1996 when ponies also grazed for two months. Since then it has predominantly reverted back to cattle, except a short period in 2003 when six horses grazed there (WWT, 2003[b]).

Southstoke

Overview and History

The site is within a small village, approximately two miles south of Bath. The fields are part of the Manor Farm holding at Southstoke and are currently managed under Higher Level Stewardship (Magic, 2013). The field sampled had a grass-dominated semi-improved sward situated within a gently sloping valley with a north facing aspect.

Figure V 2. Southstoke (cut & aftermath). Looking at the sample site from the north. (Source: Author, 2009)

Site Characteristics

Situated within the Cotswolds NCA, the geology here is [formed] from the Great Oolite Group and lies between Fuller's Earth Formation Mudstone and Chalfield Oolite Formation Limestone (Natural England, 2013[b]; NERC, 2013). The soilscape number of 9, indicates lime-rich loamy and clayey soils with impeded drainage (Magic, 2013). From the soil sampling reported in Chapter 6 it was found that this was a calcareous loam with a median pH of 7.43, high mean organic matter (22.55%). Although it had the lowest mean calcium content (44543ppm) within the calcareous soils, this was still a high amount compared to the neutral soils.

Grassland Management

For at least 10 years before the seed collection was made the field had been managed as a hay cut in July/August with aftermath grazing by cattle until late October (Thompstone, 2009).

Woodborough Hill

Overview and History

This site is a semi-improved grassland hilltop situated in the Pewsey Vale, privately owned and entered into Higher Level Stewardship (Magic, 2013). Part of the site is accessed by a public footpath and commonly receives visitors interested in the crop circles often seen in the surrounding arable land. Strip lynchets, unimproved rough grassland, a small woodland hanging and an old chalk pit dominate the south side of the hill which is approximately 205m above sea level, the grassland slopes cover the north, west and east facing sides of the hill (Magic, 2013). The survey area has a grass-dominated sward, with frequent *Bromus erectus* Huds., but also sedges and agricultural grasses such as *Lolium perenne* L. with occasional herbs including *Sanquisorba minor* Scop., and *Galium verum* L.

Figure V 3. Woodborough Hill, view towards south-west side of hill.
(Source: Author, 2009)

Site Characteristics

This site lies within 116 Berkshire and Marlborough Downs NCA (Natural England, 2013[c]) and is part of the Greensand Vale, in the Vale of Pewsey (Landuse Consultants, 2005). This area is part of the Soilscape 3 ‘Shallow lime-rich soils’, freely-draining (Magic, 2013), over a bedrock of the Grey

Chalk subgroup, described as a clayey chalk without flints (NERC, 2013). During the sampling it was noticed that the soil became deeper nearer the top of the hill. The soil sample results indicated this to be calcareous loam with a median pH of 7.41, and contained the highest calcium content of the chosen sites (mean 158887ppm).

Grassland Management

This hilltop is managed by taking a hay cut in July followed by cattle grazing of the re-growth until late October (Carson, 2009; Smart, 2009). As this site had the highest phosphate level of the chosen sites it is thought this may be reflected in higher levels of farm yard manure (FYM) application during the winter than the other ecotype sites.

Folly Farm

Overview and History

Folly Farm originated as a medieval deer park, later divided into farmland and in the late 18th century converted into a *ferme ornée.* The estate was later owned by Sutton Court and managed by a succession of tenant farmers until 1987 when the estate was dismantled and Avon Wildlife Trust gained ownership (Avon Wildlife Trust, 1999; 2012[a]), part of the site is now designated as SSSI. The two fields sampled ['Great Wall Close and 'Plain Hill'] are non-SSSI grassland and have a south-west facing aspect, approximately 160m above sea level (Magic, 2013). These fields are semi-improved neutral grassland with a grass-dominated sword and frequent *Ranunculus repens* L. but more species-rich areas occur with *Carex flacca* Schreber, *Centaurea nigra* L., and *Leucanthemum vulgare* Lam.

Figure V 76. Folly Farm. The more northerly field of the two, looking east. (Source: Author, 2009)

Site Characteristics

Neutral soils are formed here from a bedrock of the Mercia Mudstone Group, a sedimentary rock formed in the Triassic period, part of the New Red Sandstone Supergroup (NERC, 2013). With a soilscape of 8 this area is known to have a marginally acidic clay loam soil with slightly impeded natural drainage. Soil sample results indicated this as having the lowest median pH (6.24) of the chosen ecotypes, it also showed lowest levels of phosphate (mean 4.23ppm), possibly an indicator of the organic status of the site. The slopes and valleys of this site as well as the impeded drainage are characteristic of the Bristol Avon & Valleys NCA in which it is situated.

Grassland Management

Avon Wildlife Trust manage the farm with the aid of a tenant grazier, the two meadows sampled are situated on the north-east side of the reserve. These are cut for hay and aftermath grazed by cattle (Glazebrook, 2009[a]; Glazebrook, 2009[b]; Avon Wildlife Trust, 2012[b]). The current Organic Environmental Stewardship agreement stipulates that cutting should be done after 15th July and aftermath grazing at low density until 31st October, application of fertilizers or organic manures are not permitted (Haplin, 2013).

Although the current stewardship agreement didn't start until 2009 the cut and aftermath grazing was in place prior to this (Glazebrook, 2009[a]). It is likely the same requirements would have been met as the management regime has been undertaken with a view to restore the semi-improved grasslands to a more species-rich sword (Avon Wildlife Trust, 1999). The extent of past management is unclear. The previous Avon Wildlife Trust management plan (1999) states the grassland has been sheep-grazed since entry into a Countryside Stewardship Agreement in 1992, however, the management plan list of fields included doesn't mention either of those sampled. It is thought that when the first Countryside Stewardship Agreement ended in 2002 (Avon Wildlife Trust, 1999), the cut and aftermath grazing (if not already in place) would have started shortly after. There is also evidence of a long history of haymaking at Folly Farm (though actual fields are uncertain) from resident recollections back to 1940 (Brain, 1994; Avon Wildlife Trust, 2012[a]).

Hellenge Hill

Overview and History

Hellenge Hill was purchased in 1998 by the Avon Wildlife Trust (Avon Wildlife Trust, 2011), it largely comprises calcareous unimproved grassland and scrub, although the main area sampled followed permissive guidelines (Glazebrook, 2009[a]) and therefore was concentrated on the deeper soils nearer the north of the reserve which consisted more of semi-natural grassland. The sample area had a shortly-cropped sward and more tussocky patches with a south-westly facing aspect, approximately 100m above sea level (Magic, 2013). The site is predominantly surrounded by semi-improved and unimproved grassland and scrub. During the sampling it was noted that the soil was very shallow, and cattle were present.

Figure V 5. Hellenge Hill, looking south from the Roman Road.
(Source: Langford Evangelical Church, 2013)

Site Characteristics

The bedrock geology is an underlying Black Rock Limestone Subgroup, part of the Pembroke Limestone Group (NERC, 2013), and which would influence areas of calcareous grassland such as that present on site. Although part of the soil sample was collected from areas mapped as BAP calcareous grassland (Magic, 2013) the neutral pH results instead reflect the character of the deeper soils nearer the northern end of the site. As the site had a soilscape of 7 this may also explain the lower than expected pH (median 6.5) as this soilscape is often slightly acidic, but base-rich and freely-draining (Magic, 2013). In addition, the site is situated within the Mendip Hills NCA which has a mosaic of calcareous, neutral and acid habitats due to the underlying limestone and sandstone geology (Natural England, 2013[h])

Grassland Management

Since the Avon Wildlife Trust bought the reserve it has been grazed mainly by cattle but also horses and more recently sheep as well (Avon Wildlife Trust, 2001; Glazebrook, 2009[b]; Martin, 2009[b]). Grazing is carried out for a period of at least 10 weeks annually, between 1st June and 28th February. Grazing is regulated to remove grass growth without poaching to achieve areas of closely-grazed turf scattered with taller tussocks. The northern area of the reserve where sampling was focused is semi-improved, interpretation from the management plan (2001) is that this partial agricultural improvement is possibly from historical management of overwintering cattle here. The site does have some scrub encroachment which is thought to be due to undergrazing in the past and therefore, since the site was sampled, the grassland has been topped and the grazing increased (Avon Wildlife Trust, 2011).

Salisbury Plain

Overview and History

Salisbury Plain Military Training Area in south Wiltshire was bought by the Ministry of Defence over a long period between 1897 and 1933 (Clarke-Smith, 1969). It is split between schedule I (agriculturally-improved) and schedule III (unimproved) land, the latter generally being within the live firing ranges and only allowed to be grazed by tenant farmers under tight restrictions (Clarke-Smith, 1969). Due to the army activity on the plain a large area has been retained and conserved for wildlife. The movements of tanks and other military activity disturbing the ground occasionally can also create microhabitats suitable for specialist species such as the fairy shrimp (*Chirocephalus diaphanous)* which exists in temporary ponds created by tank tracks (Natural England, 1975[b]). The sample area had an elevation of approximately 98m with a flat aspect. Vegetation here was tussocky with areas of thick thatch from *Festuca rubra* L. dominated grasses and sedges. Tall ruderals including *Heracleum sphondylium* L. and *Pastinaca sativa* L. were present, as well as frequent *Plantago lanceolata* L, and occasional *Helianthemum nummularium* Mill. Although on neutral soil, also present were the calcicoles; *Sanquisorba minor* Scop., and *Filipendula vulgaris* Moench.

[Image redacted in this digitized version due to potential copyright issues]

Figure V 6. Biodiversity Action Plan (BAP) Calcareous Grassland map of sample area and surrounding land on Salisbury Plain to illustrate extent of grassland mosaic. Green overlay indicates calcareous grassland, uncoloured areas are agriculturally-improved or neutral grassland. The red circle shows the sample area. (Source: Magic, 2013)

Figure V 7. Salisbury Plain sample area, looking south. (Source: Author, 2009)

Site Characteristics

Salisbury Plain is situated on the White Chalk subgroup of sedimentary bedrock (NERC, 2013) as also shown from the 132 (Sailsbury Plain and West Wiltshire Downs) NCA within which it is situated (Natural England, 2013[e]) and forms the largest area of chalk grassland in north-west Europe (Natural England, 1975; 2013[f]), however, there is also a mosaic of more neutral soils present. This is often as a result of past or present agricultural improvement but also due to environmental factors such as alluvial deposits when situated in valley bottoms (Natural England, 2013[e]). The site appeared to be situated in a dry river valley (‘Bourne Bottom’) which may have been historically connected to the winterbourne ‘Nine Mile River’ approximately 1km south-east (Magic, 2013). This could indicate the low median pH of 6.74, and low mean calcium level (7476ppm) where alluvial drift has neutralised the soil (Ahmad, 2011), masking the chalk bedrock. Alluvial deposits have also been shown to decrease soil phosphate levels (Ahmad, 2011) which could be the reason for such a low mean here (3.75ppm). Soilscape of this area is classed as 3 which is thought to reflect the typical calcareous soils surrounding this location, rather than the small areas of neutral influence.

Grassland Management

Historically, the high number of rabbits and sheep grazing on Salisbury Plain created the shortly-cropped chalk grassland, of which large remnants still remain (Natural England, 2013[e]). However, factors such as the myxomatosis outbreak in the mid 1950s (Ash, *et al.,* 2005) [which decreased the rabbit population] resulted in large areas becoming unmanaged as has happened within this sample area. Small amounts of scattered scrub present indicated that grazing here at least in the recent past has been minimal.

Figure illustrates the extent of calcareous grassland mosaics on Salisbury Plain and shows the small pockets of either agriculturally-improved or neutral land.

Berrow Dunes

Overview and History

Situated on the Somerset coast, the Sedgemoor District Council-owned Local Nature Reserve lies within the larger Berrow Dunes SSSI. The site was sampled within the area of stable dune grassland, dominated by *Festuca rubra* L., also present were tall sedges, *Ononis repens* L. and *Galium verum* L. The site is 5m above sea level and surrounded by coastal habitats, a holiday park and golf course and therefore receives high levels of human disturbance.

Figure V 8. Berrow Dunes.
(Source: Author, 2009)

Site Characteristics

The geology here is part of the Charmouth Mudstone Formation of the Lias parent unit (NERC, 2013) which helps form the lime-rich sand dune soils (Magic, 2013), giving the area a soilscape of 4 (Sand Dune Soils). The median pH was 7.42 with slightly higher organic matter of the three sandy soils though still low compared to the other chosen soils (mean 6.75%), it also had one of the highest phosphate means (30.85ppm). The descriptive features of the NCA classification (Somerset Levels and Moors) within which this site lies, encompasses the lowland flat landscapes found inland of this area, reflecting little landscape character of this coastal site.

Grassland Management

There is no formal grazing or cutting management at this site. Some of the fixed dune system here was grazed by cattle and sheep historically but this practice ceased during the beginning of the twentieth Century. Management here is now limited to chemical treating and cutting back and clearance of invasive vegetation, as well as grazing by high numbers of rabbits (Sedgemoor District Council, undated).

Woolacombe Warren

Overview and History

These sand dunes are situated on the north Devon coast and are owned and managed by the National Trust. On a steep climb from the top of the grey dunes the site is approximately 20m above sea level at no specific aspect. Above (east) is 'Marine Drive' a road used as a public car park and then Woolacombe Down and below (west) is the long beach stretching three miles from Woolacombe to Putsborough (Magic, 2013). The dunes are used by holiday makers, dog walkers and horse riders. The sample site was a mosaic of long grassy areas with *Ammophila arenaria* L., and *Festuca rubra* L., and shorter, rabbit grazed areas with herbs including *Hypochaeris radicata* L., *Rubia peregrina* L., *Thymus polytrichus* Kern., and *Ameria maritima* Mill.

Figure V 9. Woolacombe Warren.
(Source: Author, 2009)

Site Characteristics

This area is part of the Pickwell Down Sandstones Formation with the parent unit of Aeolian Deposits (NERC, 2013). This, together with the breakdown of coastal deposits, has formed this series of calcareous grey dunes, the alkalinity of which was the highest of the sites tested (median pH 8.01) and in sharp contrast to much of the rest of the Exmoor NCA in which Woolacombe lies (Natural England, 2012[e]). The dry course sand here as shown in the particle analysis (mean 47.05%) is also reflected in the lowest organic matter content (mean 2.78%) and indicates the semi-successional stage these dunes are in, at an earlier stage than the sample sites of Berrow and Dawlish. Soilscape here is 14 (freely-draining very acid and loamy soils) which does not match the highly calcareous pH of this coastal sandy soil.

Grassland Management

Historically these dunes were used to train troops during the Second World War, especially for the D-Day landings, after which the north dunes were badly damaged. Therefore *Ammophila arenaria* L., was planted with some areas fenced off to try and decrease dune erosion and encourage natural vegetation growth once again (North Devon Coast AONB, 2013). The only management now present is occasional scrub clearance and rabbit grazing (North Devon Biosphere, 2011).

Dawlish Warren

Overview and History

Dawlish Warren NNR is a small seaside family resort of south Devon with a mixture of important habitats including shifting dunes, fixed dunes, salt marsh and humid dune slacks (Teignbridge District Council, undated). The sample site was part of the humid dune slacks, 5m above sea level, a flat aspect, adjacent to a large pond surrounded by *Salix caprea* L. During the time of sampling it was noted that vegetation had tall, tussocky patches with frequent *Juncus effusus* L,. and *Mentha aquatica* L. reflecting the more waterlogged aspect here.

Figure V 10. Dawlish Warren.
(Source: Author, 2009)

Site Characteristics

During sampling it was noted that soil here was shallow and damp. It is thought that coastal features such as periodic flooding, desiccation and salt spray of this site could account for the highest sodium level of the ecotype soils (mean 38.75ppm) (Castillo *et al.,* 1991). Additionally, the low organic matter content (mean 5.04%) is typical of dune slack formation (Roulston, 2003) which in turn would account for the lowest amounts of nitrate (mean 6.12ppm) and phosphate (mean 3.02ppm). Soilscape of this area is classed as 4 (sand dune soils) as expected. The area lies within the Exeter Group of sandstone and subordinate Breccia sedimentary bedrock, which is part of the New Red Sandstone Supergroup (NERC, 2013). The NCA (Devon Redlands) landscape description here focuses on more inland features influenced by underlying red sandstone and agriculture, though sand dunes are mentioned as an important habitat of the character landscape (Natural England, 2013[j]).

Grassland Management

The sample area had been grazed with Dartmoor ponies in the winters only since October 2004 (Chambers, 2009; Grazing Animals Project, 2009).

APPENDIX VI - Analysis of Treatment Soils Raw Data

Chemical Analysis of Treatment Soils

Table VI 1. Chemical analysis of treatment soils

		Replicates								
		1	2	3	4	5	6	Median	Min	Max
pH	Win Green	7.22	7.08	7.14	7.09	7.20	7.33	7.17	7.08	7.33
	Avis	5.47	5.48	5.59	5.65	5.75	6.32	5.62	5.47	6.32
	Woolacombe	7.87	7.78	7.76	7.59	7.86	7.79	7.79	7.59	7.87
	Silk Hill*	8.24	8.02	8.15	8.22	8.28	8.25	8.23	8.02	8.28
		1	2	3	4	5	6	Mean	St.Error	
Cond.mS	Win Green	0.23	0.17	0.15	0.17	0.18	0.17	0.18	0.01	
	Avis	0.03	0.07	0.05	0.06	0.12	0.10	0.07	0.01	
	Woolacombe	0.09	0.09	0.10	0.09	0.09	0.14	0.10	0.01	
	Silk Hill*	0.28	0.17	0.21	0.18	0.17	0.18	0.20	0.02	
N (ppm)	Win Green	40.09	30.18	36.11	48.85	35.51	41.94	38.78	2.39	
	Avis	11.42	4.10	3.86	4.58	6.37	5.57	5.98	1.05	
	Woolacombe	1.54	1.56	4.78	5.57	1.54	1.55	2.76	0.70	
	Silk Hill	42.09	30.31	29.83	22.86	15.74	19.03	26.64	3.55	
P (ppm)	Win Green	26.01	20.26	14.07	19.19	23.24	15.67	19.74	1.67	
	Avis	10.87	14.50	15.99	14.07	15.78	15.03	14.37	0.69	
	Woolacombe	1.04	2.77	1.79	1.79	0.81	1.27	1.58	0.26	
	Silk Hill*	26.79	34.34	31.70	31.70	21.13	36.98	30.44	2.12	
OM %	Win Green	27.18	27.55	30.89	31.31	31.64	31.05	29.94	0.75	
	Avis	13.57	13.34	15.90	14.81	12.24	12.44	13.72	0.53	
	Woolacombe	0.65	0.60	0.95	0.83	0.61	0.69	0.72	0.05	
	Silk Hill*	13.06	13.40	12.35	12.44	13.16	11.95	12.73	0.21	
K (ppm)	Win Green	100.60	84.70	57.30	49.20	51.35	54.85	66.33	7.89	
	Avis	60.50	92.00	69.25	69.65	72.30	69.00	72.12	3.92	
	Woolacombe	20.20	18.75	15.65	14.65	19.75	18.50	17.92	0.84	
	Silk Hill*	85.05	74.15	71.55	83.95	76.30	74.35	77.56	2.09	
Na (ppm)	Win Green	18.00	16.23	23.45	26.23	23.70	24.32	21.99	1.47	
	Avis	22.14	23.80	24.54	27.35	23.19	23.37	24.06	0.67	
	Woolacombe	23.66	22.18	20.60	19.81	21.11	21.24	21.44	0.50	
	Silk Hill*	12.23	13.22	13.76	13.09	12.35	13.55	13.03	0.23	

7.29 Table VI 2. Calcium results of treatment soils

Ca (ppm) 11628.42	1	2	3	4	5	6	Mean	St.Error
Win Green 30715.24	18772.18	23954.82	11284.20	14134.45	9866.02	11221.32	14872.16	2037.92
Brigmerston*	223297.56	409956.48	338097.24	360998.76	422175.12	453942.48	368077.94	7.29
Avis Meadow	503.21	505.26	489.91	452.27	476.42	484.10	485.20	11628.42
Woolacombe	92362.85	83912.93	11129.63	80163.71	89221.25	88504.75	74215.85	30715.24

NB. * indicates a second calcareous site which was considered at this point but not used

Table VI 3. Particle analysis of treatment soils.

Site	Sample	% A	% B	% C	% <63µm & OM	From previous OM results: Potential % OM	From previous OM results: Potential % <63µm
1. Wingreen	1	1.96	0.83	90.22	2.76	6.12	8.88
Calcareous loam	2	1.84	0.40	90.49	2.54	6.47	9.01
	3	1.71	1.53	91.33	2.69	4.58	7.27
	4	1.87	0.59	89.68	2.71	7.01	9.72
	5	1.72	0.58	92.03	2.51	4.87	7.38
	6	2.35	0.68	88.89	2.69	7.23	9.92
mean		**1.91**	**0.77**	**60.50**	**36.82**	**29.94**	**6.88**
2. Avis Meadow	1	1.80	1.34	82.97	1.91	13.29	15.20
Neutral loam	2	2.33	1.28	86.04	1.47	9.90	11.37
	3	1.74	2.03	82.43	3.46	12.70	16.16
	4	2.67	1.63	83.58	1.71	11.58	13.29
	5	1.62	1.31	85.68	1.60	10.89	12.49
	6	1.77	1.42	85.74	1.49	10.60	12.09
mean		**1.99**	**1.50**	**70.69**	**25.82**	**13.72**	**12.11**
3. Woolacombe	1	0.07	10.25	89.06	0.09	0.59	0.68
Calcareous sand	2	0.08	11.22	87.60	0.05	1.09	1.14
	3	0.04	9.85	89.26	0.07	0.83	0.90
	4	0.03	10.19	88.94	0.11	0.80	0.91
	5	0.03	6.87	91.57	0.07	1.51	1.58
	6	0.02	8.40	90.62	0.10	0.94	1.04
means		**0.04**	**9.46**	**88.79**	**1.71**	**0.72**	**0.98**

APPENDIX VII – Glasshouse Layout

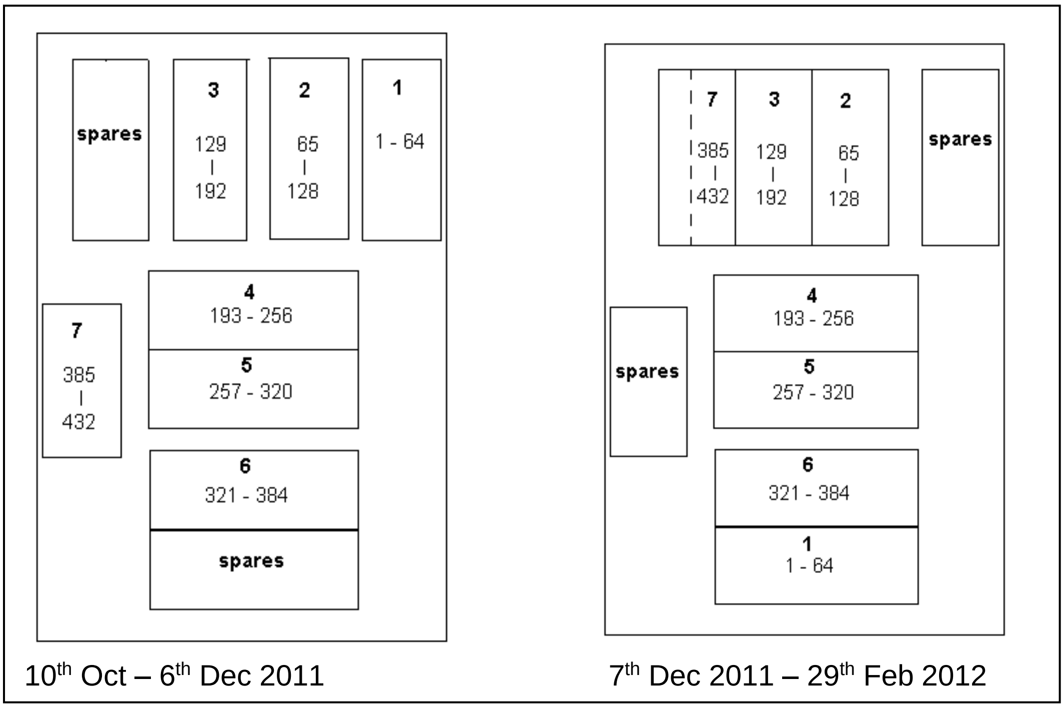


Figure VII 1. Glasshouse bench lay-out. Bold numbers are 'Bench numbers', the other numbers refer to plant numbers.

