The influence of plant diversity on soil physical properties in grasslands

Iain James Gould

MSc. BSc. (Hons)

Lancaster Environment Centre

Lancaster University

Submitted for the degree of Doctor of Philosophy

September 2014

Declaration

I herewith declare that I have produced the work for this thesis by myself, and that it has not previously been presented to obtain a degree of any form. Collaborations with other researchers are properly acknowledged.

Iain James Gould

Lancaster University, September 2014

Statement of authorship

This thesis has been prepared in the alternative format, as a set of three papers intended for submission to peer-reviewed journals. The papers are presented as intended for submission, with the exception of a consolidated bibliography at the end of the thesis, and in instances where the papers refer back to previous chapters. All three papers have multiple authors, and their contributions to each paper are detailed and certified by my supervisors below. Chapters 1 and 2 are review chapters, not intended for paper submission.

Chapter 3 is intended for submission as:

Gould, I.J., Quinton, J.N., and Bardgett, R.D (2014). The influence of plant diversity on soil physical properties in a model grassland community

IJG designed and set up the experiment, organised and executed all sampling and laboratory work, analysed the data, and prepared the revised manuscript. JNQ and RDB gave advice on experimental design and analysis, and contributed significantly to revisions of the manuscript, and provided laboratory space.

Chapter 4 is intended for submission as:

Gould, I.J., Quinton, J.N., Weigelt, A., De Deyn, G.B., and Bardgett, R.D (2014). Title

Plant species richness and functional identity impacts on soil aggregate stability

IJG and AW organised the field sampling, IJG executed the field sampling, analysed the data, and prepared the revised manuscript. IJG completed all laboratory work, with the exception of glomalin analysis (GBD) and root scanning (AW). JNQ and RDB gave advice at all stages of the experiment, and contributed significantly to revisions of the manuscript, and provided laboratory space.

Chapter 5 is intended for submission as:

Gould, I.J., Quinton, J.N., Ward, S.E., Quirk, H, and Bardgett, R.D (2014). The effect of management intensity and plant community composition on soil physical properties of temperate grasslands.

IJG, RDB, SEW and HQ organised the field sampling. IJG, SEW and HQ executed the field sampling. IJG undertook the laboratory work, analysed the data, prepared and revised the manuscript. JNQ and RDB gave advice at all stages of the experiment, and contributed significantly to revisions of the manuscript, and provided laboratory space.

I hereby agree with the above statements:

John Quinton

Richard Bardgett

Acknowledgements

This thesis would not have been possible without the support and guidance of many people. I would particularly like to thank my supervisors Prof. John Quinton and Prof. Richard Bardgett for all of their help and advice over the past few years. I would also like to thank the members of my advisory panel, Prof. Andy Binley and Dr. Carly Stevens, for their constructive feedback throughout the project. This thesis also benefitted from valuable contributions from Dr. Sue Ward, Helen Quirk, Dr. Alexandra Weigelt and Dr. Gerlinde De Deyn. Special mention must go to James Paul Stride, for his assistance in setting up the mesocosm experiment, and to John Huddleston. The project was funded by a BBSRC studentship for which I am very grateful, and I would also like to thank the British Society of Soil Science, and the British Ecological Society for grants allowing me to attend training courses and conferences.

I would also like to thank all of the amazing friends I have made at Lancaster University; Catherine, Sarah, Tom, Hannah, Mike, Caley, Kathy, Helen, Sue, Ali, Roger, Fran, Victor, Beth, George, Danny, and many, many more; not to mention Craig, Howard and Erik, and Phil, from the early days. Special thanks to all of you for your valuable help and friendship over the last few years.

I am forever grateful to the great friendship I have always had from 'Squad X' back home, in particular; Phil, Jason, Mike, Matt, Rickie- thank you for keeping my spirits high and my bank balance low. Above all, I would not have been able to undertake this challenge without my great friendship with Andy Bayfield, and the wonderful Quixotic world of Robin Williams-fuelled whimsical imagination we created. Finally, I would like to thank my family – Mum, Dad, Rachel, Fraser, my Grandparents, and my late Granny and Uncle John.

Abstract

The scale of biodiversity loss facing our planet has prompted many scientists to explore the potential consequences for ecosystems, and the goods and services that they provide. A favoured approach for investigating such impacts of species loss is to experimentally address the result of a reduction in species numbers on multiple ecosystem functions. Such studies of biodiversity-ecosystem function (BEF) relationships have generated a wealth of knowledge on the consequences of diversity loss for a range of ecosystem processes, such as primary productivity, nutrient cycling and the stability of communities under environmental change. However, virtually nothing is known about the response of soil physical properties to plant biodiversity change, which represents a serious gap in our knowledge given the key role soil physical structure has in providing an essential medium for plant growth; microbial activity; carbon storage; nutrient cycling; water retention; and gas flow. The potential negative effects of species loss on the degradation of soil physical properties could have adverse consequences for a host of ecosystem functions, and thus conservation of both biodiversity and soil physical integrity has potential to work hand in hand to regulate services essential to our survival. The overarching goal of this thesis is to address this gap in our understanding, by investigating the impact of shifts in plant biodiversity on a range of soil physical properties.

Grassland plant communities influence soil erosion factors through their rooting properties. Plant roots can act to stabilise the soil, create hydrological pathways and release organic exudates to benefit soil aggregation. Different grassland species exhibit contrasting root traits. For example, some species produce vast expanses of fine roots, enmeshing the soil and supporting binding mechanisms, whilst other species invest in fewer, yet thicker, roots, which create anchorage and aid water flow. Grassland communities that encapsulate a large variety of plant species will exhibit a wider array of root traits, and therefore have potential for multiple beneficial effects on soil stability.

Here, a pot experiment, experimental plot sampling, and a field survey, were employed, alongside an extensive review, to investigate the influence of plant diversity, and grassland community dynamics, on soil physical properties. Plant species richness was found to have strong effects over soil aggregate stability. Plant functional group and species identity also impacted on soil strength and hydraulic regimes, often with legumes and grasses displaying contrasting behaviour. The impact of changes in rooting structure, and their associated inputs to the soil, was significant in all of these relationships. This represents the first time such a relationship have been revealed at a range of scales, and provide valuable insight into a new direction for biodiversity-ecosystem function studies.

Table of Contents

Declaration 2

Statement of authorship 3

Acknowledgements 6

Abstract 8

List of figures 14

List of tables 17

1. Literature Review 18

1.1 Biodiversity-ecosystem function: an introduction 18

1.2 Soil physical properties: functions and threats 21

1.3 The influence of plants on soil physical properties 23

1.3.1 Soil texture 24

1.3.2 Soil aggregation 25

1.3.3 Bulk density 27

1.3.4 Soil water flow 28

1.3.5 Root reinforcement of soil strength 29

1.3.6 Aboveground protection of the soil surface 29

1.4 Mechanisms for plant diversity to impact on soil physical properties 30

1.4.1 Influence through root properties 30

1.4.2 Influence through microbial communities and organic residue 33

1.4.3 Influence through aboveground properties 34

1.5 Measuring biodiversity-ecosystem function 34

1.5.1 Grasslands as a study site 36

1.6 Thesis aims and objectives 37

2 Measurement of biodiversity impacts on soil shear strength: reviewing and developing a methodology 41

2.1 Introduction 41

2.2 Measurement of soil shear strength: a review 42

2.2.1 Equipment 44

2.2.2 Methodologies 45

2.2.3 Outputs 46

2.3 Development of a custom-made device to test root reinforcement of soil strength 47

2.3.1 Equipment 47

2.3.2 Methodology 49

2.3.3 Output 50

3 Plant diversity impacts on soil physical properties: a mesocosm study 58

3.1 Introduction 59

3.2 Methods 61

3.2.1 Experimental set up 61

3.2.2 Sampling 63

3.2.2.1 Soil properties 63

3.2.2.2 Plant properties 68

3.2.2.3 Microbial properties 70

3.2.3 Data analysis 70

3.3 Results 72

3.3.1 Community effects on soil physical properties 72

3.3.2 Plant trait effects on soil physical properties 74

3.3.3 Community effects on plant properties 75

3.4 Discussion 77

3.4.1 Plant species interactions and soil aggregate stability 78

3.4.2 Plant function, soil hydraulic conductivity and strength 80

3.4.3 Aboveground properties and canopy effects 82

3.5 Conclusion 83

4 Plant species richness and functional identity impacts on soil aggregate stability: an experimental plot study 94

4.1 Introduction 95

4.2 Methods 98

4.2.1 Experimental design 98

4.2.2 Sampling 99

4.2.3 Soil analysis 99

4.2.4 Root analysis 100

4.2.5 Data analysis 100

4.3 Results 102

4.3.1 Soil aggregate stability 102

4.3.2 Soil biotic activity: root properties, organic matter, and glomalin 103

4.4 Discussion 104

4.4.1 Impact of plant diversity on soil aggregate stability 105

4.4.2 Impact of functional group identity on soil aggregate stability 106

4.4.3 Disparity between aggregate breakdown measures 108

4.5 Conclusion 109

5 The influence of management intensity and plant community composition on soil physical properties of temperate grassland: a field study 116

5.1 Introduction 117

5.2 Methods 119

5.2.1 Study sites and sampling 119

5.2.2 Root analysis 120

5.2.3 Soil analysis 121

5.2.4 Statistical analysis 122

5.3 Results 123

5.4 Discussion 125

5.5 Conclusion 130

6 General Discussion 135

6.1 The impact of plant diversity on soil processes 135

6.1.1 Aggregate stability 135

6.1.2 Soil hydraulic conductivity 140

6.1.3 Root reinforcement of soil strength 141

6.1.4 Diversity impacts on soil biological properties 141

6.2 Plant identity and function 143

6.3 Conclusion 144

6.4 Future work 145

7 **References** **147**

Appendix A1 **178**

List of Figures

Figure 2.1 Example Mohr-Coulomb relationship given by conventional laboratory shearboxes. Repeat measures of stress at failure are plotted in order to derive cohesion (c) and angle of internal friction (ϕ) of a soil. 46

Figure 2.2 Example Force-Displacement graph from the output of measuring root reinforcement on soil strength. Often, the strength rises to a peak, followed by returning to a residual strength measurement. 47

Figure 2.3 Direct shearing device. Diagram shows (a) sketch of the original design, (b) the finalised rig compatibility with mesocosm pots and (c,d) the shearing process. 51

Figure 2.4 Actual output from sheared mesocosm pot from Chapter 3. Displaying the three parameters used in our analysis: (i) Peak force – the maximum strength reached; (ii) Force reading at 10 cm displacement; (iii) Average force over the entire shear process. 53

Figure 3.1 Diagram of mesocosm set up for measurement of hydraulic conductivity. See Ksat equation for details. 64

Figure 3.2 Plant diversity effects over soil physical properties. Displaying the significant positive impact of species richness upon all three aggregate stability measures: slaking (a); microcracking (b); and mechanical breakdown (c); and the significant positive impact of increasing functional group richness upon soil strength (d). Letters denote significant difference between treatments P<0.05. 74

Figure 3.3 The influence of *Lolium perenne* on the root length effects over soil aggregates. Relationship between root length density (RLD) on three aggregate stability measures: slaking (a); microcracking (b); and mechanical breakdown (c) in the presence and absence of *Lolium perenne*. Black dots indicate *L. perenne* absent in a community, white dots indicate *L. perenne* absent. 75