APPENDIX VIII – Glasshouse Randomisation

Table VIII 1. Randomisation generated in R, used in location of glasshouse plants. Mgmt (management) key: G=grazed, U=Unmanaged. Soil key: C=calcareous loam, N=neutral loam, S=calcareous sand. See Table 17 in Chapter 13 for ecotype key.

Mgmt	Soil	Eco-type	Rep	R original code combination	Plant no.
G	C	wb	1	1,"1","AG","3"	1
U	C	bd	1	2,"1","ANG","7"	2
U	N	wb	1	3,"1","BNG","3"	3
G	N	cd	1	4,"1","BG","1"	4
U	C	sp	1	5,"1","ANG","6"	5
U	N	ww	1	6,"1","BNG","8"	6
G	N	ww	1	7,"1","BG","8"	7
G	N	ss	1	8,"1","BG","2"	8
G	S	dw	1	9,"1","CG","9"	9
U	N	hh	1	10,"1","BNG","5"	10
U	N	ff	1	11,"1","BNG","4"	11
G	S	sp	1	12,"1","CG","6"	12
U	C	hh	1	13,"1","ANG","5"	13
G	S	ff	1	14,"1","CG","4"	14
U	S	dw	1	15,"1","CNG","9"	15
G	C	sp	1	16,"1","AG","6"	16
G	C	ww	1	17,"1","AG","8"	17
U	S	hh	1	18,"1","CNG","5"	18
G	N	dw	1	19,"1","BG","9"	19
G	C	ss	1	20,"1","AG","2"	20
G	S	cd	1	21,"1","CG","1"	21
G	C	ff	1	22,"1","AG","4"	22
U	C	dw	1	23,"1","ANG","9"	23
U	S	bd	1	24,"1","CNG","7"	24
G	S	hh	1	25,"1","CG","5"	25
U	C	ff	1	26,"1","ANG","4"	26
G	N	sp	1	27,"1","BG","6"	27
U	C	cd	1	28,"1","ANG","1"	28
G	C	dw	1	29,"1","AG","9"	29
U	N	sp	1	30,"1","BNG","6"	30
U	N	cd	1	31,"1","BNG","1"	31
U	S	cd	1	32,"1","CNG","1"	32
G	C	hh	1	33,"1","AG","5"	33
U	C	ss	1	34,"1","ANG","2"	34
U	S	wb	1	35,"1","CNG","3"	35
G	C	cd	1	36,"1","AG","1"	36
U	S	ff	1	37,"1","CNG","4"	37
U	N	dw	1	38,"1","BNG","9"	38
G	S	bd	1	39,"1","CG","7"	39
U	N	bd	1	40,"1","BNG","7"	40
U	C	wb	1	41,"1","ANG","3"	41
U	N	ss	1	42,"1","BNG","2"	42
G	C	bd	1	43,"1","AG","7"	43
U	S	ww	1	44,"1","CNG","8"	44
G	N	hh	1	45,"1","BG","5"	45
G	S	ss	1	46,"1","CG","2"	46
G	S	ww	1	47,"1","CG","8"	47
G	N	wb	1	48,"1","BG","3"	48
U	C	ww	1	49,"1","ANG","8"	49
U	S	ss	1	50,"1","CNG","2"	50
G	N	bd	1	51,"1","BG","7"	51
G	S	wb	1	52,"1","CG","3"	52
G	N	ff	1	53,"1","BG","4"	53
U	S	sp	1	54,"1","CNG","6"	54
U	N	bd	2	55,"2","BNG","7"	55
G	S	sp	2	56,"2","CG","6"	56
G	S	hh	2	57,"2","CG","5"	57
G	C	bd	2	58,"2","AG","7"	58
G	N	ff	2	59,"2","BG","4"	59
G	S	ss	2	60,"2","CG","2"	60
G	S	wb	2	61,"2","CG","3"	61
U	S	cd	2	62,"2","CNG","1"	62
G	S	cd	2	63,"2","CG","1"	63
U	C	ww	2	64,"2","ANG","8"	64
G	S	ff	2	65,"2","CG","4"	65
G	C	dw	2	66,"2","AG","9"	66
G	N	dw	2	67,"2","BG","9"	67
G	C	ww	2	68,"2","AG","8"	68
U	S	dw	2	69,"2","CNG","9"	69
U	N	sp	2	70,"2","BNG","6"	70
U	S	bd	2	71,"2","CNG","7"	71
U	C	hh	2	72,"2","ANG","5"	72
U	N	wb	2	73,"2","BNG","3"	73
G	N	cd	2	74,"2","BG","1"	74

Mgmt	Soil	Eco-type	Rep	R original code combination	Plant no.
G	C	ff	2	75,"2","AG","4"	75
U	C	wb	2	76,"2","ANG","3"	76
U	S	hh	2	77,"2","CNG","5"	77
U	S	ff	2	78,"2","CNG","4"	78
G	C	wb	2	79,"2","AG","3"	79
G	S	bd	2	80,"2","CG","7"	80
G	C	sp	2	81,"2","AG","6"	81
G	N	ww	2	82,"2","BG","8"	82
G	S	dw	2	83,"2","CG","9"	83
U	N	dw	2	84,"2","BNG","9"	84
U	N	ff	2	85,"2","BNG","4"	85
G	S	ww	2	86,"2","CG","8"	86
U	C	ff	2	87,"2","ANG","4"	87
G	N	bd	2	88,"2","BG","7"	88
U	C	bd	2	89,"2","ANG","7"	89
U	N	hh	2	90,"2","BNG","5"	90
G	N	hh	2	91,"2","BG","5"	91
U	N	cd	2	92,"2","BNG","1"	92
U	C	dw	2	93,"2","ANG","9"	93
U	S	sp	2	94,"2","CNG","6"	94
G	C	cd	2	95,"2","AG","1"	95
G	C	ss	2	96,"2","AG","2"	96
G	N	sp	2	97,"2","BG","6"	97
U	S	ss	2	98,"2","CNG","2"	98
U	C	cd	2	99,"2","ANG","1"	99
U	N	ww	2	100,"2","BNG","8"	100
U	S	wb	2	101,"2","CNG","3"	101
G	N	wb	2	102,"2","BG","3"	102
G	C	hh	2	103,"2","AG","5"	103
U	C	ss	2	104,"2","ANG","2"	104
U	C	sp	2	105,"2","ANG","6"	105
U	N	ss	2	106,"2","BNG","2"	106
G	N	ss	2	107,"2","BG","2"	107
U	S	ww	2	108,"2","CNG","8"	108
U	N	dw	3	109,"3","BNG","9"	109
U	N	bd	3	110,"3","BNG","7"	110
U	C	sp	3	111,"3","ANG","6"	111
G	C	dw	3	112,"3","AG","9"	112
G	S	sp	3	113,"3","CG","6"	113
G	C	sp	3	114,"3","AG","6"	114
G	C	ss	3	115,"3","AG","2"	115
U	C	ss	3	116,"3","ANG","2"	116
U	N	ww	3	117,"3","BNG","8"	117
U	C	dw	3	118,"3","ANG","9"	118
U	N	wb	3	119,"3","BNG","3"	119
U	C	wb	3	120,"3","ANG","3"	120

Mgmt	Soil	Eco-type	Rep	R original code combination	Plant no.
G	C	ff	3	121,"3","AG","4"	121
U	C	cd	3	122,"3","ANG","1"	122
G	S	ww	3	123,"3","CG","8"	123
G	C	hh	3	124,"3","AG","5"	124
G	C	cd	3	125,"3","AG","1"	125
G	N	sp	3	126,"3","BG","6"	126
G	N	hh	3	127,"3","BG","5"	127
G	N	cd	3	128,"3","BG","1"	128
U	S	ww	3	129,"3","CNG","8"	129
G	S	bd	3	130,"3","CG","7"	130
U	S	wb	3	131,"3","CNG","3"	131
U	C	ff	3	132,"3","ANG","4"	132
G	S	hh	3	133,"3","CG","5"	133
G	C	ww	3	134,"3","AG","8"	134
G	S	ff	3	135,"3","CG","4"	135
G	S	ss	3	136,"3","CG","2"	136
G	N	ww	3	137,"3","BG","8"	137
U	N	sp	3	138,"3","BNG","6"	138
G	C	wb	3	139,"3","AG","3"	139
U	S	ss	3	140,"3","CNG","2"	140
U	N	ff	3	141,"3","BNG","4"	141
U	C	ww	3	142,"3","ANG","8"	142
G	S	cd	3	143,"3","CG","1"	143
G	S	wb	3	144,"3","CG","3"	144
G	N	wb	3	145,"3","BG","3"	145
U	C	bd	3	146,"3","ANG","7"	146
G	S	dw	3	147,"3","CG","9"	147
U	S	hh	3	148,"3","CNG","5"	148
U	S	cd	3	149,"3","CNG","1"	149
U	S	sp	3	150,"3","CNG","6"	150
G	N	ss	3	151,"3","BG","2"	151
G	N	dw	3	152,"3","BG","9"	152
G	N	ff	3	153,"3","BG","4"	153
U	S	bd	3	154,"3","CNG","7"	154
U	S	ff	3	155,"3","CNG","4"	155
G	N	bd	3	156,"3","BG","7"	156
U	N	hh	3	157,"3","BNG","5"	157
U	S	dw	3	158,"3","CNG","9"	158
U	N	cd	3	159,"3","BNG","1"	159
U	C	hh	3	160,"3","ANG","5"	160
U	N	ss	3	161,"3","BNG","2"	161
G	C	bd	3	162,"3","AG","7"	162
G	N	hh	4	163,"4","BG","5"	163
U	C	dw	4	164,"4","ANG","9"	164
G	S	ss	4	165,"4","CG","2"	165
G	C	ww	4	166,"4","AG","8"	166

Mgmt	Soil	Eco-type	Rep	R original code combination	Plant no.
G	S	dw	4	167,"4","CG","9"	167
U	N	wb	4	168,"4","BNG","3"	168
U	S	ff	4	169,"4","CNG","4"	169
U	S	hh	4	170,"4","CNG","5"	170
U	N	dw	4	171,"4","BNG","9"	171
G	N	wb	4	172,"4","BG","3"	172
G	C	hh	4	173,"4","AG","5"	173
U	S	wb	4	174,"4","CNG","3"	174
G	N	cd	4	175,"4","BG","1"	175
G	C	dw	4	176,"4","AG","9"	176
G	S	bd	4	177,"4","CG","7"	177
U	C	bd	4	178,"4","ANG","7"	178
G	S	ff	4	179,"4","CG","4"	179
G	S	ww	4	180,"4","CG","8"	180
G	N	bd	4	181,"4","BG","7"	181
G	N	ss	4	182,"4","BG","2"	182
U	N	sp	4	183,"4","BNG","6"	183
U	S	bd	4	184,"4","CNG","7"	184
U	C	ss	4	185,"4","ANG","2"	185
U	C	sp	4	186,"4","ANG","6"	186
G	C	sp	4	187,"4","AG","6"	187
U	S	dw	4	188,"4","CNG","9"	188
U	N	hh	4	189,"4","BNG","5"	189
U	C	ff	4	190,"4","ANG","4"	190
U	N	ff	4	191,"4","BNG","4"	191
G	N	dw	4	192,"4","BG","9"	192
U	S	ww	4	193,"4","CNG","8"	193
U	S	ss	4	194,"4","CNG","2"	194
G	C	ff	4	195,"4","AG","4"	195
G	S	cd	4	196,"4","CG","1"	196
G	S	wb	4	197,"4","CG","3"	197
G	S	hh	4	198,"4","CG","5"	198
U	C	ww	4	199,"4","ANG","8"	199
U	N	cd	4	200,"4","BNG","1"	200
G	C	wb	4	201,"4","AG","3"	201
G	N	ff	4	202,"4","BG","4"	202
U	N	ss	4	203,"4","BNG","2"	203
G	C	cd	4	204,"4","AG","1"	204
G	N	sp	4	205,"4","BG","6"	205
G	C	ss	4	206,"4","AG","2"	206
G	N	ww	4	207,"4","BG","8"	207
G	C	bd	4	208,"4","AG","7"	208
G	S	sp	4	209,"4","CG","6"	209
U	S	sp	4	210,"4","CNG","6"	210
U	C	cd	4	211,"4","ANG","1"	211
U	N	bd	4	212,"4","BNG","7"	212

Mgmt	Soil	Eco-type	Rep	R original code combination	Plant no.
U	C	wb	4	213,"4","ANG","3"	213
U	S	cd	4	214,"4","CNG","1"	214
U	C	hh	4	215,"4","ANG","5"	215
U	N	ww	4	216,"4","BNG","8"	216
U	N	ss	5	217,"5","BNG","2"	217
U	S	dw	5	218,"5","CNG","9"	218
U	C	wb	5	219,"5","ANG","3"	219
G	N	hh	5	220,"5","BG","5"	220
G	S	cd	5	221,"5","CG","1"	221
G	N	ww	5	222,"5","BG","8"	222
U	N	ff	5	223,"5","BNG","4"	223
G	C	ww	5	224,"5","AG","8"	224
G	S	bd	5	225,"5","CG","7"	225
G	N	ff	5	226,"5","BG","4"	226
G	N	bd	5	227,"5","BG","7"	227
U	S	hh	5	228,"5","CNG","5"	228
U	N	wb	5	229,"5","BNG","3"	229
G	C	dw	5	230,"5","AG","9"	230
G	C	hh	5	231,"5","AG","5"	231
G	S	ss	5	232,"5","CG","2"	232
G	N	sp	5	233,"5","BG","6"	233
G	S	sp	5	234,"5","CG","6"	234
U	S	cd	5	235,"5","CNG","1"	235
U	C	hh	5	236,"5","ANG","5"	236
U	S	ww	5	237,"5","CNG","8"	237
U	C	bd	5	238,"5","ANG","7"	238
G	S	wb	5	239,"5","CG","3"	239
G	N	wb	5	240,"5","BG","3"	240
U	S	ff	5	241,"5","CNG","4"	241
U	N	ww	5	242,"5","BNG","8"	242
G	C	ff	5	243,"5","AG","4"	243
G	S	ww	5	244,"5","CG","8"	244
U	C	sp	5	245,"5","ANG","6"	245
U	N	bd	5	246,"5","BNG","7"	246
U	S	ss	5	247,"5","CNG","2"	247
U	C	ww	5	248,"5","ANG","8"	248
G	C	wb	5	249,"5","AG","3"	249
G	S	dw	5	250,"5","CG","9"	250
U	N	cd	5	251,"5","BNG","1"	251
U	N	sp	5	252,"5","BNG","6"	252
U	C	ss	5	253,"5","ANG","2"	253
G	N	ss	5	254,"5","BG","2"	254
G	S	hh	5	255,"5","CG","5"	255
G	C	cd	5	256,"5","AG","1"	256
U	C	dw	5	257,"5","ANG","9"	257
G	N	cd	5	258,"5","BG","1"	258

Mgmt	Soil	Eco-type	Rep	R original code combination	Plant no.
U	N	dw	5	259,"5","BNG","9"	259
G	S	ff	5	260,"5","CG","4"	260
G	C	ss	5	261,"5","AG","2"	261
U	S	sp	5	262,"5","CNG","6"	262
G	C	bd	5	263,"5","AG","7"	263
U	C	ff	5	264,"5","ANG","4"	264
G	N	dw	5	265,"5","BG","9"	265
U	C	cd	5	266,"5","ANG","1"	266
U	S	bd	5	267,"5","CNG","7"	267
G	C	sp	5	268,"5","AG","6"	268
U	N	hh	5	269,"5","BNG","5"	269
U	S	wb	5	270,"5","CNG","3"	270
U	S	ww	6	271,"6","CNG","8"	271
G	N	ww	6	272,"6","BG","8"	272
U	N	ff	6	273,"6","BNG","4"	273
G	N	hh	6	274,"6","BG","5"	274
G	S	sp	6	275,"6","CG","6"	275
U	N	ww	6	276,"6","BNG","8"	276
U	C	sp	6	277,"6","ANG","6"	277
U	S	ff	6	278,"6","CNG","4"	278
G	S	hh	6	279,"6","CG","5"	279
G	S	bd	6	280,"6","CG","7"	280
U	N	cd	6	281,"6","BNG","1"	281
G	S	ww	6	282,"6","CG","8"	282
G	S	ff	6	283,"6","CG","4"	283
U	C	cd	6	284,"6","ANG","1"	284
G	N	wb	6	285,"6","BG","3"	285
U	S	sp	6	286,"6","CNG","6"	286
G	N	ss	6	287,"6","BG","2"	287
U	C	wb	6	288,"6","ANG","3"	288
G	S	dw	6	289,"6","CG","9"	289
G	C	dw	6	290,"6","AG","9"	290
U	C	ff	6	291,"6","ANG","4"	291
U	S	wb	6	292,"6","CNG","3"	292
U	S	ss	6	293,"6","CNG","2"	293
U	N	bd	6	294,"6","BNG","7"	294
G	S	wb	6	295,"6","CG","3"	295
U	N	dw	6	296,"6","BNG","9"	296
G	N	ff	6	297,"6","BG","4"	297
G	C	cd	6	298,"6","AG","1"	298
U	C	hh	6	299,"6","ANG","5"	299
G	N	sp	6	300,"6","BG","6"	300
G	N	cd	6	301,"6","BG","1"	301
U	S	bd	6	302,"6","CNG","7"	302
U	S	dw	6	303,"6","CNG","9"	303
G	C	bd	6	304,"6","AG","7"	304

Mgmt	Soil	Eco-type	Rep	R original code combination	Plant no.
G	N	dw	6	305,"6","BG","9"	305
U	C	dw	6	306,"6","ANG","9"	306
G	C	sp	6	307,"6","AG","6"	307
G	S	cd	6	308,"6","CG","1"	308
G	C	ww	6	309,"6","AG","8"	309
G	C	hh	6	310,"6","AG","5"	310
U	N	ss	6	311,"6","BNG","2"	311
G	C	ss	6	312,"6","AG","2"	312
U	C	ss	6	313,"6","ANG","2"	313
U	S	hh	6	314,"6","CNG","5"	314
U	C	bd	6	315,"6","ANG","7"	315
U	S	cd	6	316,"6","CNG","1"	316
G	C	wb	6	317,"6","AG","3"	317
U	C	ww	6	318,"6","ANG","8"	318
U	N	hh	6	319,"6","BNG","5"	319
G	N	bd	6	320,"6","BG","7"	320
U	N	wb	6	321,"6","BNG","3"	321
U	N	sp	6	322,"6","BNG","6"	322
G	C	ff	6	323,"6","AG","4"	323
G	S	ss	6	324,"6","CG","2"	324
U	C	bd	7	325,"7","ANG","7"	325
G	C	ff	7	326,"7","AG","4"	326
U	N	ss	7	327,"7","BNG","2"	327
G	C	ss	7	328,"7","AG","2"	328
G	S	hh	7	329,"7","CG","5"	329
G	S	sp	7	330,"7","CG","6"	330
G	C	hh	7	331,"7","AG","5"	331
G	N	wb	7	332,"7","BG","3"	332
G	S	wb	7	333,"7","CG","3"	333
G	S	dw	7	334,"7","CG","9"	334
U	C	ff	7	335,"7","ANG","4"	335
U	N	ff	7	336,"7","BNG","4"	336
G	C	dw	7	337,"7","AG","9"	337
U	S	wb	7	338,"7","CNG","3"	338
U	C	ww	7	339,"7","ANG","8"	339
G	S	ff	7	340,"7","CG","4"	340
U	N	dw	7	341,"7","BNG","9"	341
U	C	wb	7	342,"7","ANG","3"	342
G	N	dw	7	343,"7","BG","9"	343
G	C	sp	7	344,"7","AG","6"	344
G	N	bd	7	345,"7","BG","7"	345
G	N	sp	7	346,"7","BG","6"	346
G	C	bd	7	347,"7","AG","7"	347
U	S	ff	7	348,"7","CNG","4"	348
U	C	dw	7	349,"7","ANG","9"	349
G	N	ff	7	350,"7","BG","4"	350

Mgmt	Soil	Eco-type	Rep	R original code combination	Plant no.
U	N	hh	7	351,"7","BNG","5"	351
U	N	cd	7	352,"7","BNG","1"	352
U	S	ww	7	353,"7","CNG","8"	353
U	S	cd	7	354,"7","CNG","1"	354
U	C	ss	7	355,"7","ANG","2"	355
U	S	hh	7	356,"7","CNG","5"	356
U	N	bd	7	357,"7","BNG","7"	357
U	C	cd	7	358,"7","ANG","1"	358
G	C	cd	7	359,"7","AG","1"	359
U	S	bd	7	360,"7","CNG","7"	360
U	S	ss	7	361,"7","CNG","2"	361
G	N	ss	7	362,"7","BG","2"	362
U	S	sp	7	363,"7","CNG","6"	363
U	S	dw	7	364,"7","CNG","9"	364
U	C	sp	7	365,"7","ANG","6"	365
G	N	cd	7	366,"7","BG","1"	366
G	S	bd	7	367,"7","CG","7"	367
G	N	hh	7	368,"7","BG","5"	368
G	S	ss	7	369,"7","CG","2"	369
G	C	wb	7	370,"7","AG","3"	370
U	N	sp	7	371,"7","BNG","6"	371
G	S	cd	7	372,"7","CG","1"	372
U	N	ww	7	373,"7","BNG","8"	373
U	N	wb	7	374,"7","BNG","3"	374
U	C	hh	7	375,"7","ANG","5"	375
G	C	ww	7	376,"7","AG","8"	376
G	N	ww	7	377,"7","BG","8"	377
G	S	ww	7	378,"7","CG","8"	378
G	N	dw	8	379,"8","BG","9"	379
G	N	cd	8	380,"8","BG","1"	380
G	S	ff	8	381,"8","CG","4"	381
G	N	sp	8	382,"8","BG","6"	382
G	N	hh	8	383,"8","BG","5"	383
U	S	cd	8	384,"8","CNG","1"	384
U	N	bd	8	385,"8","BNG","7"	385
U	C	wb	8	386,"8","ANG","3"	386
U	N	cd	8	387,"8","BNG","1"	387
U	C	hh	8	388,"8","ANG","5"	388
G	C	bd	8	389,"8","AG","7"	389
G	N	ww	8	390,"8","BG","8"	390
U	C	ff	8	391,"8","ANG","4"	391