Figure 3.4 Plant community effects on plant productivity. Displaying the influence of a) species identity (*Lolium perenne,* Lp, *Anthoxanthum oderatum,* Ao, *Trifolium repens,* Tr, *Lotus corniculatus,* Lc, *Achilliea millefolium,* Am, *Plantago Lanceolata,* Pl), b) functional group identity (Grass, Legume, Forb), c) species richness, and d) functional group richness on aboveground biomass. Letters denote significant difference between treatments P <0.05. 79

Figure 3.5 Plant diversity impacts on root traits. Displaying the significant influence of species richness on: root length density (RLD) (a); average root diameter

(RDIAM) (c); and root dry matter content (DMC) (e); and the significant influence of raising functional group richness from one to two groups upon: root mass density (RD) (b); specific root length (SRL) (d); and root tissue mass density (TMD) (f). Letters denote significant difference between treatments P<0.05. 80

Figure 4.1 Relationship between sown species richness (log transformed) (a-c) and functional group richness (d-f) and the mean weight diameter (MWD) of each of the aggregate breakdown measures (slaking, microcracking, mechanical breakdown). Trendlines indicate significant effect of richness on aggregate stability (P < 0.05). 101

Figure 4.2 Regressions between aggregate stability (as represented by mean weight diameter, MWD) resistance to slaking (a-d), microcracking (e-h) , and mechanical breakdown (i-l) with root length density (RLD) (a,e,i), root mass density (RD) (b,f,j), glomalin-related protein (GRP) (c,g,k) and organic matter content of aggregates (OM) (d,h,l). 102

Figure 4.3 Path analysis of plant community impacts on slaking (B), microcracking (C) and mechanical breakdown (D), whilst (A) shows the initial model before selection of optimal pathways for each aggregate stability measure. Exogenous variables used were presence of grasses and legumes, and species richness. Endogenous variables were root mass, organic matter, root length, and glomalin-related protein. Solid (positive relationship) and dashed (negative relationship) arrows represent those that remained in optimal model, those in bold represent a significant relationship between variables (P<0.05). Numbers on arrows are path coefficients, whilst percentage values in circles represent variance explained of relevant variable by model. Circles in initial model (A) represent error terms. 103

Figure 5.1 Impact of grassland management on the three measures of soil aggregate stability (slaking, microcracking, and mechanical breakdown) at each of the five sites studied. Dark shading represent intensive management, light shading bars represent extensive management. Bars signify ± 1 S.E. Significant differences between managements are denoted by (*) P<0.1, * P<0.05, ** P<0.01, *** P<0.001. 124

List of Tables

Table 1.1 Potential mechanisms for biodiversity effect on soil physical properties 38

Table 2.1 Design specifications for a custom built shearing rig 48

Table 3.1 Species effects on soil physical properties. 72

Table 3.2 ANCOVA results to investigate whether traits explain any species richness effect. 76

Table 3.3 Individual species variation in root traits. 81

Table 4.1 Summary of ANOVA analysis of plant community properties on soil biological and physical properties. 100

Table 4.S1: Supplementary path analysis model selection information 110

Table 5.1 ANOVA results for the effects of grassland management on soil and root properties 122

Table 5.2: Relationship between plant community, soil, and root properties and soil aggregate stability and hydraulic conductivity 123

1. Literature Review

1.1 Biodiversity-ecosystem function: An introduction

Over the last century, we have witnessed a dramatic reduction in species diversity throughout the globe (Millennium Ecosystem Assessment 2005, Rockström et al. 2009). The impact of land use change, invasive species, pollution and climate change have all contributed towards significant extinction rates and species loss across all ecosystems worldwide (Mack et al. 2000, Stoate et al. 2001, Stevens et. al. 2004, Tylianakis et al. 2008). Faced with this dramatic shift in equilibria, ecological research has begun to focus on the potential impact this continual species loss will have upon ecosystem processes, and ultimately the ecosystem goods and services which we rely on. This field of research, prominent since the 1990s, is known as the study of biodiversity-ecosystem function (BEF) (Tilman, 1997).

The past 20 years has seen significant advances in our understanding of biodiversity-ecosystem function. As primary producers, much of this research has focussed on the impact of changes in plant diversity and plant community composition. Higher plant species richness has been found to increase aboveground plant productivity (Naeem et al. 1996, Cardinale et al. 2001), affect the composition of invertebrate communities (Siemann 1998, Haddad et al. 2001), alter carbon storage (Steinbess et al. 2008, Conti and Diaz 2013), cause shifts in microbial community composition (Spehn et al 2000, Eisenhauer et al. 2010), and influence many important components of soil nutrient cycling (Hector et al. 2000, Scherer-Lorenzen et al. 2007). It is now becoming evident that the promotion of biodiversity can help to maintain ecosystem multifunctionality (Maestre et al. 2012) – the ability to promote and sustain a wide range of vital functions within the environment. Therefore, it is essential that we not only continue to research the impact of BEF on an even wider range of ecosystem functions, that may have been under-looked until now, but we also attempt to gain knowledge of the underlying mechanisms that are involved.

To explain the positive effect of higher plant diversity upon a range of processes, there are two ecological concepts that are generally considered (Loreau and Hector 2001, Fargione et al. 2007). The first is the complementarity effect, whereby a mixed community facilitates ecosystem benefits that are greater than the sum of their parts. This effect is brought about by niche differentiation and facilitation between species. For example, in a high-diversity community, different species may exhibit different rooting or nutrient acquisition strategies, strategies that in a monoculture or low-diversity community would bring about greater competition. A high-diversity community therefore, encompasses a wider variety of plant species, and can thus have more efficient resource uptake, which will benefit community performance. The second concept behind BEF is known as the sampling effect. This process is based on the principal that a high-diversity community is more likely to contain one species, or functional group, that dominates the community, and provides a governing effect over the functioning of the community. Higher species richness, therefore, increases the chance that a dominant species is present the community, thus influencing ecosystem function.

A key pathway through which plant communities can impact on soil functioning is through the action of plant traits (Bardgett and Wardle 2010). Plant traits are the morphological, or physiological, characteristics of individual plant species that define an individual's functioning (Lavorel et al. 1997). In many cases, species that exhibit similar traits or life strategies can occupy similar functions within a system. Therefore, species can be categorised into functional groups, and the presence or absence of any species from within a group can potentially account for the functions of the group as a whole, giving way to research less concerned with species identity, and more interested in the role of particular plant functions on BEF feedbacks. For example, the legume functional group has been found to drive impacts on productivity (Spehn et al. 2002) or carbon and nitrogen dynamics (De Deyn et al. 2009, Scherer-Lorenzen 2003), whilst graminoid species within communities have been responsible for changes in microbial abundance (De Deyn et al. 2010) or root properties (Mommer et al. 2010). The ability to differentiate between the effects of species richness, species identity, functional group richness and functional group identity presents a challenge for experimental analysis of the BEF relationship.

Advancements in the BEF field are numerous, and we have recently seen several large meta-analyses detailing the overall state of the research, often highlighting the positive effects of higher plant diversity on a wide array of ecosystem processes (Isbell et al. 2011, Cardinale et al. 2012, Allen et al. 2013). These meta-analyses do, however, reveal that studies exploring impacts of plant diversity on belowground processes have largely focused on biological and biochemical properties of soil, rather than on the soil physical environment. In fact, virtually nothing is known of the consequences of plant biodiversity shifts on soil physical properties. This is surprising, given the fundamental importance of soil structure for ecosystem functioning; not only does it provide space for soil organisms and plant roots to grow, but it also regulates the movement of water, nutrients and gases through soil. Having been under-looked in BEF studies, the mechanisms behind the potential impact of plant diversity on soil physical structure will now be discussed in this review.

1.2 Soil physical properties: functions and threats

The breakdown of soil structure is a global concern, not only causing the loss of fertile topsoil from the landscape (Lal 1998, Lal 2001), but also contributing toward many other detrimental impacts on the environment. Soil erosion can mobilise nutrients from fields into waterways (Carpenter et al. 1998, Quinton et al. 2001), intensifying diffuse pollution (Sharpley et al. 1992, Miller et al. 2009, Ekholm and Lehtoranta 2012). Sedimentation of rivers can reduce hydraulic potential, contributing to flooding. Carbon, once stored in the soil reservoir, can be mobilised and transported throughout the environment (Lal 2003, Jacinthe et al. 2004, Quinton et al. 2010). Soil failure can occur on large scales, even inducing landslides which cause disruption to infrastructure, and pose a hazard to human life (Montrasio et al. 2012). In the past, widespread soil erosion has led to economic disaster, such as in the American dustbowl or the Russian steppes (Baveye et al. 2011). With a growing global population, and a risk of food shortages in years to come, the importance of soil research, and the way we manage the land to regulate soil physical structure, is more vital now than ever.

Soil physical properties comprise the fundamental structure of soil, and are crucial in regulating many of the essential functions that soil provides. Physical properties allow soil to play a central role in water dynamics within the environment (Hillel 1998): influencing runoff and flooding, nutrient transport, and water provisioning belowground. The interconnectivity of soil pores affects soil gas regimes, allowing oxygen flow to belowground biota, and greenhouse gas release from soil respiration (Delahaye and Alonso 2002). Soil's physical structure is also vital for food production, providing a habitat for plant, microbial, and animal life (Bardgett 2005), which in turn drives nutrient cycling and carbon storage (Six et al. 2002, van der Heijden et al. 2008). Along with all of these essential services that soil structure provides, soil also acts as the physical and structural platform for humans to build upon.

The degradation of soil physical structure constitutes a significant threat to this crucial role that soil plays in ecosystem functioning. In the UK, and throughout much of the world, water acts as a major eroding agent of soils (Morgan 2005). The relationship between soil and water is complex, and relies on intricate feedbacks between the two mediums. Before I discuss some of the mechanisms through which water breaks down soil structure, I must first mention how soil structure can determine this behaviour of water flow through the landscape. The saturated hydraulic conductivity of a soil is an important physical property, and represents the capacity for a saturated soil body to transmit water across a hydraulic gradient (Kutilek and Nielson 1994). This rate of flow in turn affects water infiltration into the soil, and therefore regulates how much water can get into the soil subsurface, and how much water remains on the surface. It is here at the soil surface that subsequent flow of water downslope, known as overland flow, poses a serious threat to soil structure. Overland flow can entrain and transport sediments and nutrients, depositing far away from their source, often into waterways (Novotny 1999).

Although the hydrological cycle can significantly influence rates of soil erosion, it is the intrinsic soil physical, biological, and chemical, properties that determine the erodibility of the soil – how susceptible the soil is to removal by water. Raindrop impact, rapid flooding, and rising water tables all exert pressure on soil structural integrity, and soil aggregates respond to these forces of water by breaking down, and losing finer particles (Quinton et al. 2001). Therefore, good maintenance of soil aggregates can help to withstand structural breakdown and sediment losses. In addition to aggregate breakdown, soil water can fill pore space between aggregates, creating internal forces that may reduce soil cohesion and strength, forcing soil apart, contributing to structural failure (Gray and Sotir 1996).

With regard to the significance of soil physical degradation as a global issue, and the aforementioned declines in biodiversity we face, the remainder of this review considers the impact of plants on soil physical structure, and the potential for plant diversity to govern these dynamics.