Mgmt	Soil	Eco-type	Rep	R original code combination	Plant no.
U	C	bd	8	392,"8","ANG","7"	392
U	C	sp	8	393,"8","ANG","6"	393
G	S	ss	8	394,"8","CG","2"	394
U	S	wb	8	395,"8","CNG","3"	395
U	N	hh	8	396,"8","BNG","5"	396
G	S	hh	8	397,"8","CG","5"	397
U	N	ff	8	398,"8","BNG","4"	398
U	N	ss	8	399,"8","BNG","2"	399
G	C	ff	8	400,"8","AG","4"	400
G	S	sp	8	401,"8","CG","6"	401
G	S	cd	8	402,"8","CG","1"	402
U	S	ww	8	403,"8","CNG","8"	403
U	S	dw	8	404,"8","CNG","9"	404
G	S	ww	8	405,"8","CG","8"	405
U	N	dw	8	406,"8","BNG","9"	406
G	N	bd	8	407,"8","BG","7"	407
U	S	sp	8	408,"8","CNG","6"	408
U	S	ff	8	409,"8","CNG","4"	409
G	C	dw	8	410,"8","AG","9"	410
G	N	wb	8	411,"8","BG","3"	411
G	C	ss	8	412,"8","AG","2"	412
U	C	dw	8	413,"8","ANG","9"	413
G	N	ss	8	414,"8","BG","2"	414
U	S	hh	8	415,"8","CNG","5"	415
U	S	bd	8	416,"8","CNG","7"	416
G	C	sp	8	417,"8","AG","6"	417
U	S	ss	8	418,"8","CNG","2"	418
U	N	sp	8	419,"8","BNG","6"	419
U	N	wb	8	420,"8","BNG","3"	420
U	N	ww	8	421,"8","BNG","8"	421
G	N	ff	8	422,"8","BG","4"	422
G	C	hh	8	423,"8","AG","5"	423
U	C	ss	8	424,"8","ANG","2"	424
G	C	ww	8	425,"8","AG","8"	425
G	C	wb	8	426,"8","AG","3"	426
U	C	cd	8	427,"8","ANG","1"	427
G	S	dw	8	428,"8","CG","9"	428
U	C	ww	8	429,"8","ANG","8"	429
G	C	cd	8	430,"8","AG","1"	430
G	S	wb	8	431,"8","CG","3"	431
G	S	bd	8	432,"8","CG","7"	432

APPENDIX IX – Glasshouse Temperature and Humidity

Table IX 1. Mean monthly temperatures and humidity (based on 30 minute readings) in glasshouse 2011-2012 and Met Office (2013) mean temperatures for 2012-2013.

	2011 - 2012 Glasshouse Humidity (% RH)	2011 - 2012 Glasshouse Temperature (^oC)	2011 - 2012 MET office Temperature (^oC)	2012 - 2013 MET office Temperature (^oC)
November	77.0	12.4	13.7	10.6
December	69.8	10.8	10.4	9.7
January	75.5	11.1	9.7	7.5
February	74.7	10.4	8.3	6.8
March	72.7	13.8	13.6	7.4
April	76.2	13.0	12.1	12.1
May	72.5	17.5	17.1	15.5
June	81.9	16.7	17.7	19.0
July	79.5	18.2	20.0	25.3
August	84.2	18.9	20.7	22.4
September	85.8	15.0	18.0	19.2
October	86.9	13.6	14.0	16.5

APPENDIX X – GLMM (LME4) Effects Plots

Table X 1. P-values for GLMM effects plots ('Soil+mgmt' is cumulative lengths when plants were receiving both soil and management treatment)

	Management Treatment Split	Ecotype	Soil Treatment	Management Treatment	Ecotype Soil	Ecotype Management	Interaction
Main Stem Length (Harvest)	**All**		$7.3e^{-08}$	$<2e^{-16}$			
	Grazed	0.0143	0.0003				
	Unmanaged		$9.67e^{-06}$				
Main Stem Length (Soil+mgmt)	**All**		0.0024	$<2e^{-16}$			
	Grazed		0.0768				
	Unmanaged		$1.77e^{-09}$				
Stems per Plant (Harvest)	**All**	$<2e-^{16}$	$<2e^{-16}$	$<2e^{-16}$			
	Grazed	$<2e^{-16}$	$<2e^{-16}$		$<2e^{-16}$	$9.9e^{-05}$	$6.9e^{-05}$
	Unmanaged	$1.6e^{-10}$	$<2e^{-16}$		$<2e^{-16}$		$5.6e^{-06}$
Leaflets per main stem (Harvest)	**All**	$<2e^{-16}$	$<2e^{-16}$	$<2e^{-16}$			
	Grazed	$<2e^{-16}$	$<2e^{-16}$		$1.609e^{-09}$	0.0061	$<2e^{-16}$
	Unmanaged	$<2e^{-16}$			$<2.2e^{-16}$	$<2e^{-16}$	$<2e^{-16}$
Hirsuteness	**All**	$<2e^{-16}$	$8.8e^{-05}$		0.0003	$5.6e^{-06}$	
Time pod	**All**	0.0003		$< 2.2e^{-16}$			
	Grazed	$5.885e^{-05}$	$1.973e^{-07}$		$< 2.2e^{-16}$	0.0002	
	Unmanaged	0.0011	$1.767e^{-08}$				0.0050
Pods All	**All**	$<2e^{-16}$	$<2e^{-16}$	$<2e^{-16}$			
	Grazed	$7.817e^{-13}$	$<2e^{-16}$			0.0072	$8.604e^{-06}$
	Unmanaged	$<2e^{-16}$	$<2e^{-16}$		$< 2.2e^{-16}$	$7.343e^{-15}$	$<2e^{-16}$

	Management Treatment Split	Ecotype	Soil Treatment	Management Treatment	Ecotype Soil	Ecotype Management	Interaction
Pods sampled	**All**	*0.0012*	*0.0477*	*$< 2.2e^{-16}$*			
	Grazed	*$1.962e^{-15}$*	*0.0037*				
	Unmanaged						
Seed per pod	**All**	*$2.007e^{-09}$*		*$< 2.2e^{e-16}$*			
	Grazed	*$2.113e^{-14}$*	*0.0055*		*$< 2.2e^{-16}$*	*$8.806e^{-12}$*	*0.0081*
	Unmanaged	*0.0040*				*0.0065*	
Grazed biomass	**All**	*0.0353*	*$< 2.2e^{-16}$*				
Harvest biomass	**All**		*$< 2.2e^{-16}$*	*$< 2.2e^{-16}$*			
	Grazed	*0.0143*	*$8.983e^{-15}$*			*0.0269*	
	Unmanaged		*$< 2.2e^{-16}$*				
Relative moisture content	**All**		*$< 2.2e^{-16}$*	*$< 2.2e^{-16}$*			
	Grazed						
	Unmanaged	*0.0289*					
Nitrogen	**All**	*0.0340*	*$4.1e^{-09}$*	*$1.5e^{-15}$*			
	Grazed		*$6.158e^{-08}$*				
	Unmanaged	*0.0071*	*0.0035*				
HCN (Qn)	**All**	*$<2e^{-16}$*			*$4.791e^{-07}$*	*$< 2.2e^{-16}$*	
Flower time	**All**	*0.0002*	*$8.683e^{-09}$*	*$1.788e^{-06}$*			
	Grazed		*0.0061*				
	Unmanaged		*0.0034*				

	Management Treatment Split	Ecotype	Soil Treatment	Management Treatment	Ecotype Soil	Ecotype Management	Interaction
Flower no.	**All**	*<0.001*	*0.000*		*<0.001*		*Soil: P<0.001; Management P=0.002*
Flower Scent	**All**			*0.049*			
Bee visits	**All**	*<0.001*	*0.037*		*<0.001*		
Time spent (bees)	**All**	*0.054*	*0.015*				

Table X 2. P-values for GLMM effects plots of bee results using previous plant parameters as factors

	Leaf-Nitrogen	Leaf-HCN (Quantitative)	Hirsuteness	Dry Veg Biomass (harvest)	Dry flower biomass (harvest)	Flower relative moisture content (%)	Pre-harvest flower scent	Flower number (harvest)
Bee visits	*0.007*	*0.016*		*0.001*	*<0.001*	*<0.001*	*<0.001*	
Time spent (bees)	*0.059*			*0.005*	*<0.001*	*<0.001*	*<0.001*	

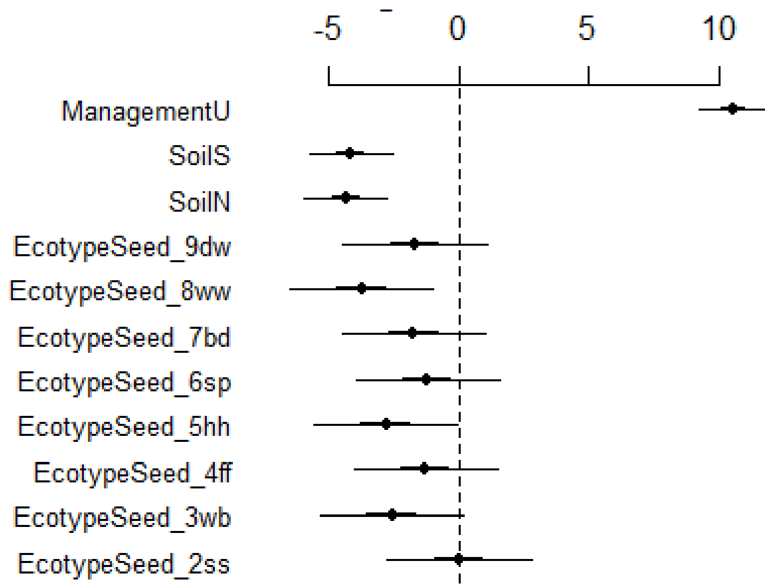


Figure X 1. Main stem length (at harvest) GLMM effects plot. Factors with capital letter (S, N) are treatments, those with lower case letters (s, n, unmanaged, cut) are ecotype factors. Cockey Down in calcareous loam + grazed treatment is used as the standard. See Table 17 in Chapter 13 for ecotype key.

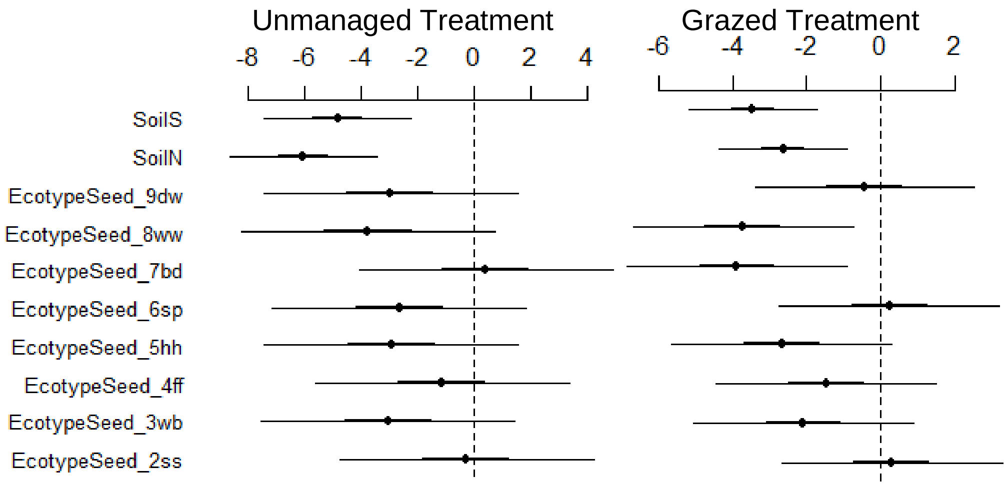


Figure X 2. Main stem length (at harvest) GLMM effects plots split by management treatment. Factors with capital letter (S, N) are treatments, those with lower case letters (s, n, unmanaged, cut) are ecotype factors. Cockey Down in calcareous loam + grazed treatment is used as the standard. See Table 17 in Chapter 13 for ecotype key.

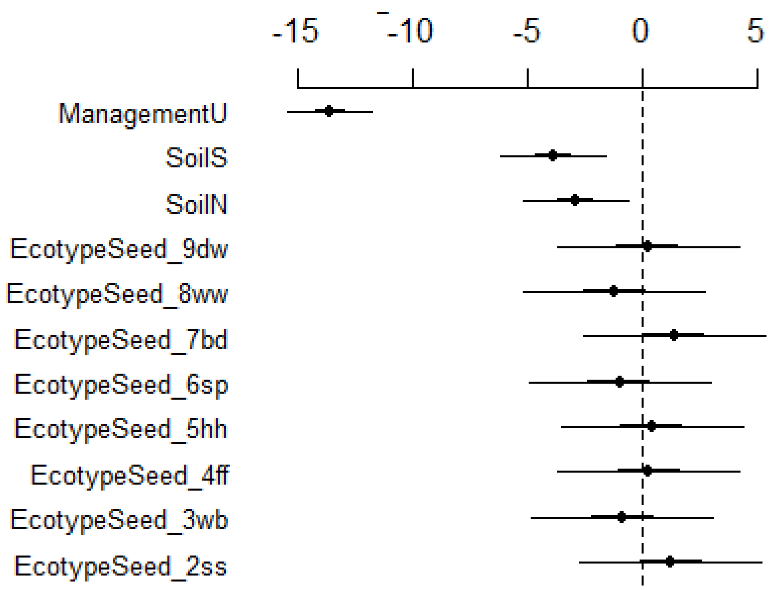


Figure X 3. Main stem length (Soil+Mgmt) GLMM effects plot. Factors with capital letter (S, N) are treatments, those with lower case letters (s, n, unmanaged, cut) are ecotype factors. Cockey Down in calcareous loam + grazed treatment is used as the standard. See Table 17 in Chapter 13 for ecotype key.

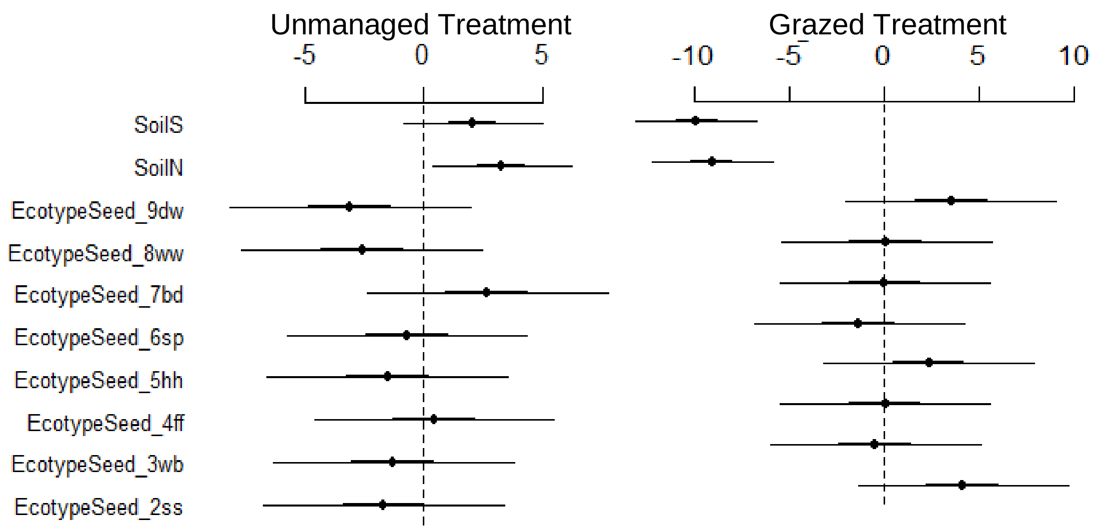


Figure X 4. Main stem length (soil+mgmt.) GLMM effects plot split by management treatment. Cockey Down in calcareous loam + grazed treatment is used as the standard. Factors with capital letter (S, N) are treatments, those with lower case letters (s, n, unmanaged, cut) are ecotype factors. See Table 17 in Chapter 13 for ecotype key.

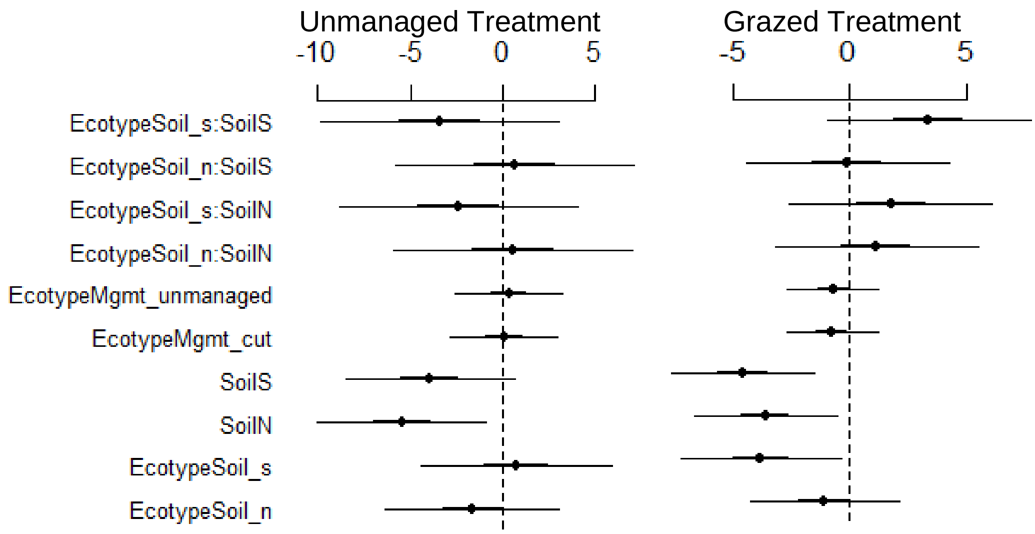


Figure X 5. Main stem length GLMM effects plot split between management treatments with interactions between ecotype and treatment. Factors with capital letter (S, N) are treatments, those with lower case letters (s, n, unmanaged, cut) are ecotype factors. Cockey Down in calcareous loam + grazed treatment is used as the standard.

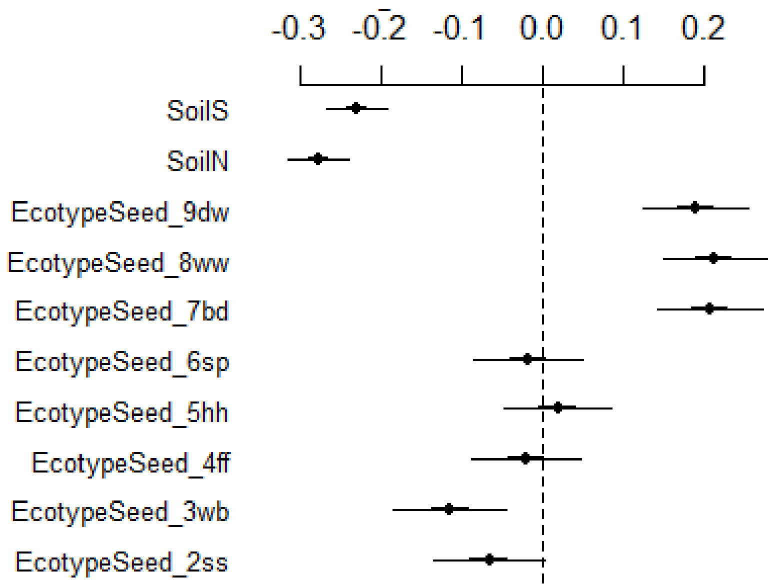


Figure X 6. Stem number per plant GLMM effects plot. Cockey Down in calcareous loam + grazed treatment is used as the standard. Factors with capital letter (S, N) are treatments, those with lower case letters (s, n, unmanaged, cut) are ecotype factors. See Table 17 in Chapter 13 for ecotype key.

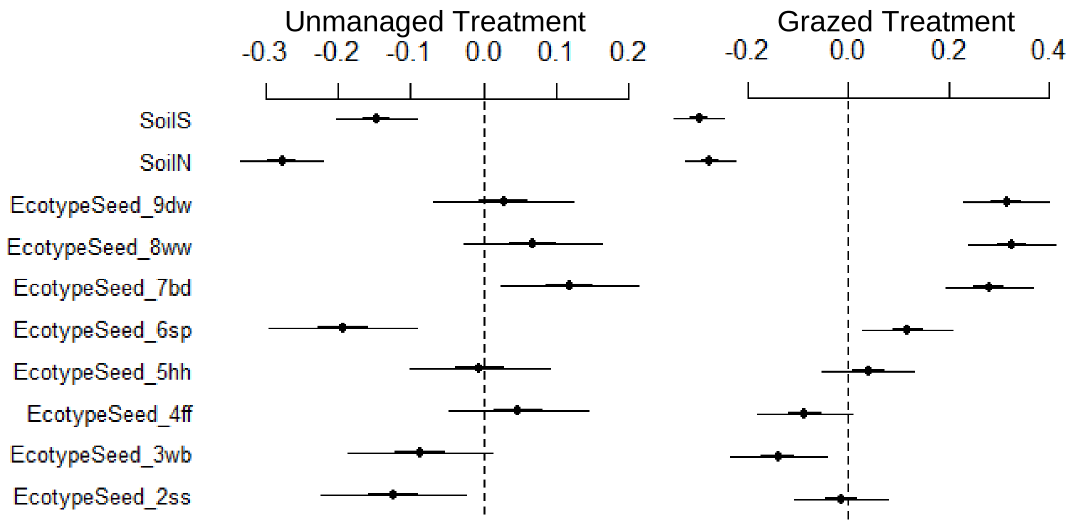


Figure X 7. Stem number per plant GLMM effects plot split by management treatment. Cockey Down in calcareous loam + grazed treatment is used as the standard. Factors with capital letter (S, N) are treatments, those with lower case letters (s, n, unmanaged, cut) are ecotype factors. See Table 17 in Chapter 13 for ecotype key.