1.3 The influence of plants on soil physical properties

The activity of plants, their associated microbial communities, and their organic matter input belowground, contribute to the formation and maintenance of soil physical structure. Here, we discuss the influence of this biotic activity on the formation of a stable soil structure, from the micro to the landscape scale.

1.3.1 Soil texture

The basic mineral composition of soil is that of clay, silt and sand particles. Clay particles constitute the finest soil fraction, having a size of no greater than 2μm (Hillel et al. 1998). Because of their platy shape and high specific surface area, clay particles exhibit a charged surface, allowing a capacity for ion exchange that serves a crucial role for nutrient and contaminant behaviour in soils. Silt occupies the size fraction 2-63 μm , and has less specific surface area than clay. Sand is the largest of the soil fractions, incorporating anything larger than silt, up to 2 mm (Hillel et al. 1998). Sand particles have low specific surface area, and therefore no charged properties, and often vary widely in size and shape. These three soil fractions provide the intrinsic make-up for soil, and the relative proportions of each of these fractions within a soil will define a soil's behaviour and functioning.

Sand, silt and clay are mineral, and are a consequence of the parent material and weathering processes that a soil has been subjected to. Although over time plant contributions to soil chemical weathering agents can indirectly induce mineral weathering (Kelly et al. 1998), there is not much direct potential for plants to impact on the intrinsic mineral composition of soil. There is, however, much potential for plant activity to directly influence the way these mineral fractions consolidate and bind together, contributing to the fundamentals of soil structure. At this stage, we must mention one further key component of soil structure: the organic soil fraction. The organic matter in a soil comprises decomposed plant residues, and interacts with the mineral soil fractions, acting as a binding agent to help develop the next level of soil structural formation to be discussed- soil aggregates.

1.3.2 Soil aggregation

Aggregate formation in soils is a hierarchical process, whereby smaller physical units bind together to form larger units across a range of scales (Oades and Waters 1991). At the nanometre scale, clay particles can flocculate and coagulate together, initiating the formation of larger structures. At around the hundred micron range, microaggregates can form when these clay structures consolidate with silt and sand particles. These microaggregates can then bind together to form millimetre-sized macroaggregates. Even after macroaggregate formation, aggregation can occur at larger scales. Aside from some aluminosilicate binding of clays at the nano-metre scale, the importance of roots, microbial, and organic residue activity is prominent at all stages of aggregation.

Root growth can entrain and enmesh soil particles, encouraging the formation of stable aggregates (Miller and Jastrow, 1990). Root penetration will re-orientate soil particles, forcing smaller particles together to form more stable microaggregates, whilst also breaking apart, and increasing the friability of, less-stable macroaggregates (Six et al. 2004, Gyssels et al. 2005). The cementation effects of mucilage and organic acids from root exudates act to bind the smaller aggregates into larger ones (Morel et al. 1991, Traoré et al. 2000), an effect often altered by the type of vegetation aboveground (Pojasok and Kay 1990, Degens et al. 1994).

Root growth also encourages microbial activity in soil, which in turn has significant impacts on soil aggregation, particularly the behaviour of AM fungi. Fungal hyphae can extend into the soil matrix and act to stabilise aggregates (Oades and Waters 1991, Degens 1997, Ritz and Young 2004), whilst fungal extracellular polymeric substances can contribute to the cementation of aggregates (Meadows et al. 1994, Tisdall 1994). One of the key contributions that AM fungi make to aggregation is through the glue-like extracellular compound glomalin. Glomalin is deposited in soil through the degradation of mycorrhizal hyphae, and aids aggregate binding (Wright and Upadhyaya 1998, Rillig et al. 2002, Driver et al. 2005). Studies on the effects bacterial and fungal assemblages on soil stability have found positive relationships between bacterial biomass and total hyphae lengths on soil aggregation respectively (Tisdall and Oades 1979, Tisdall 1991, Meadows et al. 1994, Edgerton et al. 1995). In addition to aggregate formation, roots and microbes can also alter the way in which aggregates breakdown, by exudating hydrophobic compounds. When adhering to aggregate surfaces, these hydrophobic compounds influence the wetting rate of soil aggregates, leading to changes in the way in which water can disrupt soil structure (Czarnes et al. 2000, Hallett 2007).

Through these actions of roots and microbial associations, plants hold a governing role over soil aggregation. These mechanisms contribute to one soil physical property that has significant implications for soil erosion and degradation processes – soil aggregate stability. In the UK, and much of Europe, water acts as a powerful eroding agent of soils. Aggregate stability represents the capacity for soil aggregates to withstand these erosive forces, of which there are a number: (i) slaking, brought about by flooding and rapid wetting of dry soils, can cause the release of entrapped air within a soil aggregate to disrupt its structure; (ii) microcracking, as a result of a gradual rise in the water table below ground, may encourage differential swelling and disturbance across an aggregate structure; whilst (iii) direct raindrop impact can cause aggregates to breakdown under the mechanical stresses of impact, particularly during intense storm events (Caron et al. 1996, Le Bissonais 1996, Nearing 1997). Soil exhibiting good aggregate stability can maintain pore space, influence water flow, and prevent loss of soil and nutrients into waterways; hence this parameter represents a good indicator for soil physical stability (Le Bissonais 1996, Barthes et al. 2002, Six et al. 2004).

1.3.3 *Bulk density*

A larger structure can build up around the arrangement of these soil aggregates, forming a complex framework of clay, silt, sand, gravel, aggregates and humic substances. This arrangement and structure influences the soil bulk density, and holds an inverse relationship to the porosity, or pore space, within the soil. Through this intricate pore network, bulk density relates to the amount of air and water than can be stored within a soil, and thus has a substantial impact on many essential soil functions. A lack of aeration or water supply, as a result of insufficient porosity, will hinder biological activity. In addition, very dense soils can prevent root development, whilst soils with a very low bulk density may lose topsoil cohesion and be susceptible to water or wind erosion (Whalley et al. 1995, Goodman and Ennos 1999). Not only does bulk density, and porosity, rely on the stability of organically-bound aggregates to form a stable structure, it is also affected by rooting activity belowground. Roots play a major role in the development of soil porosity, which will be discussed in terms of soil water flow in the next section.