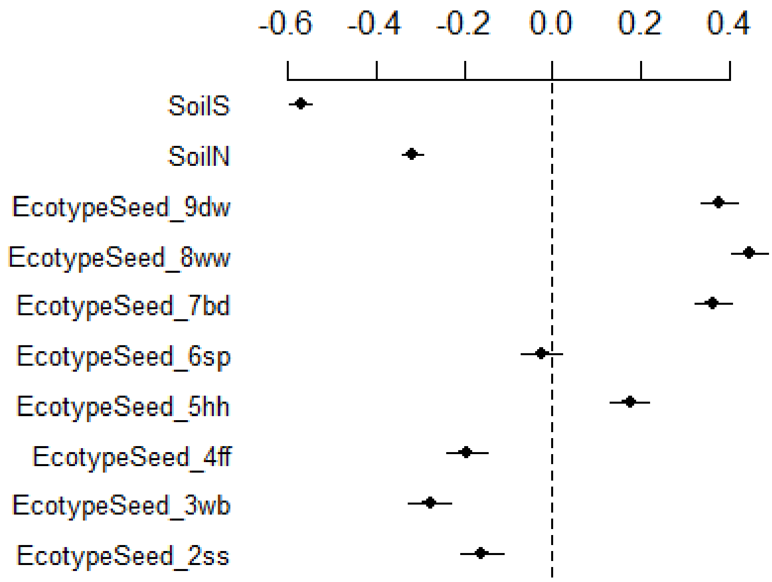


Figure X 8. Leaflet number per main stem GLMM effects plot. Cockey Down in calcareous loam + grazed treatment is used as the standard. Factors with capital letter (S, N) are treatments, those with lower case letters (s, n, unmanaged, cut) are ecotype factors. See Table 17 in Chapter 13 for ecotype key.

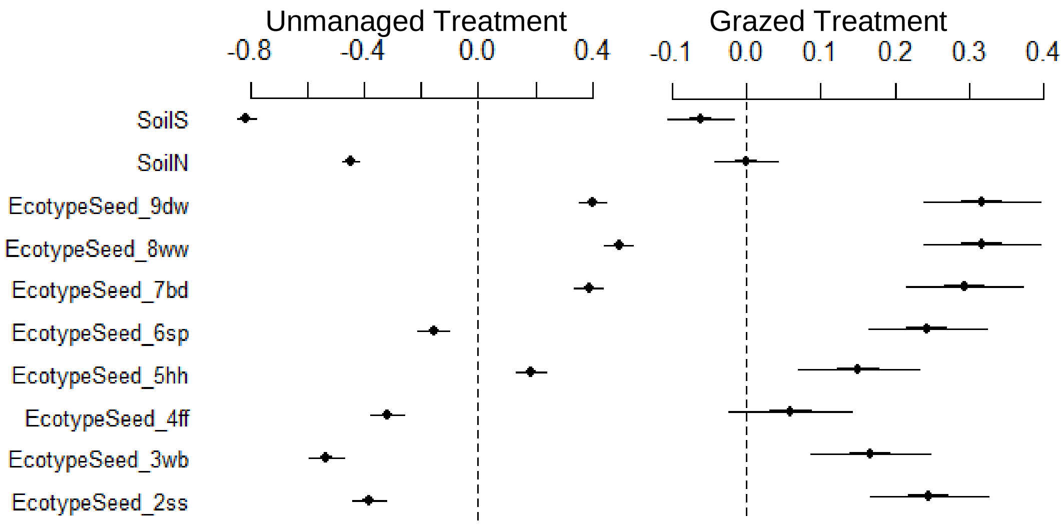


Figure X 9. Leaflet number per main stem GLMM effects plot split by management treatment. Cockey Down in calcareous loam + grazed treatment is used as the standard. Factors with capital letter (S, N) are treatments, those with lower case letters (s, n, unmanaged, cut) are ecotype factors. See Table 17 in Chapter 13 for ecotype key.

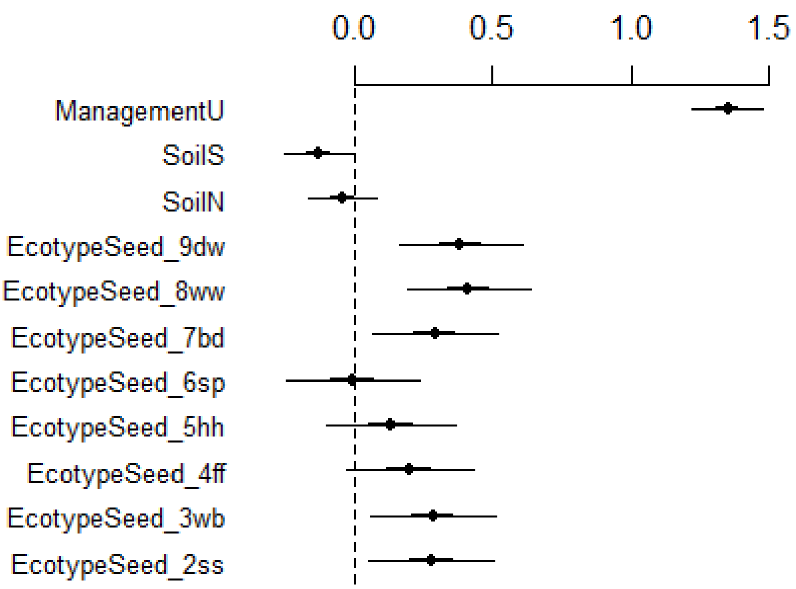


Figure X 10. Time taken to first pod formation, GLMM effects plot. Cockey Down in calcareous loam + grazed treatment is used as the standard. Factors with capital letter (S, N) are treatments, those with lower case letters (s, n, unmanaged, cut) are ecotype factors. See Table 17 in Chapter 13 for ecotype key.

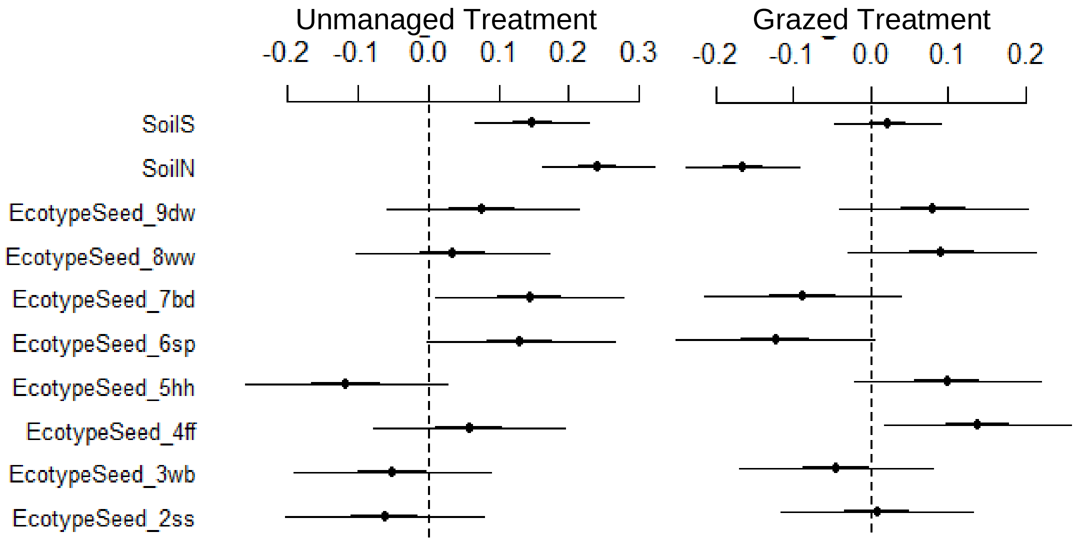


Figure X 11. Time taken to first pod formation, GLMM effects plot slit by management treatment. Cockey Down in calcareous loam + grazed treatment is used as the standard. Factors with capital letter (S, N) are treatments, those with lower case letters (s, n, unmanaged, cut) are ecotype factors. See Table 17 in Chapter 13 for ecotype key.

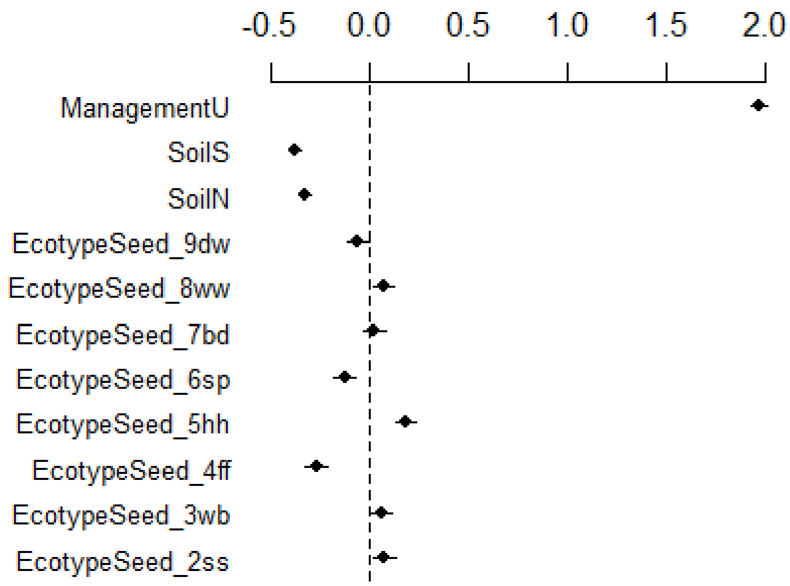


Figure X 12. Pod number from both years, GLMM effects plot. Cockey Down in calcareous loam + grazed treatment is used as the standard. Factors with capital letter (S, N) are treatments, those with lower case letters (s, n, unmanaged, cut) are ecotype factors. See Table 17 in Chapter 13 for ecotype key.

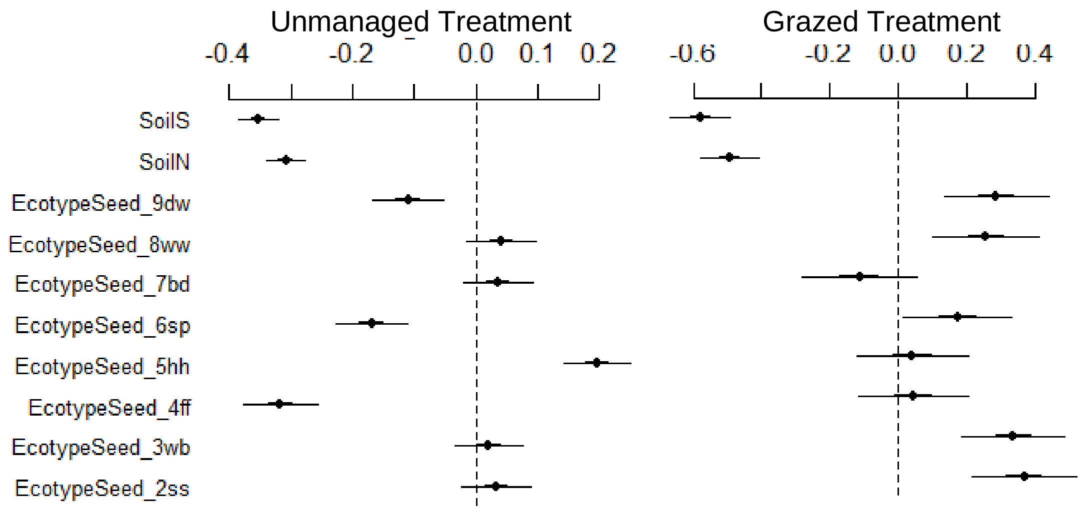


Figure X 13. Pod number from both years, GLMM effects plot split by management treatment. Cockey Down in calcareous loam + grazed treatment is used as the standard. Factors with capital letter (S, N) are treatments, those with lower case letters (s, n, unmanaged, cut) are ecotype factors. See Table 17 in Chapter 13 for ecotype key.

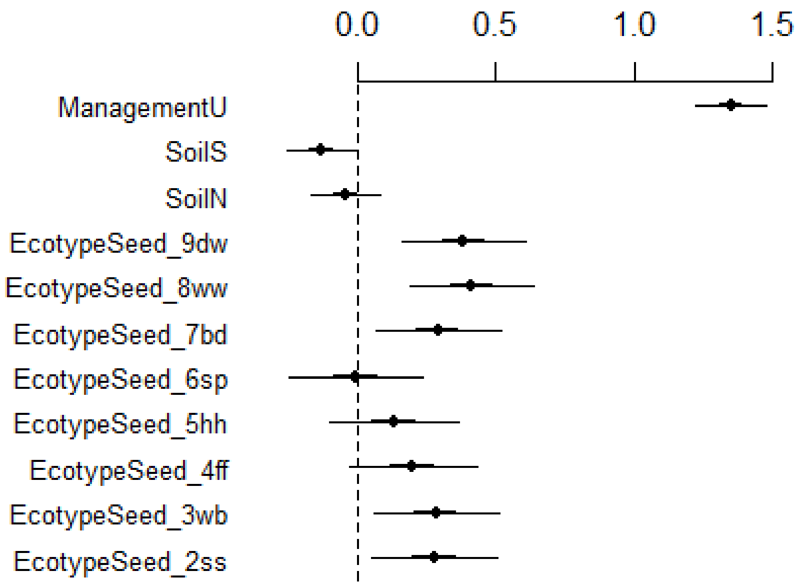


Figure X 14. Pods sampled [in calculating average seed per pod]. Cockey Down in calcareous loam + grazed treatment is used as the standard. Factors with capital letter (S, N) are treatments, those with lower case letters (s, n, unmanaged, cut) are ecotype factors. See Table 17 in Chapter 13 for ecotype key.

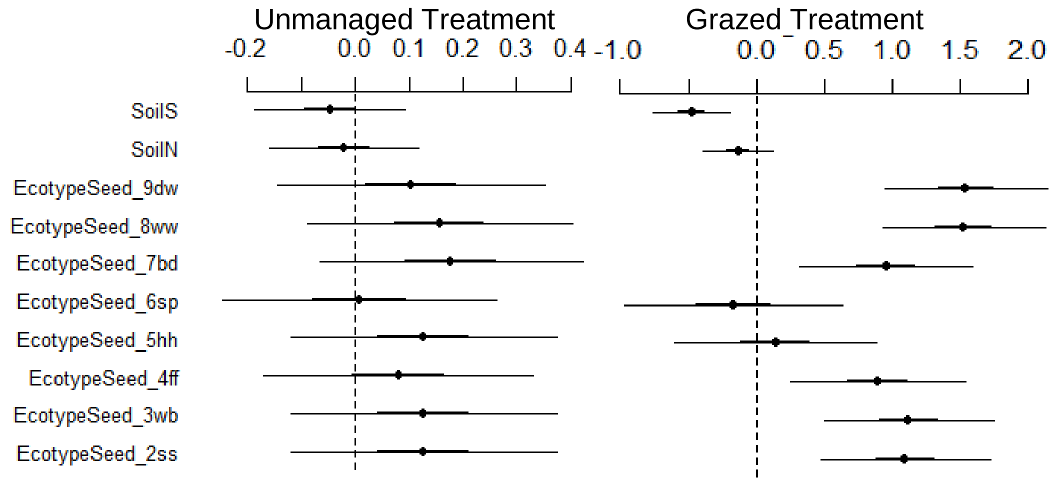


Figure X 15. Pods sampled in calculating average seed per pod. GLMM effects plot, split by management treatment. Cockey Down in calcareous loam + grazed treatment is used as the standard. Factors with capital letter (S, N) are treatments, those with lower case letters (s, n, unmanaged, cut) are ecotype factors. See Table 17 in Chapter 13 for ecotype key.

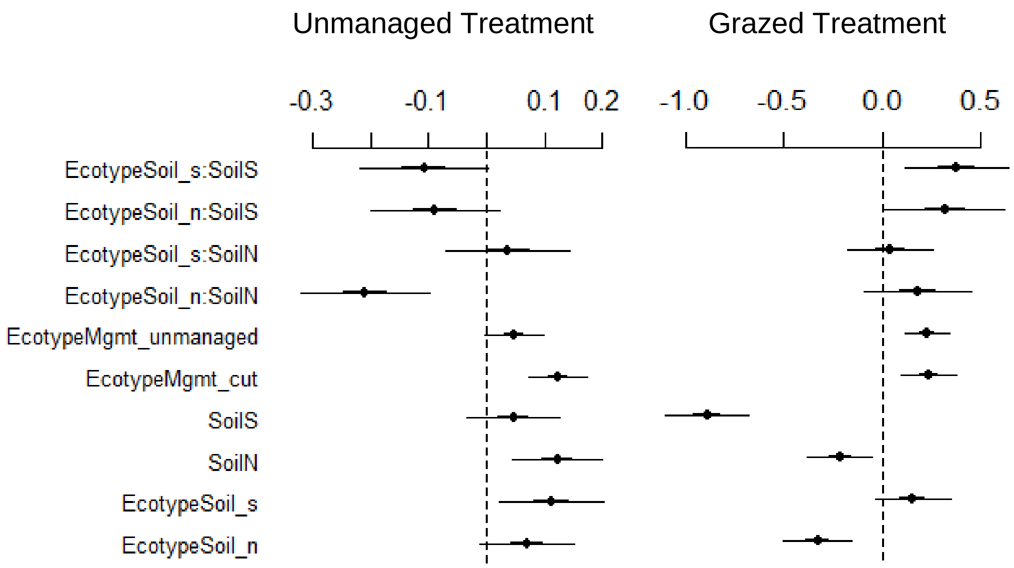


Figure X 16. Pods sampled in calculating average seed per pod, GLMM effects plot, split between management treatments with interactions between ecotype and treatment. Factors with capital letter (S, N) are treatments, those with lower case letters (s, n, unmanaged, cut) are ecotype factors. Cockey Down in calcareous loam + grazed treatment is used as the standard.

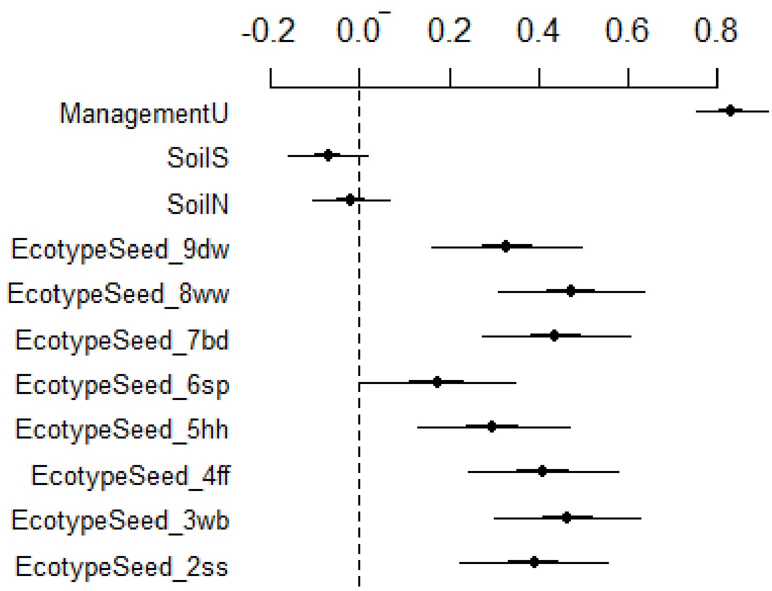


Figure X 17. Mean seed per pod GLMM effects plot. Cockey Down in calcareous loam + grazed treatment is used as the standard. Factors with capital letter (S, N) are treatments, those with lower case letters (s, n, unmanaged, cut) are ecotype factors. See Table 17 in Chapter 13 for ecotype key.

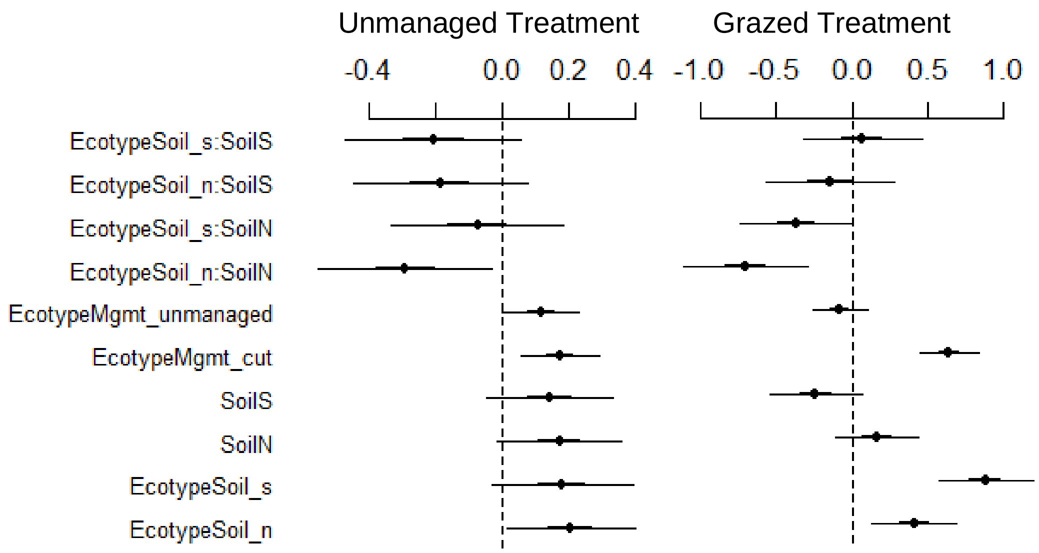


Figure X 18. Mean seed per pod GLMM effects plot, split between management treatments with interactions between ecotype and treatment. Factors with capital letter (S, N) are treatments, those with lower case letters (s, n, unmanaged, cut) are ecotype factors. Cockey Down in calcareous loam + grazed treatment is used as the standard.

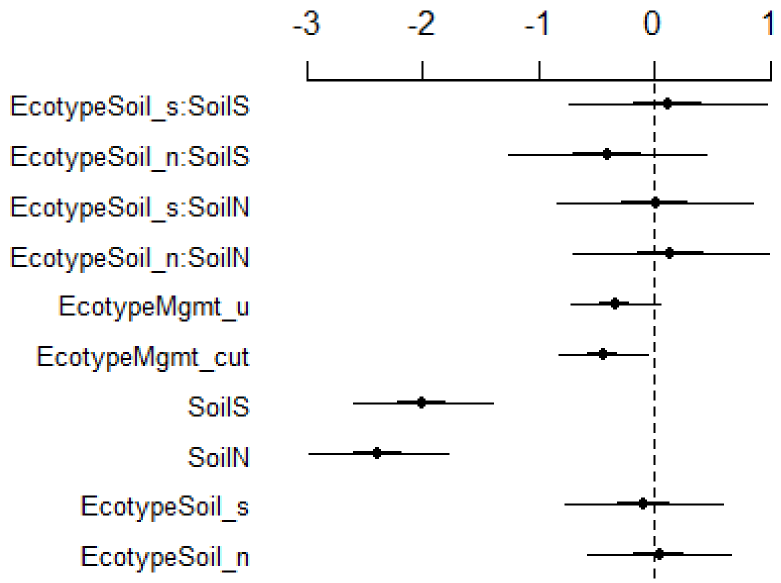


Figure X 19. Grazed biomass GLMM effects plot. Mean seed per pod GLMM effects plot. Cockey Down in calcareous loam + grazed treatment is used as the standard. Factors with capital letter (S, N) are treatments, those with lower case letters (s, n, unmanaged, cut) are ecotype factors. See Table 17 in Chapter 13 for ecotype key.