Antrhopogenic use of the land will also have an effect on bulk density. Intensification of grassland systems, in order to maximise yield, often involves denser stocking rates, and greater fertilisation rates. This increased trampling and machinery usage compacts the soil, which in turn reduces pore space and increases bulk density (Greenwood and Mckenzie 2001, Zhou et al. 2010).

1.3.4 Soil water flow

The nature, size and interconnectivity of the pores belowground will influence one vitally important property: the hydraulic conductivity of a soil (Beven and Germann 1982). Poor hydraulic conductivity can cause excess surface runoff; leading to erosion, flooding and river pollution. In relation to soil hydrology, plant root characteristics are complex and can either work to aid or impede water flow through soil (Powis et al. 2003, Macleod et al. 2013). Thick, tap rooted systems can provide a vertical platform to benefit water flow through the soil (Morgan et al. 1995), whilst expansive finer rooting behaviour may exploit and block pore space, allowing less water flow (Edwards et al. 1997). Along with this impact of living roots, the death and senescence of belowground biomass can create a legacy of hydraulically effective channels throughout the soil (Barley 1954, Schäffer et al. 2008).

Alongside roots, microbial activity can also influence soil water properties. Re-orientation of clay particles at the soil-microbe interface can be caused by extending fungal hyphae (Tisdall 1991, Ritz and Young 2004) or by the negatively-charged exudates of bacteria (Falchini et al. 1996) which will influence the effective porosity of the soil and thus the internal water flow regime. Microbial secretions may also affect water flow regimes, for example melanin from fungi, or bacterial extra-cellular polysaccharides (Ritz and Young 2004), both of which can cause pore clogging in moist conditions and become hydrophobic in dry conditions (Morales et al. 2010). These hydrophobic-induced changes in soil induce preferential flow of water (Lin 2010, Morales et al. 2010) and thus create uneven wetting fronts of the subsurface, and reduce the water-holding capacity of the soil (Jeffery et al. 2010).

1.3.5 Root reinforcement of soil strength

The complex interaction between roots, pores, aggregates and water contribute to a further soil physical property: soil strength. Operating at the slope or landscape scale, soil strength properties control plant growth (Masle and Passioura 1987), mass movement (Waldron 1977, Clark and Howell 1992) and the erosive nature of a soil (Al-Durrah and Bradford 1982a,b, Tengbeh 1989, Mouzai and Bouhadef 2011). Like the other physical properties discussed, root traits can play a key role in defining soil strength, increasing soil cohesion (Mamo and Bubenzer 2001a, b), although the relationship is again complex (Coppin and Richards 1990). Thick rooting species will provide anchorage, potentially preventing landslips and preserving structure across shear planes (Abdullah et al. 2011). In contrast to this, finer rooting species can develop a vast expansive array of roots in the topsoil, known as matting, which creates greater cohesion near the soil surface (Tobias 1994). Both the thick rooting anchorage, and the finer rooting cohesion are desirable traits for increasing soil strength. Root traits also behave differently in their internal tensile strength;

numerous narrow roots can exhibit greater tensile strength than fewer larger roots, resulting in varying contributions from different species to the strength of the root-soil matrix (De Baets et al. 2008, Loades et al. 2010).

1.3.6 *Aboveground protection of the soil surface*

As mentioned previously, one of the major agents contributing to soil erosion in much of Europe is rainfall impact on the soil surface (Morgan et al. 1995). The provisioning of good canopy cover by plant growth can, therefore, help topsoil conservation. Acting as a protective layer against rainsplash, an increased canopy cover has often shown reductions in surface runoff and sediment loss (Quinton et al. 1997, Casermeiro et al. 2004, Puigdefabregas 2005, Bautista et al. 2007, Merz et al. 2009, Martin et al. 2010). Above-ground vegetation also has a role to play in reducing overland flow velocity, through the presence of stem structures at the soil surface, thus allowing more time for water to infiltrate into the soil (Kouwen and Li 1980, Kouwen 1988, Ligdi and Morgan 1995). This creates less potential for soil and nutrient mobilisation and runoff.

1.4 Mechanisms for plant diversity to impact on soil physical properties

BEF studies have yet to fully explore how shifts in plant diversity could impact on soil physical properties. However, as discussed, soil physical properties are maintained by rooting activity, microbial associations, and organic input to soils; all of which are biological components known by past BEF research to be influenced by changes in plant diversity. Therefore, this review will now suggest potential for soil physical properties to be influenced by changes in plant diversity, through some of these mechanisms.

1.4.1 Influence through root properties

Plant species differ in the root traits they exhibit, and because of this plant community root properties often respond to changes in plant diversity. Fast growing species (predominantly from high-nutrient, or disturbed environments) tend to have low-density tissue, expansive rooting, and are adapted for rapid nutrient uptake. Slow-growing species (predominantly from low-nutrient, or undisturbed environments) display higher-density tissue for mechanical protection, and less expansive rooting strategies (Craine et al. 2001, Hummel et al. 2007). The difference between species' root traits suggests that when grown in mixed assemblages, communities could exhibit a wide array of root characteristics, altering the overall belowground structure of the soil. In addition to this, one of the ways plants are thought to co-exist in multi-species mixtures is through vertical root niche differentiation, whereby different species exploit different areas of soil space, and thus different sources of water and nutrients, through contrasting root architecture (Berendse 1979, Berendse 1982). This results in a more even filling of soil space by the roots (Brisson and Reynolds 1994, McLaren et al. 2004, Stokes et al. 2009). In search of resources, root spatial arrangement in competitive environments can also lead to shifts in belowground structure depending on the composition of plant communities. Plant roots can proliferate toward nutrient-rich, or competitor-poor, patches of soil (Robinson et al. 1999, Smilauerova 2001), creating local increases in root biomass, in otherwise sparse soil (Fransen et al. 1998). Plants can also allocate more biomass to fine roots in order to increase the specific surface area, and thus increase nutrient uptake, in the presence of competition (Mommer et al. 2011). Therefore, any community that imposes competitive conditions, i.e. monocultures whereby all individuals seek the same form of resource, will thus alter the physical arrangement of the root system within the soil. These changes to root properties, brought about by shifts in plant community composition, have potential to influence soil physical structure.

The first potential mechanism of plant diversity influencing soil physical structure is through increased biomass production belowground. The mass, and length, of roots provides a significant contribution to the maintenance of the soil physical properties discussed earlier. Increased root mass will: allow greater microbial biomass and organic exudation, helping to glue aggregates; provide more influence over pore generation, reducing bulk density and creating hydraulically effective pathways; help to anchor the soil, and reinforce the soil shear strength. Increased root length will allow more expansive coverage belowground, leading to a greater extent from which these mechanisms can benefit soil physical properties. Higher plant diversity has the capacity to increase these root properties belowground (Reich et al. 2004, Mueller et al. 2013), and thus has potential to benefit soil physical properties.

Higher plant diversity may not just impact on soil physical properties through increased production belowground, but could also have an effect by exhibiting a wider array of influential root traits within a mixed community. Fine rooting species can exhibit high decomposition rates, exploit soil pores, and show great expansion belowground. Thicker rooting species may decompose slower, encourage deeper water penetration, and exhibit high tensile strength for root anchorage of soil strength. With a more functionally diverse plant community, a larger variety of root traits can be expected (McLaren et al. 2004, Stokes et al. 2009, Pohl et al. 2011), which in turn could heighten the degree of soil structural formation, by applying several of these different beneficial effects (Fattet et al. 2011), although this relationship remains yet unexplored.

1.4.2 Influence through microbial communities and organic residue

The size and structure of microbial communities belowground will greatly affect soil physical processes, in particular the formation of stable aggregates. Some studies have shown that greater plant diversity can lead to larger microbial assemblages belowground, and also promote more AM fungal growth, which have a particularly notable effect. This may, however, be due to the indirect influences from increased plant productivity (De Deyn et al. 2009), which has been found to correlate positively with total microbial biomass (Bardgett et al. 1999). The presence of certain plant species in a community, for example legumes, rather than increased diversity itself, may also be more important at determining microbial biomass (Hedlund et al. 2003). Along with microbial biomass, microbial community composition can also change in response to plant community composition (Bardgett 2005). Shifts in bacterial- to fungal-dominated microbial communities have been seen across land management gradients with different plant species compositions (Bardgett and McAlister 1999), and microbial composition can respond to the quality of plant input to the soil (De Deyn et al. 2011). Because of their influential role in determining soil aggregation and soil hydrophobicity, any changes to microbial communities, as a result of shifts in plant species richness, could have the potential to significantly change soil structure.

1.4.3 Influence through aboveground properties

Aboveground plant productivity is often shown to increase in higher diversity assemblages (Marquad et al. 2009, Cardinale et al. 2012). This will no doubt have positive consequences for soil protection, providing more canopy to intercept the potentially harmful erosive energy of raindrops. Along with increased abundance, greater diversity may also benefit soil protection by exhibiting a wider variety of aboveground structures, acting to intercept rainfall at different canopy heights.

1.5 Measuring biodiversity-ecosystem function

Since the development of the biodiversity-ecosystem function as a central theme in ecology, a wide range of experimental studies have been carried out (Cardinale et al. 2012). These studies often involve the manipulation of plant communities to assess the impacts on a variety of ecosystem properties, and are usually conducted at a range of spatial scales, from the laboratory to the field.

Laboratory studies consist of plant growth in controlled environments, often investigating the influence of single species in comparison to multi-species plant communities. The tightly controlled nature of these studies allows us to single out the effects of single species plant functions, and their contributions in plant mixtures. There are some drawbacks, however, to laboratory studies. The controlled nature allows no environmental context for the relationships studied, and the time period is often too short (weeks or months) to allow multi-trophic investigations.

The next spatial scale up in BEF studies generally allows the incorporation of some environmental fluctuations. These experiments are often conducted outdoors in mesocosms, or on experimental plots, and involve the manipulation of plant communities exposed to natural variations in rainfall, temperature, light, and native biota (Roscher et al. 2004). By trading off the tightly controlled nature of laboratory growth studies, these experiments can allow the establishment of larger plant communities, and monitoring over greater timescales. Over the past few decades, mesocosm studies have provided us with valued insight into BEF feedbacks (Emery and Goss 2007, De Deyn et al. 2009, Harrison and Bardgett 2010), whilst large scale experimental plots have been established to allow exploration of these relationships at greater temporal scales (Tilman et al. 1996, Roscher et al. 2004).

Although laboratory, mesocosm, and experimental plot studies are an essential part of BEF investigation, it is also important that we explore the subject in a true environmental context. Studying BEF processes in natural systems, from fields to forests, can reveal whether these relationships hold true in the real world (Troumbis and Memtsas 2000, Pohl et al. 2009). However, with no or very little experimental control over natural systems, these studies are often correlative, and can rarely separate cause from effect.

It is clear that as the spatial scale increases, from laboratory to natural systems, we gain valued environmental context, at the expense of precision and control over studied mechanisms. Therefore, the most effective way of investigating BEF is for a project to incorporate complementary studies at all experimental levels, encompassing the benefits of each.