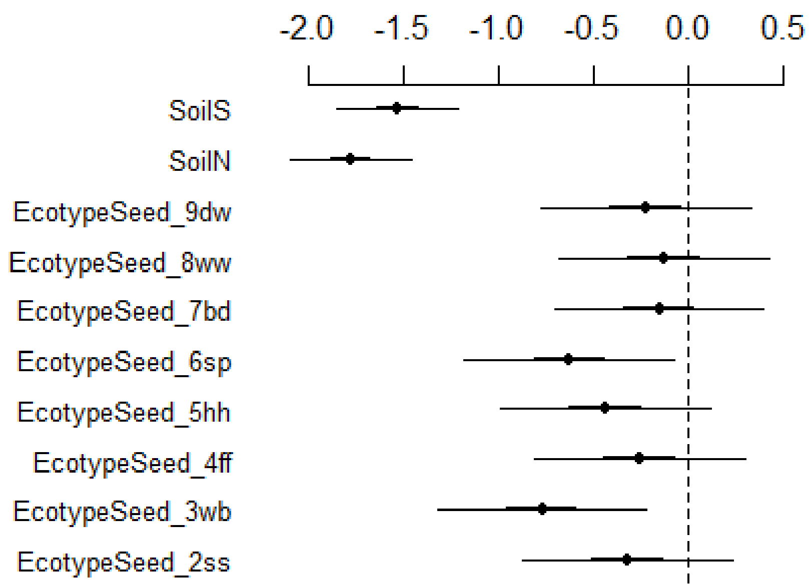


Figure X 20. Vegetation dry biomass GLMM effects plot. Cockey Down in calcareous loam + grazed treatment is used as the standard. Factors with capital letter (S, N) are treatments, those with lower case letters (s, n, unmanaged, cut) are ecotype factors. See Table 17 in Chapter 13 for ecotype key.

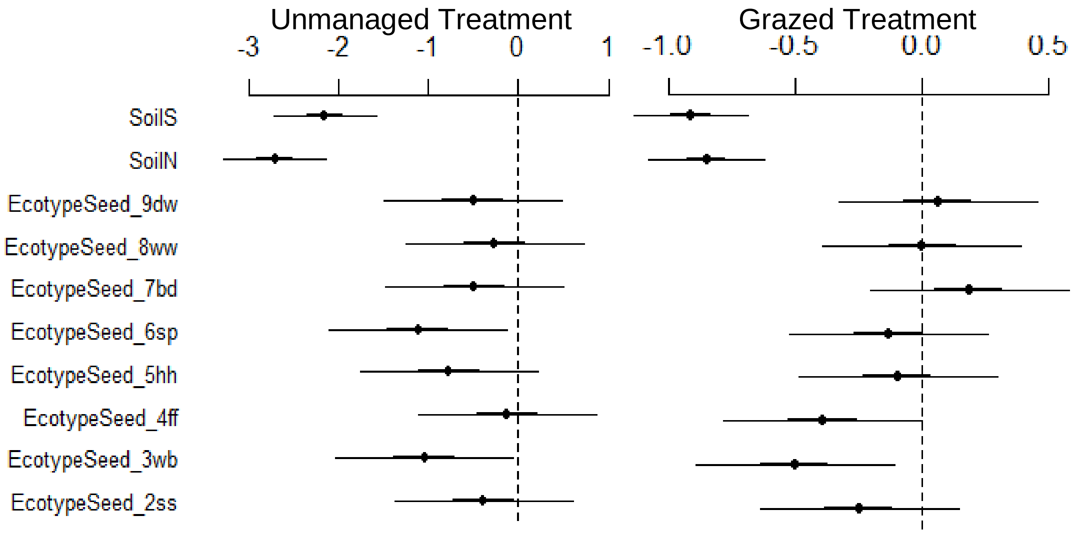


Figure X 21. Vegetation dry biomass GLMM effects plot split by management treatment. Cockey Down in calcareous loam + grazed treatment is used as the standard. Factors with capital letter (S, N) are treatments, those with lower case letters (s, n, unmanaged, cut) are ecotype factors. See Table 17 in Chapter 13 for ecotype key.

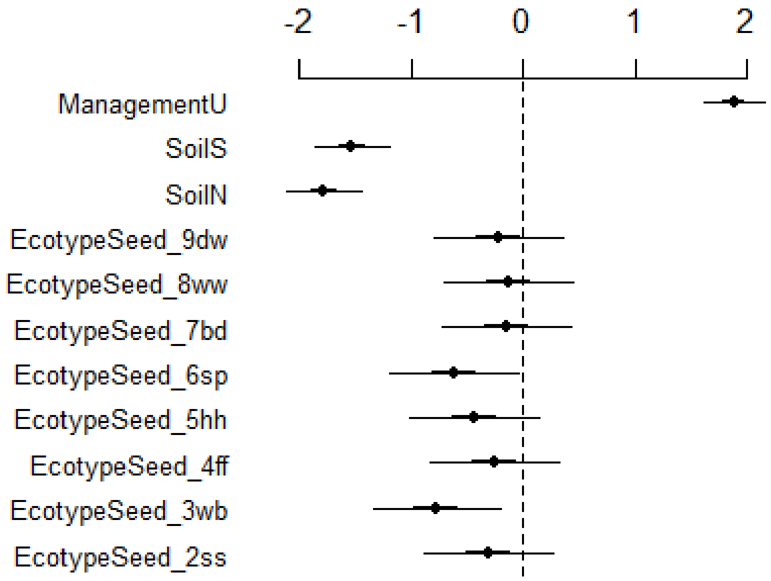


Figure X 22. Vegetation relative moisture content, GLMM effects plot. Cockey Down in Grazed + Calcareous loam treatment is used as the standard. Factors with capital letter (S, N) are treatments, those with lower case letters (s, n, unmanaged, cut) are ecotype factors. See Table 17 in Chapter 13 for ecotype key.

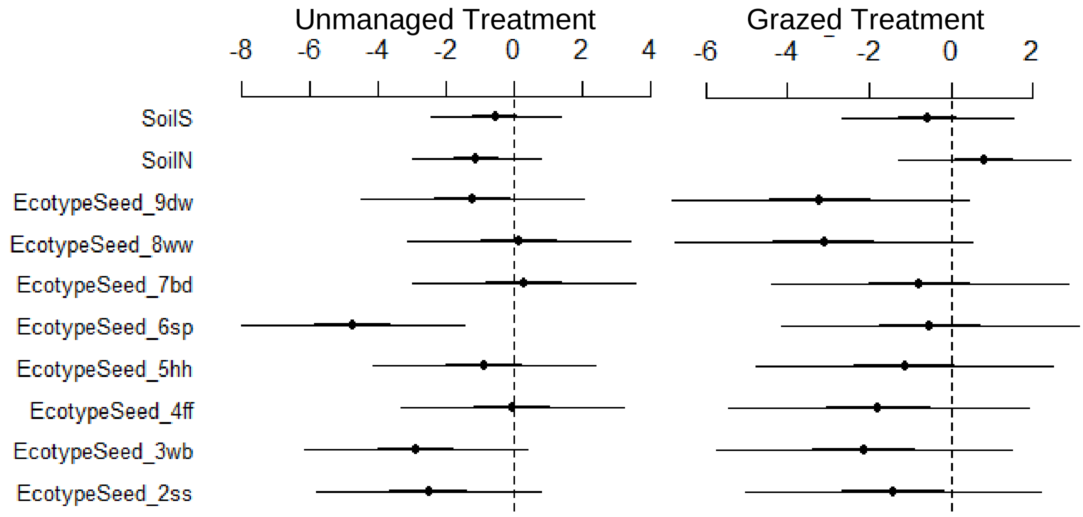


Figure X 23. Vegetation relative moisture content split by management treatment. Cockey Down in calcareous loam + grazed treatment is used as the standard. Factors with capital letter (S, N) are treatments, those with lower case letters (s, n, unmanaged, cut) are ecotype factors. See Table 17 in Chapter 13 for ecotype key.

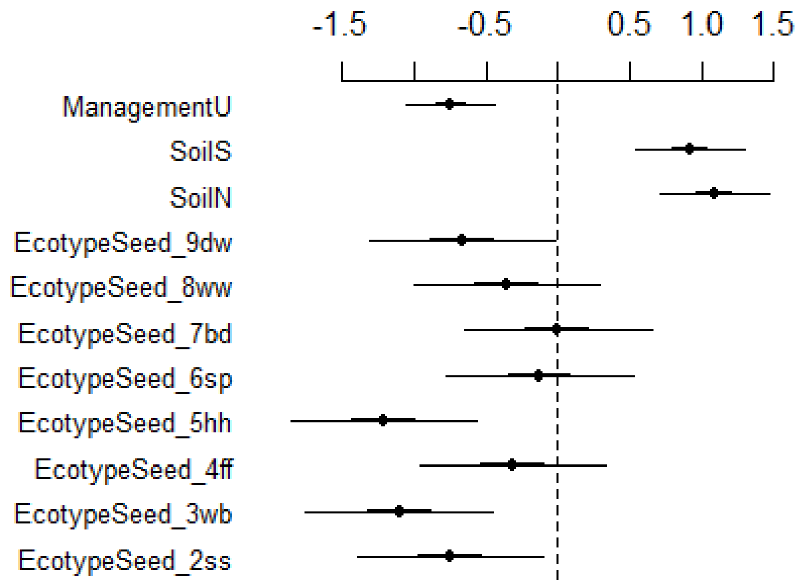


Figure X 24. Time to first flower, GLMM effects plot. Cockey Down in calcareous loam + grazed treatment is used as the standard. Factors with capital letter (S, N) are treatments, those with lower case letters (s, n, unmanaged, cut) are ecotype factors. See Table 17 in Chapter 13 for ecotype key.

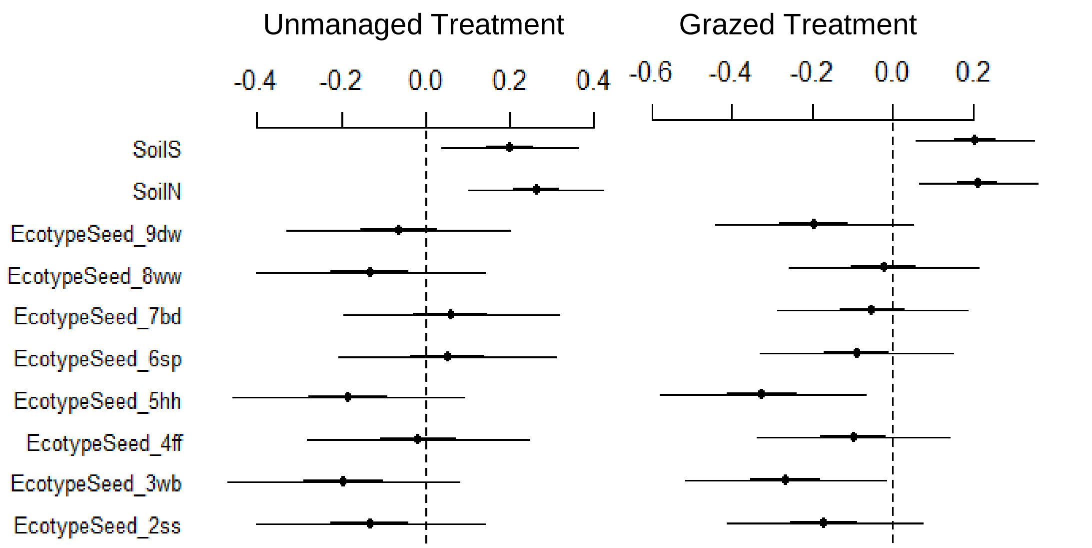


Figure X 25. Time to first flower GLMM effects plot, split between management treatments. Cockey Down in calcareous loam + grazed treatment is used as the standard. Factors with capital letter (S, N) are treatments, those with lower case letters (s, n, unmanaged, cut) are ecotype factors. See Table 17 in Chapter 13 for ecotype key.

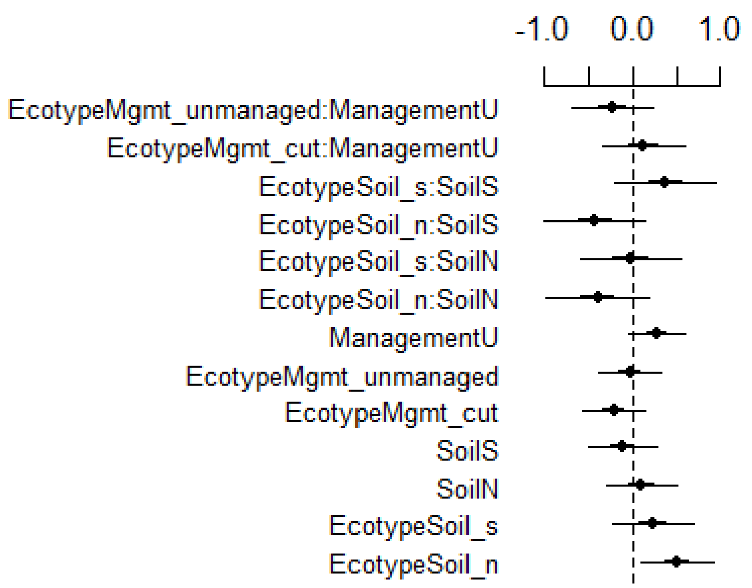


Figure X 26. Pre-harvest flower-scent GLMM effects plot with interactions. Cockey Down in calcareous loam + grazed treatment is used as the standard. Factors with capital letter (S, N, U) are treatments, those with lower case letters (s, n, unmanaged, cut) are ecotype factors. See Table 17 in Chapter 13 for ecotype key.

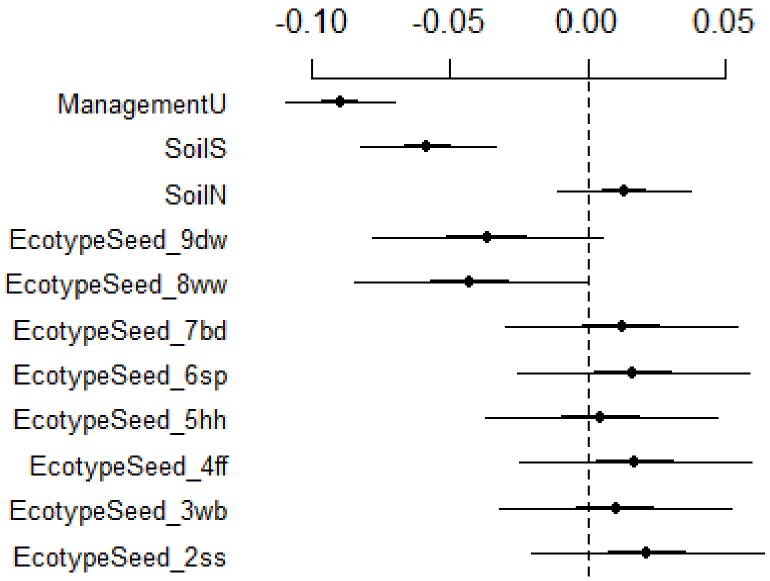


Figure X 27. Leaf-nitrogen GLMM effects plot. Cockey Down in calcareous loam + grazed treatment is used as the standard. Factors with capital letter (S, N) are treatments, those with lower case letters (s, n, unmanaged, cut) are ecotype factors. See Table 17 in Chapter 13 for ecotype key.

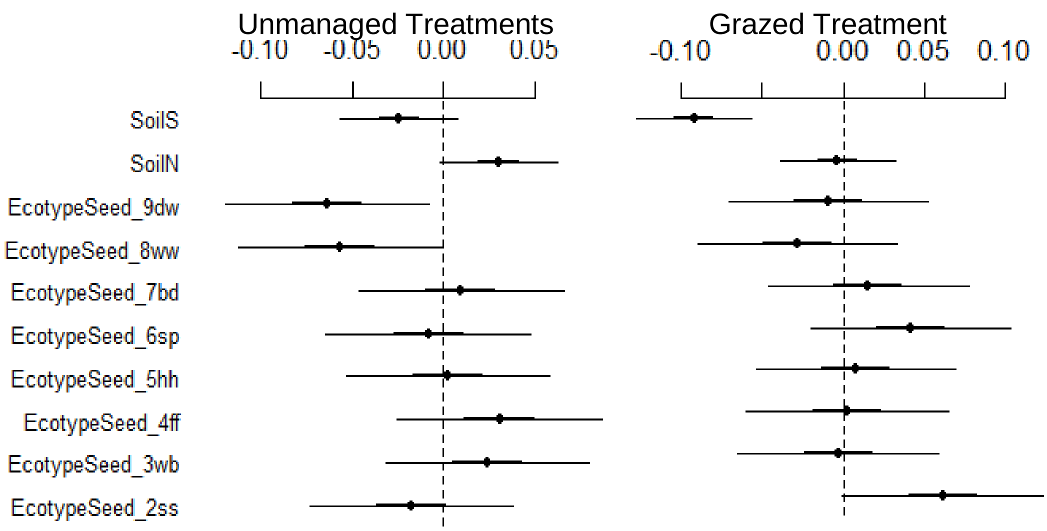


Figure X 28. Leaf-nitrogen GLMM effects plot with management split. Cockey Down in calcareous loam + grazed treatment is used as the standard. Factors with capital letter (S, N) are treatments, those with lower case letters (s, n, unmanaged, cut) are ecotype factors. See Table 17 in Chapter 13 for ecotype key.

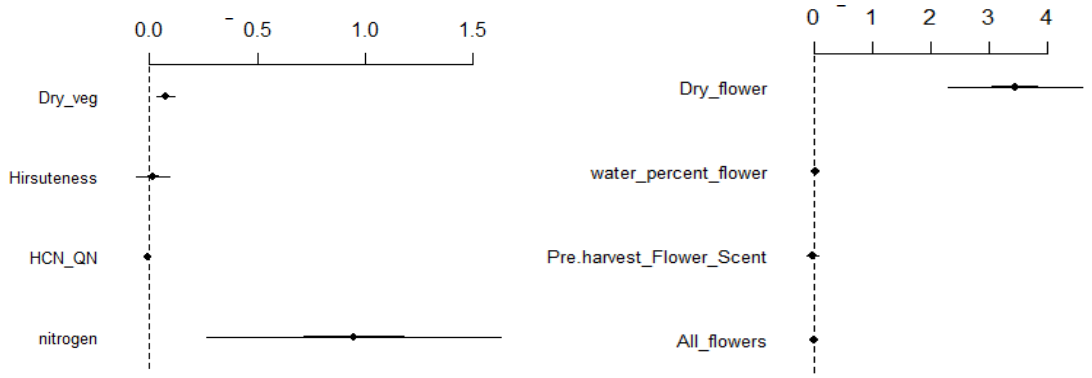


Figure X 29. Bee flower visits, GLMM effects plots. Factors with capital letter (S, N) are treatments, those with lower case letters (s, n, unmanaged, cut) are ecotype factors.

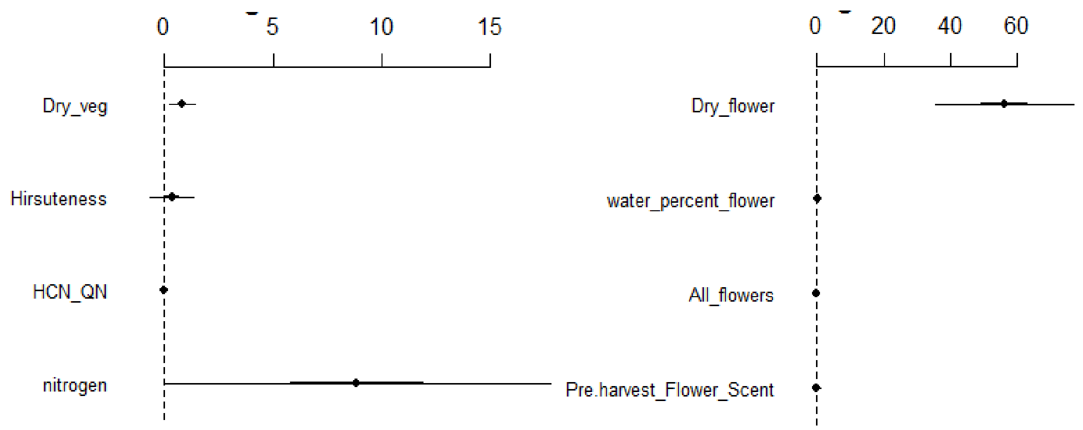


Figure X 30. Time spent (bees) on plant flowers, GLMM effects plots. Factors with capital letter (S, N) are treatments, those with lower case letters (s, n, unmanaged, cut) are ecotype factors.

APPENDIX XI – GLMM (NLME) Spatial Autocorrelation

Table XI 1. AIC numbers and P values from comparison of ANOVA between nlme and spatial nlme.

		All	Grazed	Unmanaged
Main Stem Length (Soil+Mgmt)	nlme	3208	1591	1553
	Spatial	3212	1595	1557
	P	*0.9996*	*1*	*1*
Main Stem Length (Harvest)	nlme	2906	1331	1508
	Spatial	2910	1335	1512
	P	*1*	*1*	*0.986*
Stems (Harvest)	nlme	3664	1839	1769
	Spatial	3668	1843	1773
	P	*1*	*1*	*1*
Leaflets (Harvest)	nlme	5106	2091	2630
	Spatial	5110	2095	2634
	P	*1*	*1*	*0.7204*
Time to first pod	nlme	3040	1579	1364
	Spatial	3044	1583	1367
	P	*1*	*0.7899*	*1*
Pod number (harvest)	nlme	4365	1709	2276
	Spatial	4369	1713	2280
	P	*1*	*1*	*0.6939*
Seeds per pod	nlme	2440	1265	1140
	Spatial	2444	1269	1143
	P	*1*	*1*	*0.5297*
Grazed clippings dry biomass	nlme	671.0	N/A	N/A
	Spatial	674.7		
	P	*0.8691*		
Dry vegetation biomass (Harvest)	nlme	1589	502.8	888.3
	Spatial	1593	506.8	892.1
	P	*1*	*1*	*0.8802*
Vegetation Percent moisture (Harvest)	nlme	2925	1508	1375
	Spatial	2928	1508	1379
	P	*0.9005*	*0.0908*	*1*
Hydrogen Cyanide (HCN)	nlme	3474	N/A	N/A
	Spatial	3478		
	P	*1*		
Nitrogen	nlme	-580.8	-229.9	-303.9
	Spatial	-576.8	-225.9	-300.9
	P	*1*	*1*	*0.6204*
Time to first flower	nlme	1689	886.2	817
	Spatial	1693	890.2	820
	P	*1*	*1*	*0.591*
Pre-harvest flower scent	nlme	1265	N/A	N/A
	Spatial	1269		
	P	*1*		
Flower number (both years)	nlme	4053	N/A	N/A
	Spatial	4057		
	P	*1*		

Variograms - Plant Fitness Results

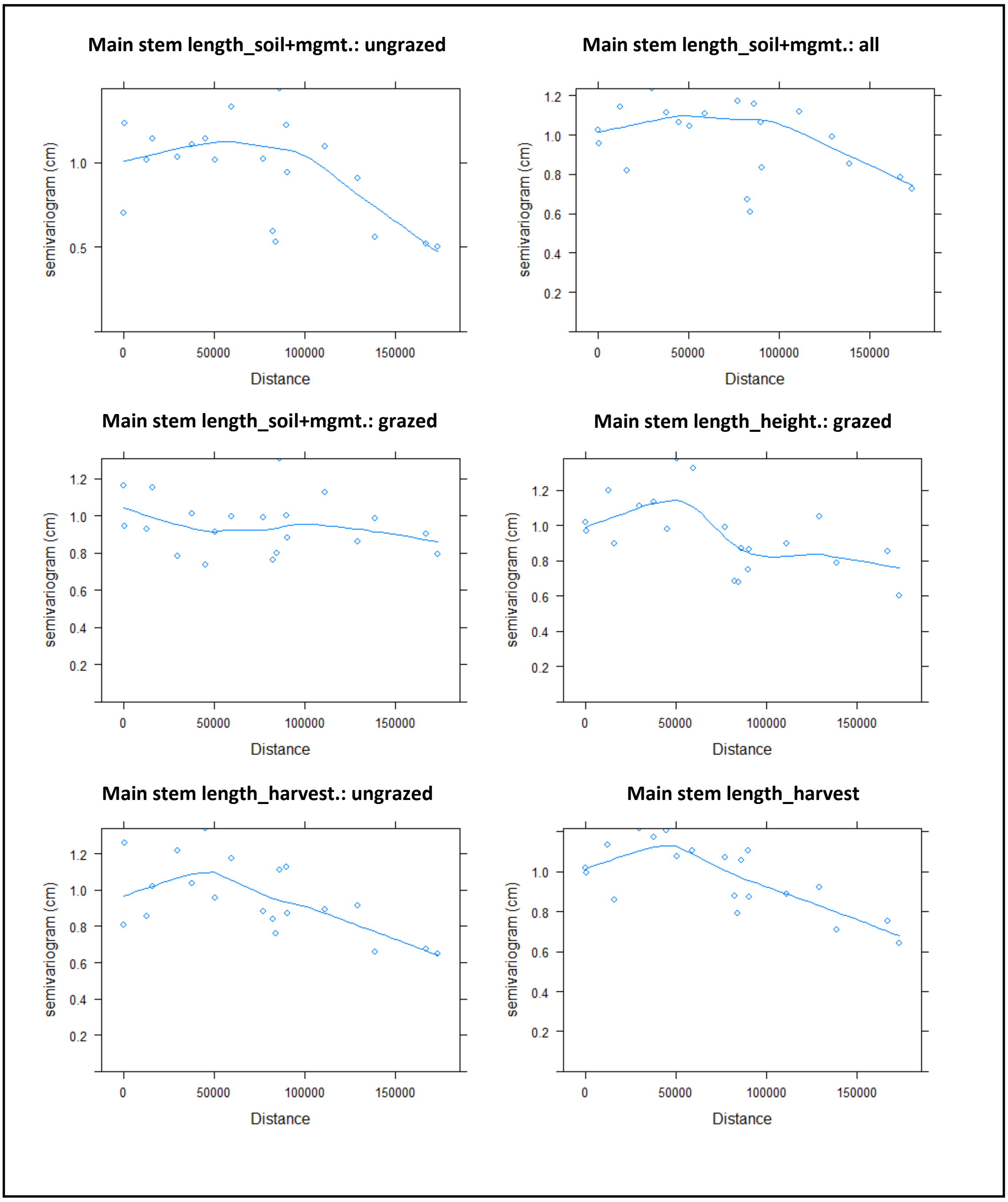


Figure XI 1. Spatial nlme variograms of main stem length.

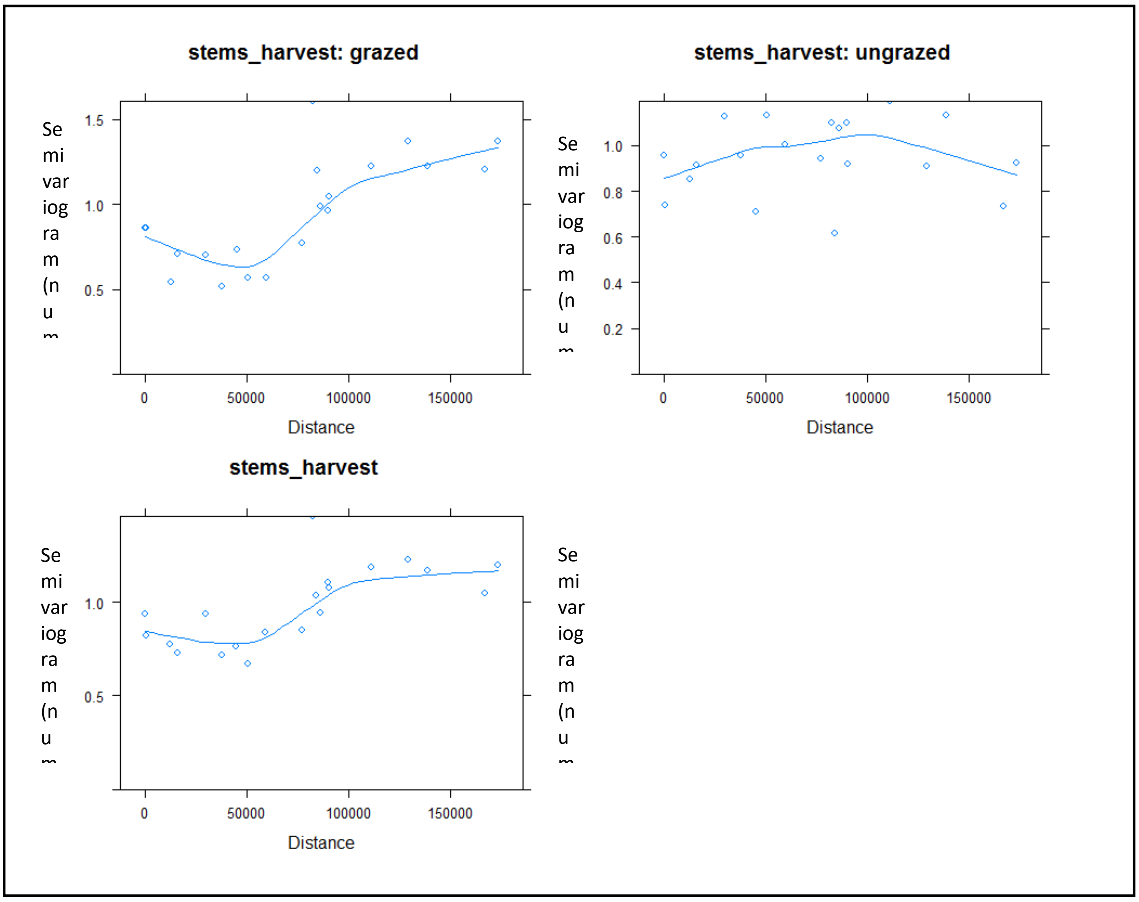


Figure XI 2. Spatial nlme variograms of stems per plant.

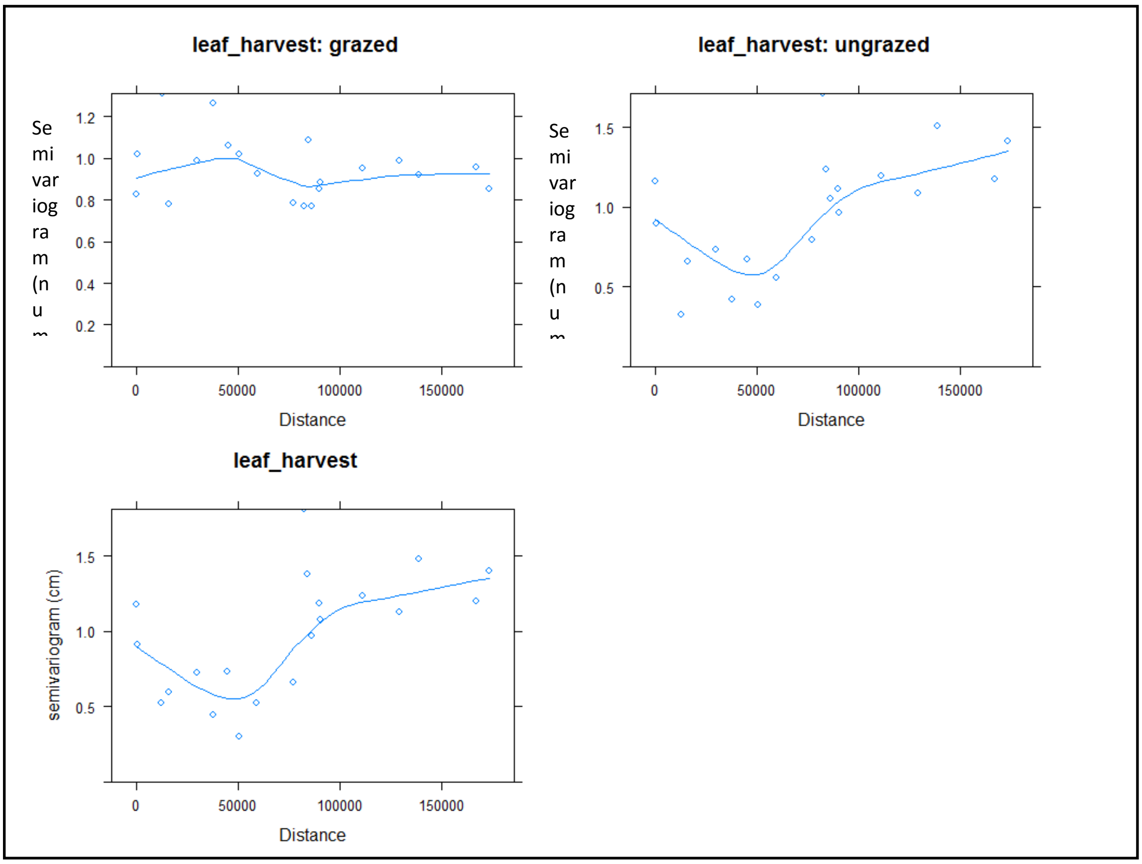


Figure XI 3. Spatial nlme variograms of leaflets per main stem.

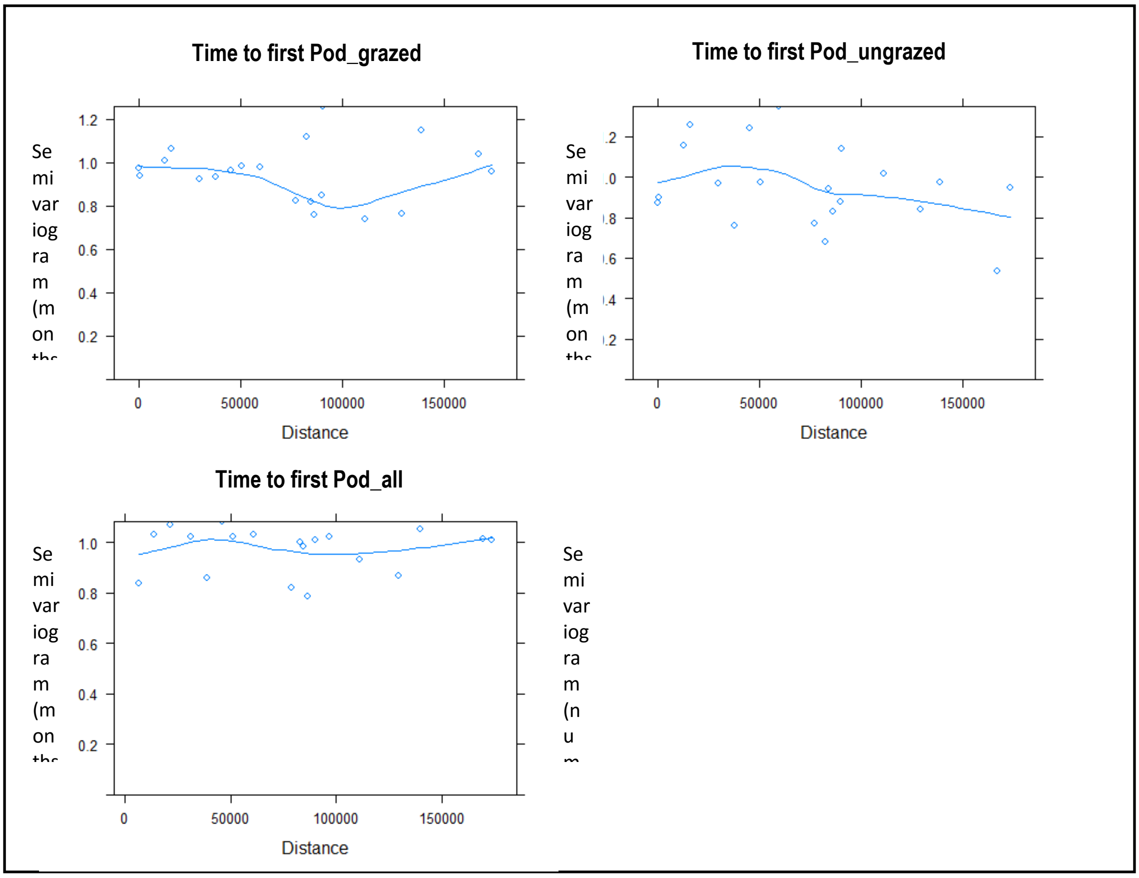


Figure XI 4. Spatial nlme variograms of time to first pod.

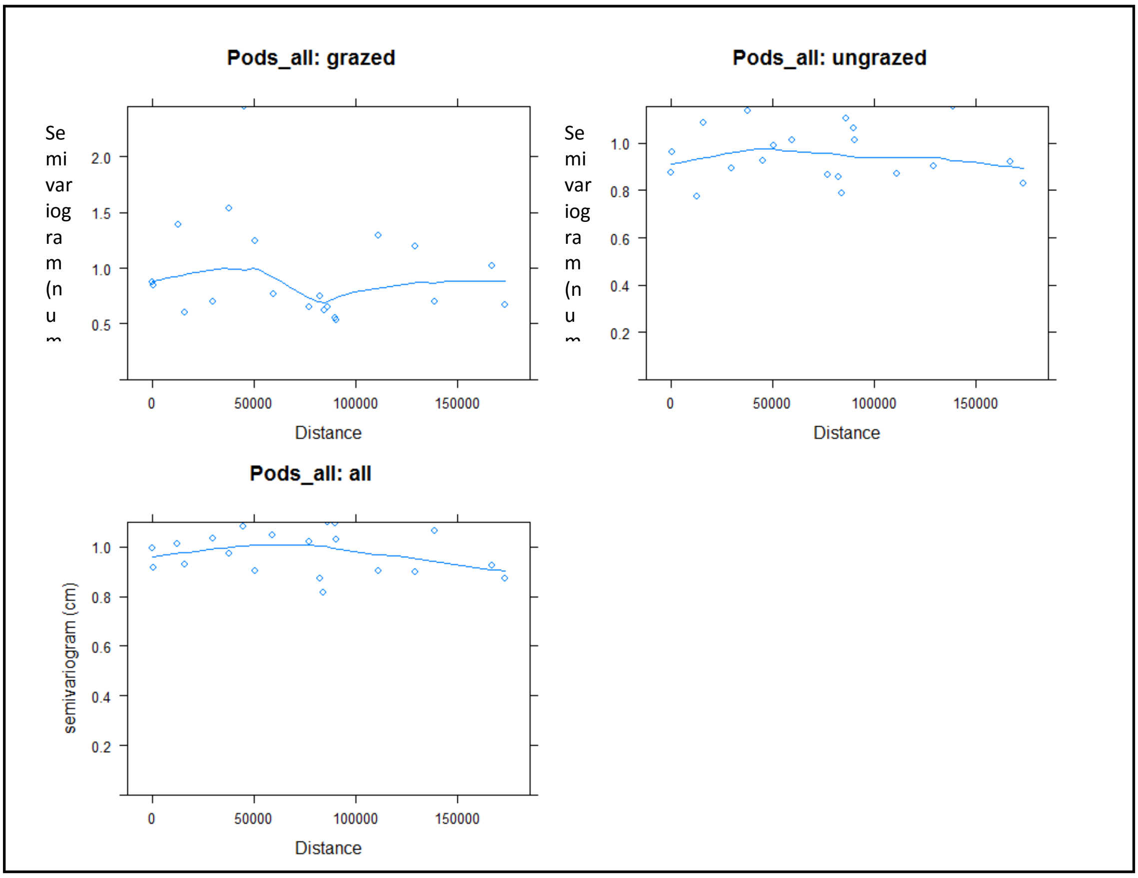


Figure XI 5. Spatial nlme variograms of pod number.

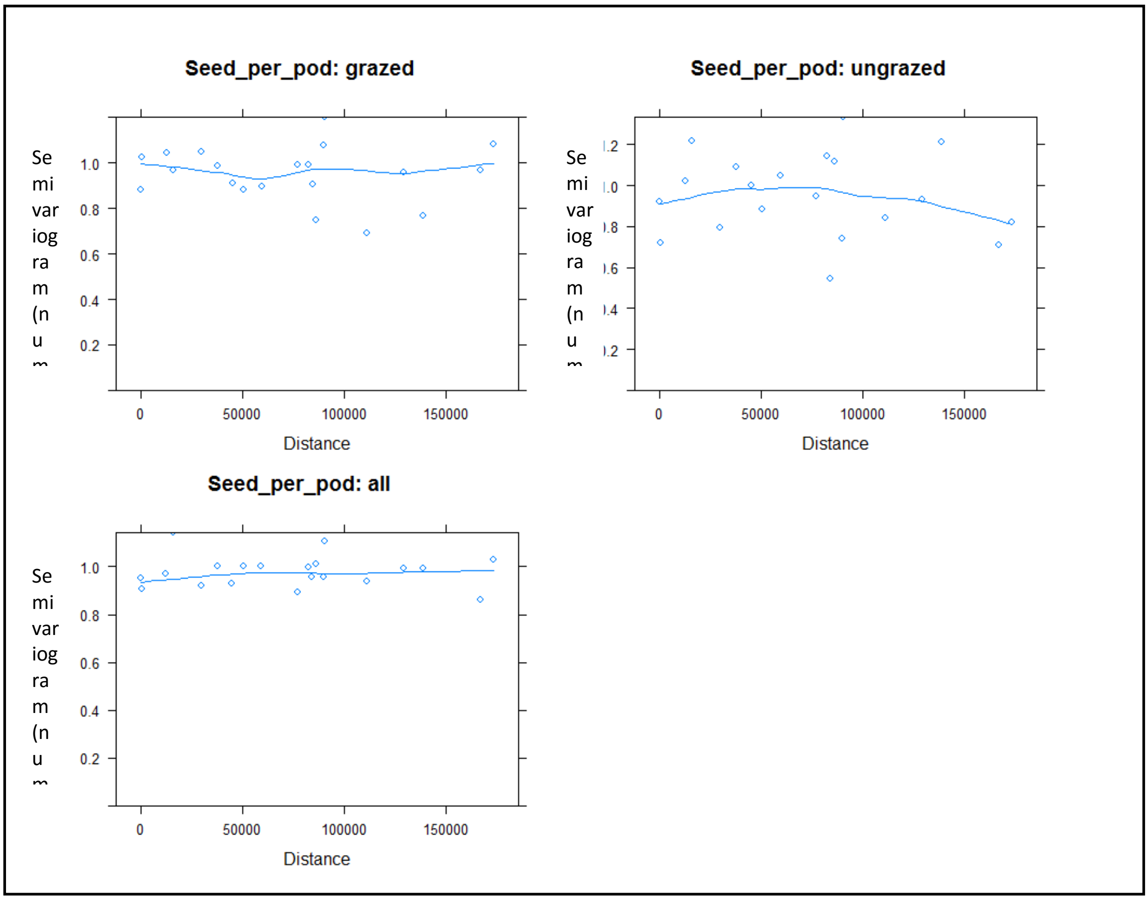


Figure XI 6. Spatial nlme variograms of seed per pod.

Variograms - Herbivore Requirement Results

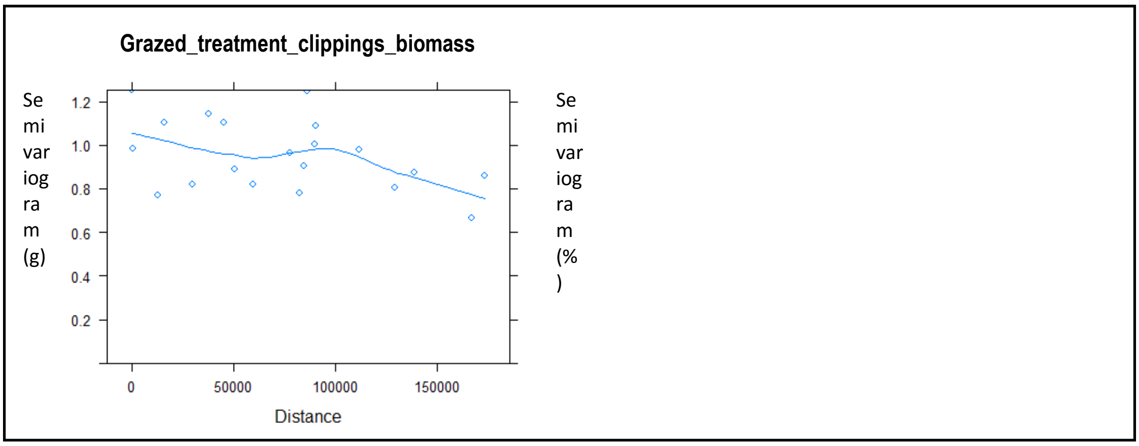


Figure XI 7. Spatial nlme variogram of grazed clippings biomass.

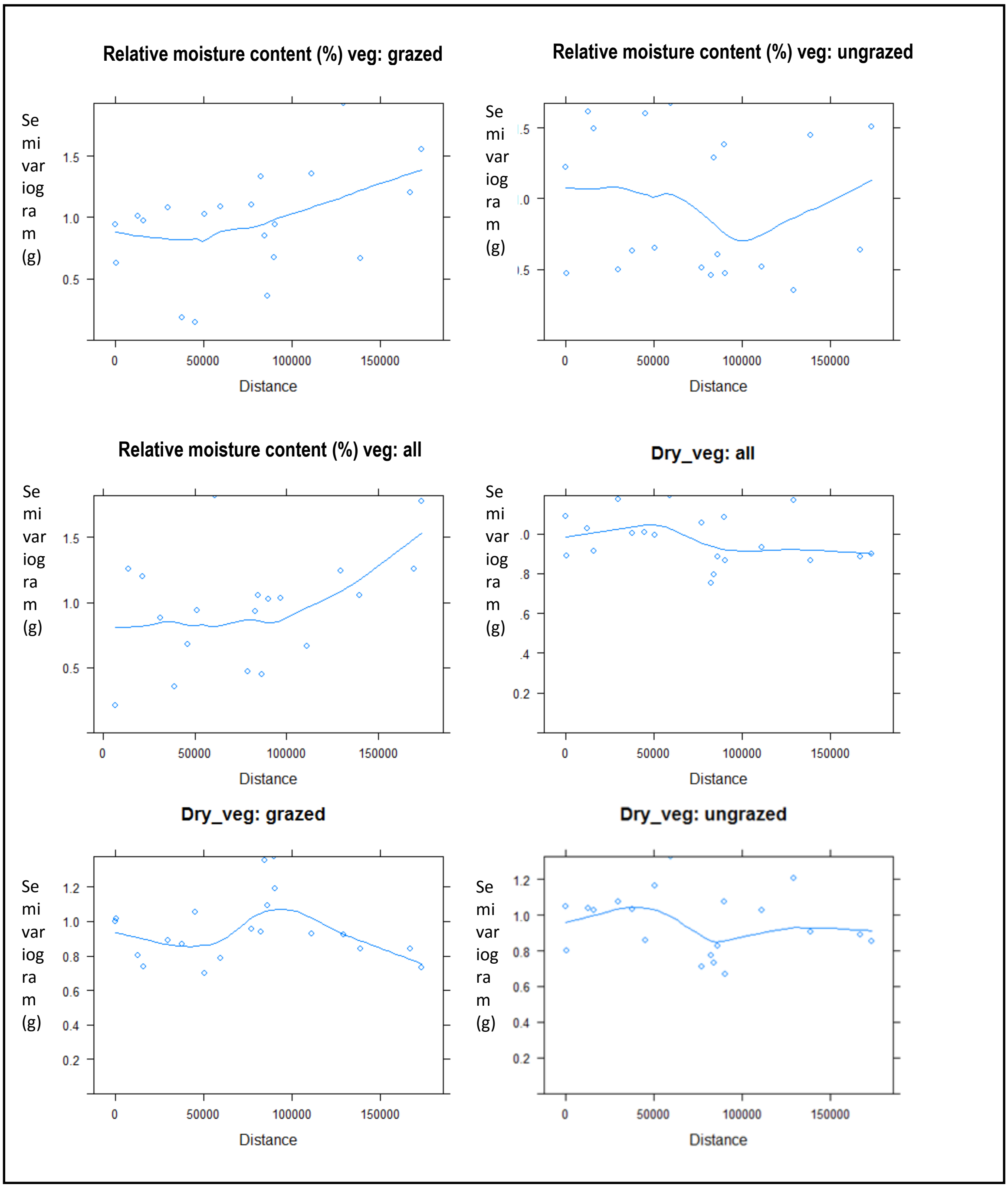


Figure XI 8. Spatial nlme variograms of harvest vegetative biomass.

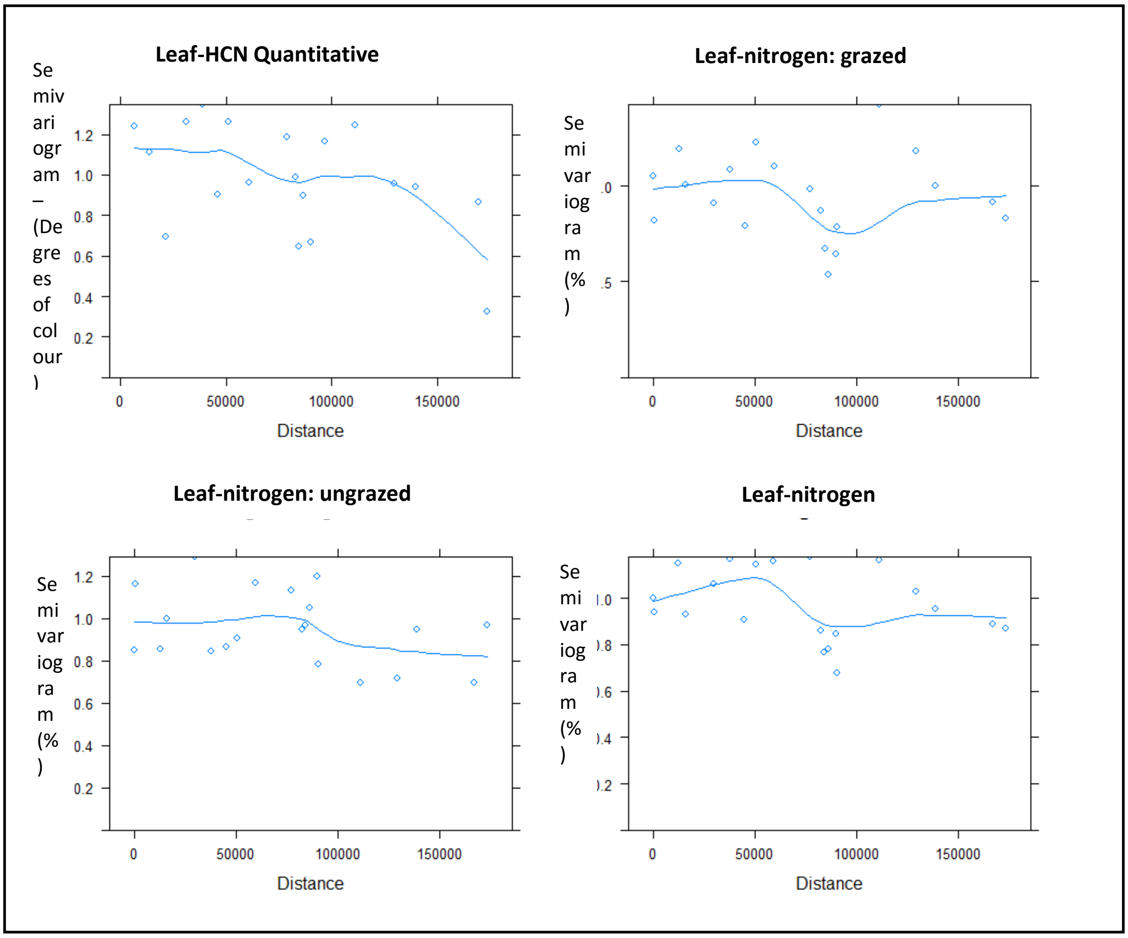


Figure XI 9. Spatial nlme variograms of chemical analysis.

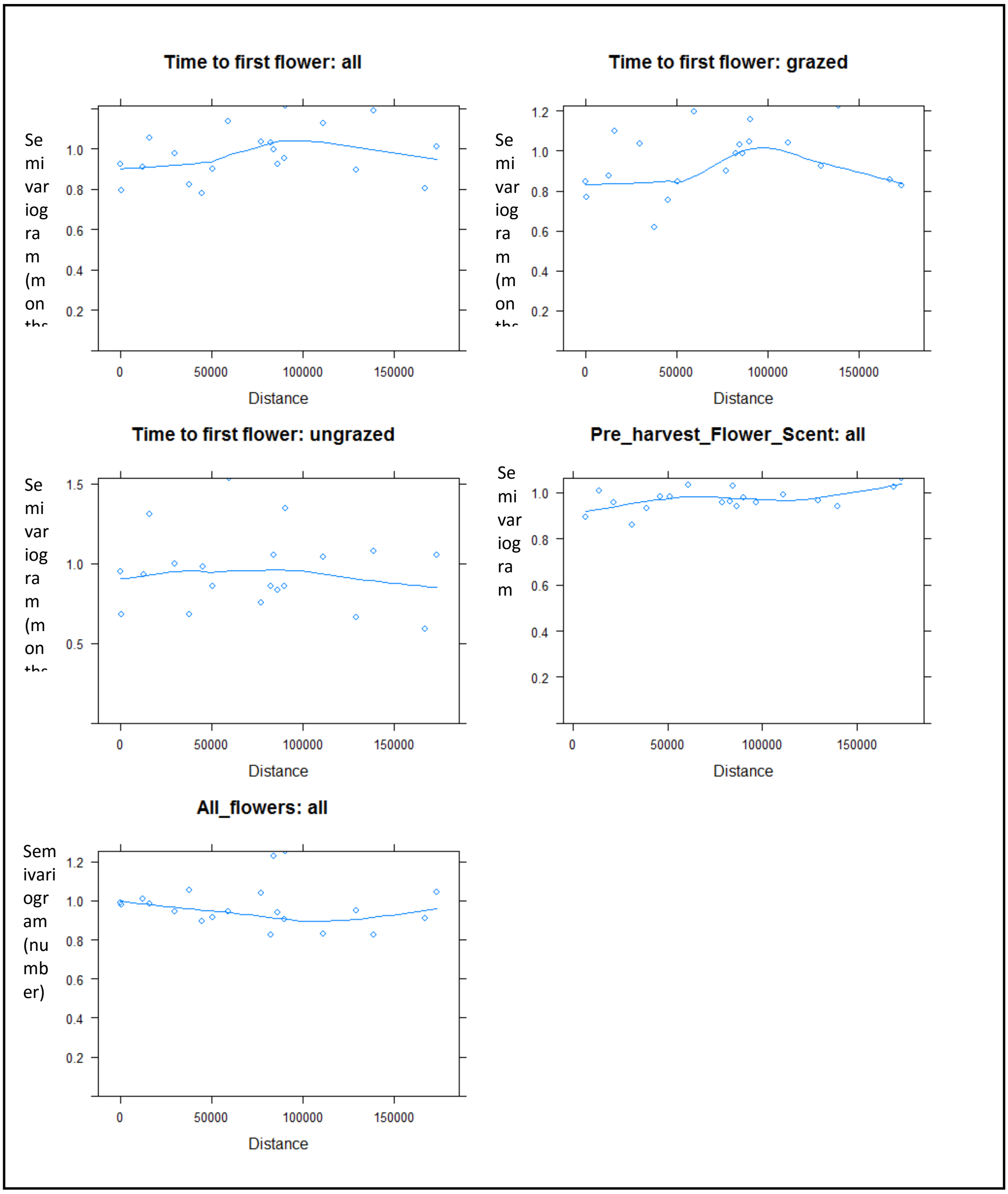


Figure XI 10. Spatial nlme variograms of flowering phenology, flower scent and flower production.

APPENDIX XII - Growth and Development Measurements

Table XII 1. Monthly means of Main Stem Length. (Ecotype n=48., Soil n=144, Management n=216). See Table 17 in Chapter 13 for ecotype key.

	Ecotype									Soil			Management	
	cd	**Ss**	**wb**	**ff**	**hh**	**sp**	**bd**	**ww**	**dw**	**C**	**N**	**S**	**G**	**U**
1.October 2011	**1.55**	**0.93**	**1.12**	**1.17**	**1.44**	**1.1**	**1.23**	**1.11**	**1.07**	**1.19**	**1.18**	**1.21**	**1.17**	**1.22**
St. Error	0.07	0.04	0.04	0.05	0.07	0.05	0.06	0.05	0.05	0.0	0.0	0.0	0.03	0.03
2.November	**1.8**	**1.72**	**1.84**	**1.53**	**1.98**	**1.81**	**2.22**	**2.15**	**1.93**	**1.87**	**1.7**	**2.09**	**1.87**	**1.9**
St. Error	0.08	0.14	0.16	0.07	0.13	0.15	0.21	0.15	0.13	0.1	0.1	0.1	0.07	0.07
3.December	**3.25**	**3.8**	**3.37**	**2.66**	**3.33**	**3.54**	**4.03**	**3.6**	**3.64**	**3.56**	**2.61**	**4.24**	**3.36**	**3.58**
St. Error	0.28	0.41	0.33	0.24	0.29	0.45	0.39	0.32	0.25	0.1	0.1	0.3	0.16	0.16
4.January 2012	**6.94**	**7.52**	**6.29**	**5.64**	**6.92**	**6.3**	**7.57**	**6.43**	**7.6**	**8.37**	**4.99**	**7.04**	**6.62**	**6.98**
St. Error	0.54	0.79	0.60	0.54	0.59	0.75	0.69	0.52	0.50	0.3	0.2	0.4	0.30	0.29
5.February	**11.17**	**11.9**	**9.74**	**9.33**	**11.8**	**10.23**	**11.25**	**10.17**	**12.27**	**15.08**	**7.33**	**10.22**	**10.56**	**11.18**
St. Error	0.97	1.22	0.94	0.88	0.97	1.06	1.07	0.77	0.86	0.5	0.3	0.6	0.47	0.46
6.March	**16.73**	**17.27**	**14.49**	**14.34**	**16.56**	**15.36**	**15.64**	**14.6**	**16.97**	**21.6**	**11.36**	**14.37**	**15.23**	**16.32**
St. Error	1.26	1.51	1.29	1.20	1.30	1.32	1.30	1.02	1.15	0.6	0.5	0.8	0.59	0.61
7.March/April	**16.95**	**17.34**	**15.69**	**16.71**	**16.96**	**15.99**	**15.93**	**16.7**	**17.56**	**20.22**	**14.04**	**15.68**	**12.78**	**20.52**
St. Error	1.12	1.17	1.24	1.04	1.13	1.09	1.06	0.91	1.11	0.7	0.5	0.6	0.29	0.57
8.April/ May	**16.41**	**17.06**	**16.11**	**15.85**	**16.31**	**15.91**	**16.24**	**16.43**	**17.18**	**19.8**	**14.1**	**15.26**	**9.89**	**22.89**
St. Error	1.40	1.39	1.46	1.20	1.29	1.38	1.30	1.21	1.28	0.9	0.6	0.8	0.16	0.60
9.May /June	**16.55**	**17.9**	**17.71**	**17.18**	**17.4**	**16.19**	**17.31**	**17.23**	**18.26**	**20.91**	**15.09**	**15.92**	**11.24**	**23.33**
St. Error	1.44	1.44	1.44	1.30	1.33	1.27	1.29	1.09	1.16	0.8	0.6	0.8	0.25	0.60
10. June/ July	**22.33**	**24.38**	**23.34**	**23.28**	**22.99**	**21.9**	**22.88**	**20.99**	**23.79**	**25.89**	**22.01**	**20.72**	**19.07**	**26.68**
St. Error	1.16	1.19	1.28	1.23	1.18	0.92	1.27	1.07	1.16	0.7	0.6	0.7	0.39	0.57
11. Harvest July/Aug	**24.24**	**24.26**	**21.67**	**22.95**	**21.44**	**23.04**	**22.51**	**20.51**	**22.55**	**25.4**	**21.06**	**21.26**	**17.29**	**27.86**
St. Error	1.27	1.42	1.30	1.43	1.49	1.13	1.53	1.08	1.14	0.8	0.6	0.8	0.39	0.61
12. Post Harvest Aug/Sept	**14.19**	**15.37**	**13.57**	**16.73**	**15.61**	**12.97**	**14.15**	**13.73**	**15.31**	**16.46**	**14.21**	**13.23**	**13.61**	**15.65**
St. Error	0.67	0.79	0.87	1.12	0.70	0.77	0.70	0.59	0.84	0.5	0.5	0.5	0.33	0.42
13. April 2013	**11.51**	**10.42**	**10.81**	**9.47**	**13.43**	**9.38**	**13.04**	**11.03**	**13.16**	**11.81**	**11.64**	**10.1**	**10.97**	**11.76**
St. Error	0.72	0.63	0.81	0.68	0.60	0.71	0.79	0.65	0.64	0.4	0.5	0.4	0.30	0.37
14. October	**9.63**	**10.46**	**9.23**	**8.71**	**10.87**	**8.37**	**9.86**	**10.64**	**10.59**	**10.81**	**9.5**	**8.68**	**8.32**	**11.32**
St. Error	0.64	0.68	0.64	0.57	0.87	0.72	0.59	0.60	0.78	0.4	0.4	0.4	0.22	0.38

Table XII 2. Monthly means of stem number per plant (Ecotype n=48., Soil n=144, Management n=216). See Table 17 in Chapter 13 for ecotype key.

	Ecotype									Soil			Management	
	cd	Ss	wb	ff	hh	sp	bd	ww	dw	C	N	S	G	U
1.October 2011	**2**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
St. Error	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2.November	**3**	**2**	**2**	**2**	**3**	**2**	**2**	**3**	**2**	**2**	**2**	**2**	**2**	**2**
St. Error	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3.December	**4**	**3**	**3**	**3**	**4**	**3**	**3**	**4**	**3**	**3**	**3**	**4**	**3**	**4**
St. Error	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4.January 2012	**6**	**5**	**5**	**5**	**6**	**5**	**6**	**6**	**6**	**6**	**5**	**5**	**6**	**6**
St. Error	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5.February	**9**	**8**	**8**	**9**	**9**	**8**	**8**	**9**	**11**	**10**	**7**	**8**	**8**	**9**
St. Error	1	1	0	1	0	1	0	1	1	0	1	0	0	0
6.March	**16**	**15**	**14**	**15**	**16**	**16**	**18**	**17**	**17**	**20**	**13**	**14**	**17**	**15**
St. Error	1	1	1	1	1	2	2	2	1	1	1	1	1	0
7.Harvest	**36**	**33**	**32**	**35**	**36**	**35**	**44**	**44**	**43**	**44**	**33**	**35**	**42**	**33**
St. Error	3	2	2	2	3	2	3	3	3	2	1	2	1	1

Table XII 3. Monthly means of leaflets per longest branch (Ecotype n=48., Soil n=144, Management n=216). See Table 17 in Chapter 13 for ecotype key.

	Ecotype									Soil			Management	
	cd	Ss	wb	ff	hh	sp	bd	ww	dw	C	N	S	G	U
1.October 2011	**7**	**3**	**4**	**5**	**7**	**4**	**5**	**3**	**4**	**5**	**5**	**5**	**5**	**5**
St.Error	0	0	0	0	0	0	1	0	0	0	0	0	0	0
2.November	**12**	**11**	**12**	**11**	**12**	**11**	**13**	**13**	**12**	**12**	**10**	**13**	**12**	**12**
St.Error	0	1	1	1	1	1	1	1	1	0	0	0	0	0
3.December	**19**	**22**	**20**	**17**	**19**	**19**	**24**	**21**	**23**	**22**	**17**	**22**	**20**	**21**
St.Error	1	1	1	1	1	1	1	1	1	1	1	1	1	1
4.January 2012	**42**	**44**	**43**	**37**	**41**	**39**	**47**	**43**	**50**	**53**	**34**	**41**	**41**	**45**
St.Error	4	3	4	5	3	4	5	4	4	2	2	2	2	2
5.February	**72**	**76**	**71**	**66**	**80**	**73**	**84**	**76**	**95**	**114**	**51**	**65**	**76**	**78**
St.Error	8	9	8	7	8	8	11	8	11	6	3	4	4	4
6.March	**124**	**122**	**122**	**116**	**160**	**130**	**134**	**114**	**173**	**224**	**82**	**92**	**127**	**138**
St.Error	13	14	15	14	19	16	18	12	25	12	5	7	7	8
7.Harvest	**80**	**68**	**60**	**66**	**95**	**78**	**115**	**125**	**116**	**117**	**85**	**66**	**56**	**122**
St.Error	15	7	6	6	15	10	18	22	22	12	8	4	2	9

Table XII 4. Monthly means of branch number per main stem (Ecotype n=48., Soil n=144, Management n=216). See Table 17 in Chapter 13 for ecotype key.

	Ecotype									Soil			Management	
	cd	**ss**	**wb**	**ff**	**hh**	**sp**	**bd**	**ww**	**dw**	**C**	**N**	**S**	**G**	**U**
1.October 2011	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
St.Error	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2.November	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
St.Error	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3.December	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
St.Error	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4.January 2012	**1**	**1**	**1**	**0**	**1**	**1**	**1**	**1**	**2**	**2**	**1**	**1**	**1**	**1**
St.Error	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5.February	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**3**	**4**	**1**	**1**	**2**	**2**
St.Error	0	0	0	0	0	0	0	0	1	0	0	0	0	0
6.March	**8**	**6**	**6**	**5**	**7**	**6**	**6**	**6**	**5**	**10**	**3**	**5**	**6**	**6**
St.Error	1	1	1	1	1	1	1	1	1	1	0	1	1	1
7.Harvest	**3**	**3**	**3**	**2**	**3**	**3**	**3**	**4**	**4**	**4**	**3**	**2**	**2**	**4**
St.Error	1	0	0	0	0	0	0	1	1	0	0	0	0	0

APPENDIX XIII – Fecundity

Table XIII 1. Means showing month of first pod formation (Ecotype N=48, Soil N=144, Management N=216). See Table 17 in Chapter 13 for ecotype key.

		2012									
	Ecotype	27th Mar	12th Apr	24th Apr	08th May	05th Jun	19th Jun	15th July	6th Sept	2013	None
Ecotype	cd	9	0	11	2	0	2	19	0	2	3
	ss	11	1	12	0	1	3	16	0	2	2
	wb	11	2	9	3	2	5	12	1	2	1
	ff	4	2	11	2	1	3	22	0	2	1
	hh	14	2	10	0	2	6	13	0	0	1
	sp	7	0	8	3	0	3	20	1	3	3
	bd	3	2	14	3	1	1	19	0	4	1
	ww	7	2	10	2	1	3	20	0	0	3
	dw	6	3	6	6	2	1	21	0	1	2
Soil treatment	Calcareous loam	33	7	37	9	2	10	41	0	3	2
	Neutral loam	15	3	24	9	3	9	60	2	11	8
	Calcareous sand	24	4	30	3	5	8	61	0	2	7
Management treatment	Grazed	8	0	24	3	6	25	119	2	13	16
	Unmanaged	64	14	67	18	4	2	43	0	3	1

APPENDIX XIV – Plant Mortality

Table XIV 1. Plant mortality details

Date	Plant Number	Ecotype	Soil treatment	Management treatment
25/10/11	46	Southstoke	Calcareous Sand	Grazed
08/11/11	354	Cockey Down	Calcareous Sand	Unmanaged
	90	Hellenge Hill	Neutral	Unmanaged
22/11/11	10	Hellenge Hill	Neutral	Unmanaged
	398	Folly Farm	Neutral	Unmanaged
03/01/12	159	Cockey Down	Neutral	Unmanaged
	318	Woolacombe	Calcareous loam	Unmanaged
	340	Folly Farm	Calcareous Sand	Grazed
17/01/12	301	Cockey Down	Neutral	Grazed
14/02/12	192	Dawlish Warren	Neutral	Grazed
27/02/12	422	Folly Farm	Neutral	Grazed
13/03/12	388	Hellenge Hill	Calcareous loam	Unmanaged
12/04/12	379	Dawlish Warren	Neutral	Grazed
08/05/12	159	Cockey Down	Neutral	Unmanaged
15/07/12	340	Folly Farm	Calcareous Sand	Grazed
23/08/12	102	Woodborough	Neutral	Grazed
	252	Salisbury Plain	Neutral	Unmanaged
Post winter 25/04/13	15	Dawlish Warren	Calcareous Sand	Unmanaged
	84	Dawlish Warren	Neutral	Unmanaged
	153	Folly Farm	Neutral	Grazed
	183	Salisbury Plain	Neutral	Unmanaged
	263	Berrow Dunes	Calcareous loam	Grazed
	307	Salisbury Plain	Calcareous loam	Grazed
	64	Woolacombe	Calcareous loam	Unmanaged
27/10/13	206	Southstoke	Calcareous loam	Grazed
	380	Woolacombe	Neutral	Grazed

Table XIV 1. Plant mortality. Kruskall-Wallis results between matching and mis-matching treatments

Ecotype	Cal loam	Neutral	Cal sand	Grazed	Unmanaged
Matching treatment	1	7	1	3	4
Mis-matching treatment	6	4	7	8	3
P	*0.2747*	*0.0111*	*0.2747*	*0.3483*	*0.4059*

APPENDIX XV – Flowering Phenology

Table XV 1. Month of first flowering 2012. Totals of each outcome variable for that month. Ecotype N=48, Soil N=144, Management N=216. See Table 17 in Chapter 13 for ecotype key.

	Ecotype	January	February	March	April	May	June	July	August	September	None
Ecotype	**cd**	0	0	6	14	4	0	16	6	0	2
	ss	0	0	17	16	1	2	10	2	0	0
	wb	1	3	9	25	2	1	5	2	0	0
	ff	0	0	8	17	2	0	15	4	0	2
	hh	1	0	15	24	1	1	5	1	0	0
	sp	0	0	6	20	2	1	10	8	0	1
	bd	0	0	8	16	2	3	14	5	0	0
	ww	0	1	11	16	3	0	13	4	0	0
	dw	1	3	12	15	1	1	12	3	0	0
Soil treatment	**Calcareous loam**	1	6	48	60	5	1	19	4	0	0
	Neutral loam	1	6	48	60	5	1	19	4	0	0
	Calcareous sand	2	1	29	48	6	3	37	17	0	1
Management treatment	**Grazed**	2	3	42	67	1	7	70	22	0	2
	Unmanaged	1	4	50	96	17	2	30	13	0	3

Table XV 2. Mean Flower number counted monthly 2012 and 2013 (Ecotype N=48, Soil N=144, Management=246). See Table 17 in Chapter 13 for ecotype key.

	Ecotype									Soil			Management	
	cd	ss	wb	ff	hh	sp	bd	ww	dw	C	N	S	G	U
January 2012	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
St.Error	0	0	0	0	0	0	0	0	0	0	0	0	0	0
February 2012	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
St.Error	0	0	0	0	0	0	0	0	0	0	0	0	0	0
March 2012	**1**	**2**	**3**	**1**	**1**	**1**	**1**	**1**	**3**	**3**	**0**	**1**	**1**	**1**
St.Error	0	1	1	0	0	0	0	0	1	0	0	0	0	0
April 2012	**4**	**6**	**7**	**4**	**6**	**5**	**4**	**5**	**5**	**8**	**3**	**4**	**4**	**7**
St.Error	1	1	1	1	1	1	1	1	1	1	0	1	0	0
May 2012	**2**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**2**	**1**	**1**	**0**	**2**
St.Error	1	0	0	0	0	0	0	0	0	0	0	0	0	0
June 2012	**0**	**1**	**2**	**0**	**1**	**0**	**1**	**0**	**1**	**1**	**0**	**1**	**1**	**0**
St.Error	0	0	1	0	0	0	1	0	0	0	0	0	0	0
July 2012	**8**	**9**	**9**	**5**	**8**	**7**	**8**	**8**	**8**	**9**	**8**	**6**	**9**	**7**
St.Error	2	1	1	1	1	1	1	1	1	1	1	1	1	0
August 2012	**7**	**3**	**5**	**4**	**3**	**4**	**4**	**5**	**4**	**6**	**4**	**4**	**6**	**3**
St.Error	1	1	1	1	1	1	1	1	1	1	0	0	0	0
April 2013	**8**	**6**	**7**	**3**	**14**	**2**	**9**	**10**	**8**	**9**	**8**	**6**	**6**	**9**
St.Error	2	1	1	1	2	1	2	2	1	1	1	1	0	1
May 2013	**1**	**3**	**4**	**2**	**17**	**1**	**9**	**11**	**6**	**7**	**7**	**4**	**5**	**7**
St.Error	1	1	1	1	2	0	2	2	1	1	1	1	0	1
June 2013	**9**	**7**	**8**	**6**	**5**	**9**	**7**	**5**	**6**	**9**	**6**	**5**	**7**	**6**
St.Error	1	1	1	1	1	1	1	1	1	1	1	0	0	0
July 2013	**2**	**2**	**1**	**1**	**2**	**2**	**1**	**1**	**1**	**2**	**1**	**1**	**2**	**1**
St.Error	1	0	0	0	0	0	0	0	0	0	0	0	0	0
August 2013	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
St.Error	0	0	0	0	0	0	0	0	0	0	0	0	0	0
October 2013	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
St.Error	0	0	0	0	0	0	0	0	0	0	0	0	0	0

APPENDIX XVI – Grazed Clippings Biomass

Table XVI 1. Monthly means of grazed clippings dry biomass (Ecotype N=24, Soil N=72). See Table 17 in Chapter 13 for ecotype key.

	Ecotype									Soil		
	cd	**ss**	**wb**	**ff**	**hh**	**sp**	**bd**	**ww**	**dw**	**C**	**N**	**S**
March 2012	**0.64**	**0.80**	**0.45**	**0.50**	**0.91**	**0.57**	**0.85**	**0.51**	**0.81**	**1.29**	**0.19**	**0.53**
	0.14	0.20	0.13	0.14	0.18	0.13	0.32	0.13	0.18	0.09	0.03	0.12
April 2012	**0.37**	**0.36**	**0.24**	**0.25**	**0.35**	**0.34**	**0.28**	**0.30**	**0.33**	**0.53**	**0.16**	**0.26**
	0.07	0.07	0.13	0.14	0.18	0.06	0.32	0.05	0.06	0.03	0.02	0.03
May 2012	**0.22**	**0.17**	**0.12**	**0.15**	**0.16**	**0.14**	**0.23**	**0.20**	**0.18**	**0.30**	**0.10**	**0.13**
	0.04	0.03	0.05	0.05	0.05	0.02	0.04	0.03	0.03	0.02	0.02	0.02
June 2012	**0.24**	**0.34**	**0.25**	**0.23**	**0.31**	**0.18**	**0.37**	**0.27**	**0.34**	**0.48**	**0.17**	**0.19**
	0.05	0.08	0.02	0.03	0.03	0.04	0.05	0.05	0.06	0.04	0.02	0.03
July 2012	**0.91**	**0.85**	**0.60**	**0.75**	**0.82**	**0.86**	**0.82**	**0.71**	**0.76**	**1.07**	**0.73**	**0.57**
	0.10	0.14	0.07	0.06	0.06	0.11	0.07	0.10	0.10	0.07	0.06	0.05
April 2013	**0.44**	**0.50**	**0.63**	**0.34**	**0.82**	**0.25**	**0.83**	**0.40**	**0.70**	**0.69**	**0.59**	**0.36**
	0.09	0.10	0.13	0.07	0.17	0.05	0.17	0.08	0.14	0.07	0.07	0.06
Total from all cuts	**2.38**	**2.52**	**1.66**	**1.89**	**2.55**	**2.10**	**2.52**	**1.99**	**2.42**	**3.67**	**1.33**	**1.68**
	0.31	0.37	0.26	0.32	0.33	0.29	0.47	0.26	0.32	0.13	0.10	0.01

APPENDIX XVII – Scattergraph Matrices

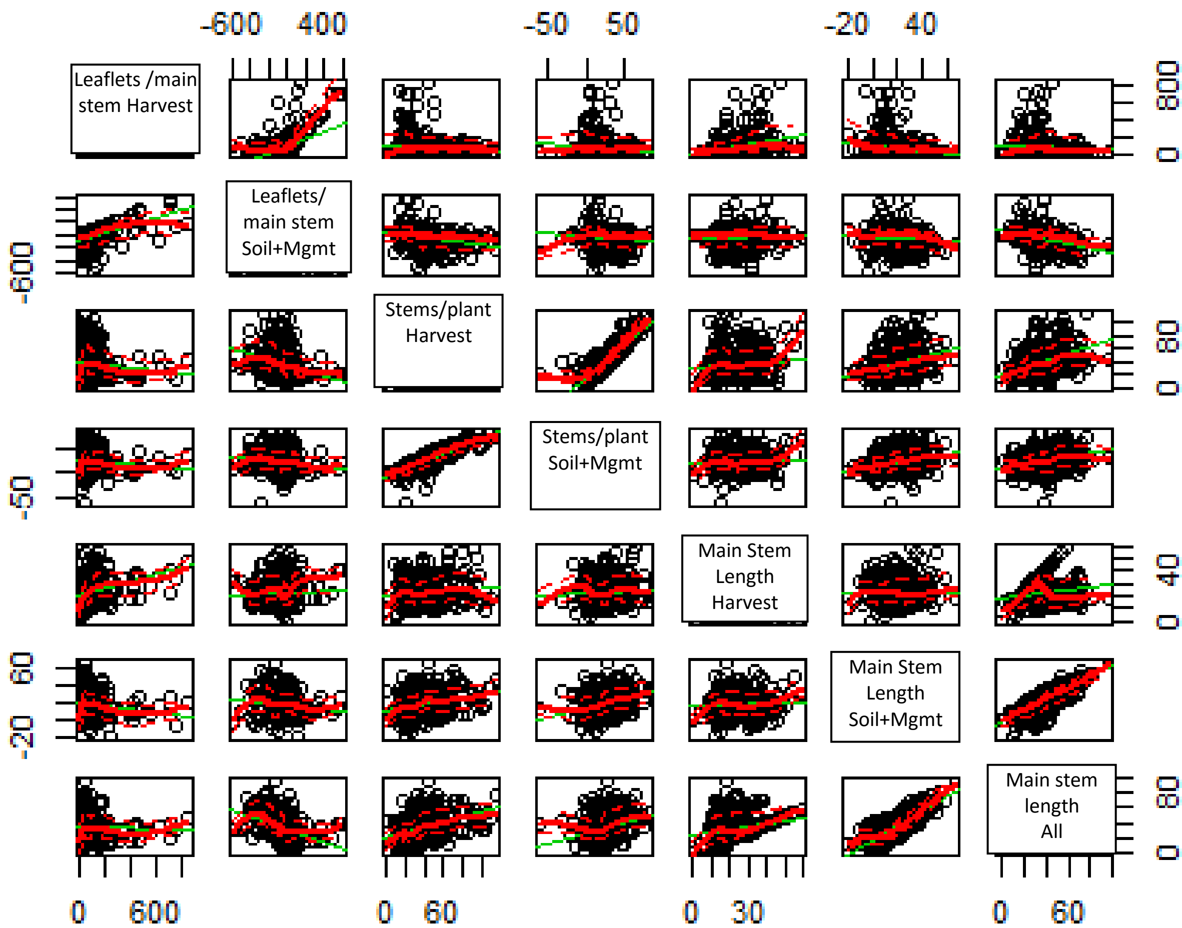


Figure XVII 1. Scattergraph matrix of main stem length (Harvest, Soil+Mgmt, and All)., number of leaflets per main stem (Harvest and Soil+Mgmt), and number of stems per plant (Harvest and Soil+Mgmt). See Figure 44 for explanation of groupings.

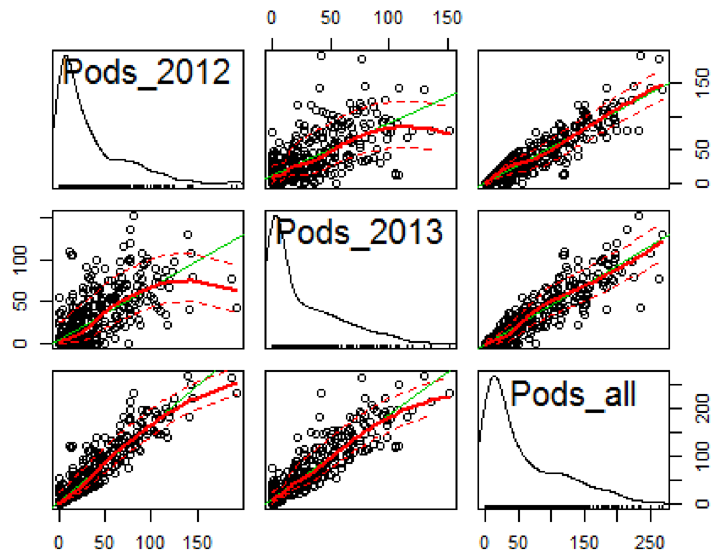


Figure XVII 2. Scattergraph matrix of seed pod count

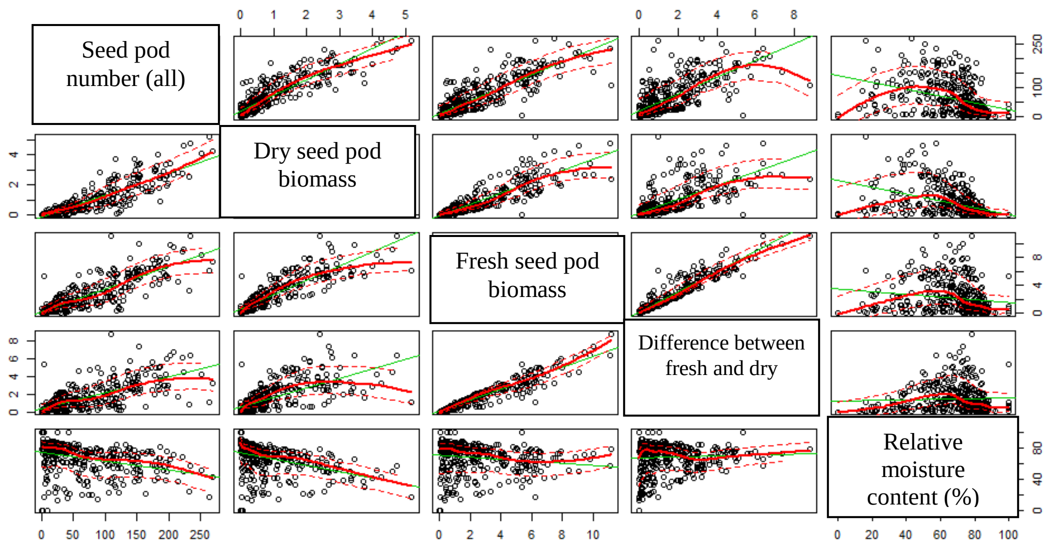


Figure XVII 377. Scattergraph matrix of seed pod biomass and total seed pod number

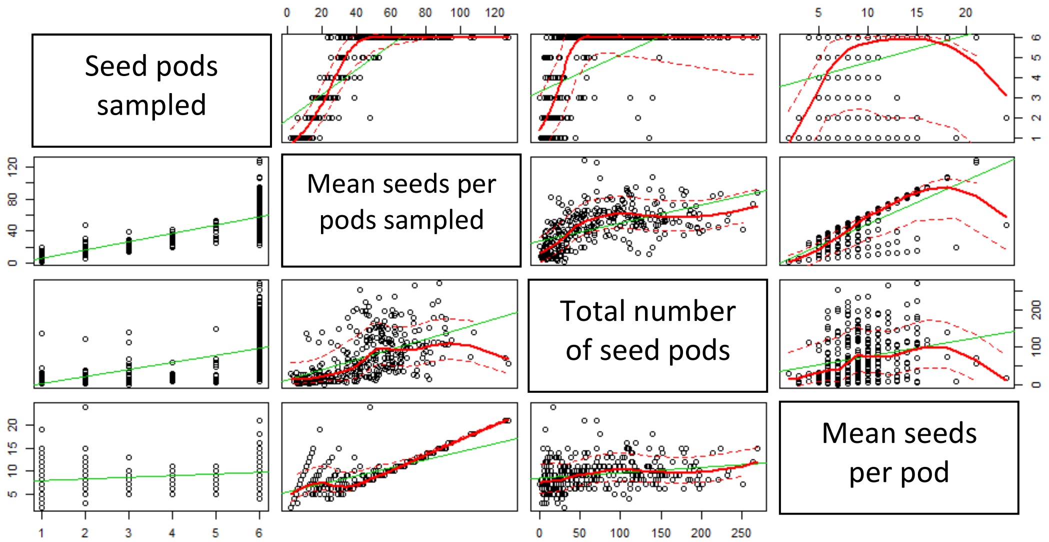


Figure XVII 4. Scattergraph matrix of seeds from six pods per plant, where less than six pods were available to sample a record of how many was also recorded ‘Seed pods sampled’.

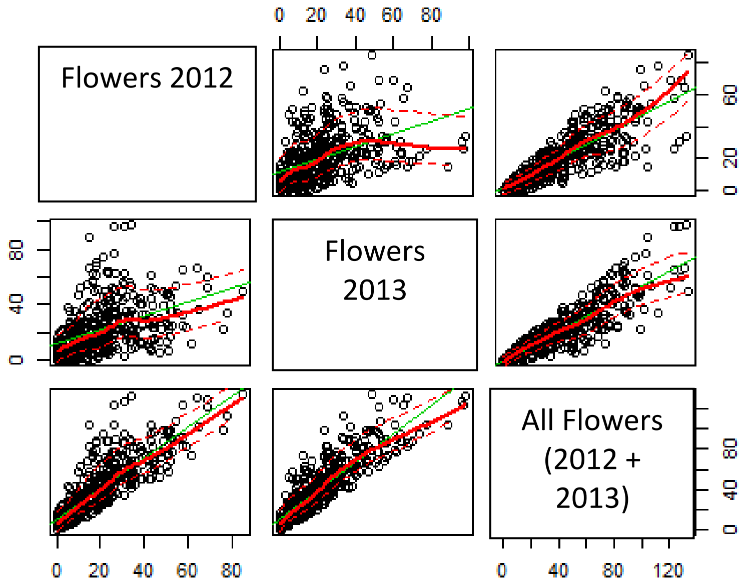


Figure XVII 5. Scattergraph matrix for flower production 2012, 2013 and all.

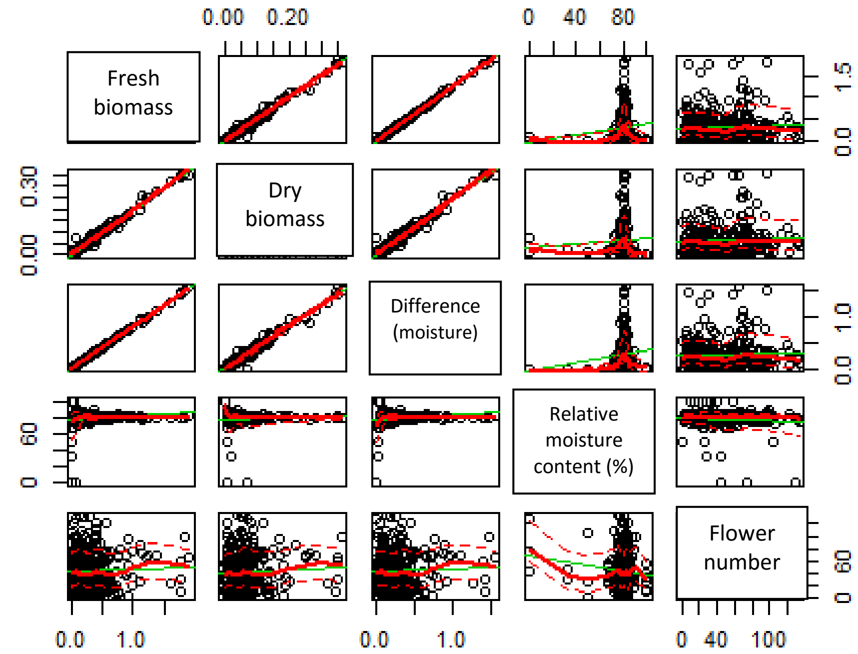


Figure XVII 6. Scattergraph matrix of flower biomass and total flower number (All_flowers).

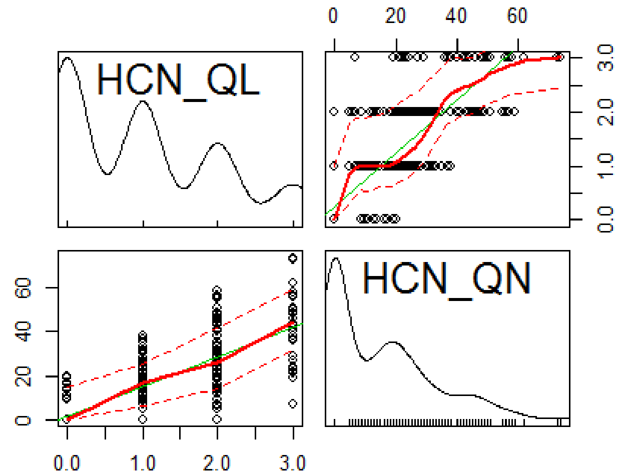


Figure XVII 7. Scattergraph matrix of leaf-HCN qualitative (QL) and quantitative (QN) results

APPENDIX XVIII – Bee Survey Details and Conditions

Table XVII 1. Bee survey details and glasshouse conditions.

Survey No.	Date	Time		Temperature (°C)		Okta
		Start	End	**Start**	**End**	
1	30/06/2012	10:40	11:40	20.16	21.43	7
2	30/06/2012	14:00	15:00	23.06	21.45	6
3	30/06/2012	15:14	16:14	21.75	23.72	6
4	03/07/2012	10.00	11.00	16.54	16:54	7
5	06/07/2012	16.23	17:23	17.72	17.13	7
6	13/07/2012	10:15	11:15	19.85	*20:24*	8
7	13/07/2012	10:30	11:30	18.7	18.6	8
8	13/07/2012	15:53	16:53	22.73	21.43	7
9	15/07/2012	13:15	14:15	19.23	18.93	8
10	15/07/2012	14:30	15:30	18.62	21.43	8
11	27/06/2013	10.00	11.00	17.8	18.6	4
12	27/06/2013	11.00	12.00	18.7	20.1	4
13	12/07/2013	12.30	13.30	23.4	24.6	3
14	27/07/2013	10.15	11.15	22.7	24.2	2
15	01/08/2013	10.00	11.00	25.6	28.6	3
16	27/08/2013	12.20	13.20	24.8	25.1	5