1.5.1 Grasslands as a study site

Often when measuring ecosystem responses to plant biodiversity, temperate grasslands provide a suitable model system. Grasslands cover large areas of the UK, occupying 65 % of total UK agricultural area (Humphreys et al. 2006), and much of the rest of the world, making up an essential component of global agricultural production (FAO 2014). We rely on them for a number of ecosystem services: from the provisioning of food, fibre, and fodder; to the regulatory role they play in biodiversity conservation, water quality management and erosion prevention. Since the Second World War, farming practices on these lands have seen intensification by means of new machinery and fertiliser production, leading to negative impacts on biological diversity (Pilgrim et al. 2010), resulting in grasslands receiving much interest from ecologists. Grasslands have been the focus of many BEF studies in relation to productivity (Hector et al. 1999, Spehn et al. 2005, Weigelt et al. 2009), carbon storage (De Deyn et al. 2009), nutrient cycling (Scherer-Lorenzen et al. 2003, Spehn et al. 2005), and land management intensity (Bardgett et al. 1996, Yeates et al. 1997). However, grassland BEF studies have rarely been investigated for soil physical responses.

Grasslands provide year-round vegetation cover, contributing toward canopy protection against soil erosion. As a result, they are not often subjected to the visually dramatic erosive events, such as gullying or rill erosion, that are commonplace in arable systems (Haygarth et el. 2006). Consequently, until recently grasslands have received much less attention in the soil erosion literature in the UK (Haygarth et al. 2006, Bilotta et al. 2008). This does not go to say that erosion from grasslands does not pose a significant threat to ecosystem functioning. In fact, the reality is far from it. Grasslands tend to occupy areas less suitable for arable crops: those of higher rainfall, and steeper topography –two factors that pose risks of greater overland flow, sediment entrainment and flooding. They also may be subjected to high nutrient input: both from fertiliser and livestock manure; and have relatively higher organic carbon content in comparison to arable soils. With a high nutrient input to the system, even relatively small levels of sediment loss, regarding the finer fractions of soil, has the potential to mobilise nutrients from the field and transport into water systems (Quinton et al. 2001, Haygarth et al. 2006). With these factors in mind, it is clear that a better understanding of physical stability and hydrological pathways in grasslands is needed. Because of the substantial vegetation cover providing adequate surface protection to the soil, we suggest that the key processes of interest will occur belowground, through the actions of plant roots.

1.6 Thesis aims and objectives

This project aims to address one of the gaps in our understanding of biodiversity-ecosystem function, by investigating the influence of grassland plant diversity on soil physical properties. In doing so, we will look at the role that belowground plant, microbial, and organic properties play in this relationship. The hypothesis is that increased biodiversity will have positive effects on a range of soil physical properties, not only as a direct effect on soil physical properties, but also through influencing both the size, and composition of root and microbial properties belowground. Table 1:1 highlights the range of potential mechanisms, by which increased biodiversity could impact on soil physical properties, which we intend to explore. In addition to revealing the impact of diversity on soil physical properties, we also have two secondary aims, to contribute further to our understanding of: (i) plant species richness impacts on root properties; and (ii) soil physical degradation in grasslands.

Table 1.1: Potential mechanisms for biodiversity effect on soil physical properties

Soil Physical Property	Biological factors that contribute to physical property	Effect of higher plant diversity on these biological factors	See Thesis Chapters
Aggregate Stability	increased root length	+	3, 4, 5
	increased root mass	+	
	organic content of soil	+	
	size of microbial community	+	
	composition of microbial community	+	
	increased glomalin content of soil	?	
Bulk Density	increased root length	+	5
	increased root mass	+	
	organic content of soil	+	
Soil Hydrology	increased root length	+	3, 5
	increased root mass	+	
	organic content of soil	+	
Soil Strength	increased root length	+	2, 3
	increased root mass	+	
	wider array of rooting depths	+	
Canopy Protection	increased aboveground biomass	+	3
	wider array of aboveground structures	+	

The range of soil physical properties explored in this project, details of some of the biological pathways which influence these physical properties, and evidence from past studies for the potential of biodiversity to impact on these biological pathways. + indicates a positive effect of biodiversity, ? Indicates an unexplored effect of biodiversity.

This thesis sets out to develop an understanding at both a mechanistic level, and in terms of wider environmental context. From a tightly controlled mesocosm study, to sampling from an experimental plot, to field observations, it will study the relationship with a set of complementary experiments at a range of scales. The thesis consists of three experimental chapters designed to address the following objectives:

(i) To experimentally determine the importance of plant species richness, species identity, functional group richness, and functional group identity, and the role of belowground plant traits, in controlling soil physical properties in a model grassland community (Chapter 3).

(ii) To test this relationship further at a long term plant manipulation field experiment (Chapter 4).

(iii) To determine, at the landscape scale, the importance of plant diversity and root trait impacts on certain soil physical properties, and the influence that land management may have on these processes (Chapter 5).

In order to address objective (i), further investigation was needed to develop a suitable methodology. As such, the thesis also includes one review and methodological chapter complementary to chapter 3:

(iv) To review soil strength testing methods, and to develop a testing device in order to measure the rooting contributions of different diversity treatments to soil strength (Chapter 2).

2. Measurement of biodiversity impacts on soil shear strength: reviewing and developing a methodology

2.1 Introduction

This thesis examines the influence of plant diversity on soil physical structure. With soil shear strength relying on the different rooting strategies and traits beneath a plant community, one of the aims of the project is to investigate the role of diversity in determining soil shear strength. Chapter 3 details a mesocosm experiment in which plant community composition was manipulated in order to reveal biodiversity impacts on a range of soil physical properties, of which shear strength was one. However, before going about this, the ways in which soil shear strength can be measured first had to be examined, and a best method for determining the strength contributions of mixed diversity plant communities must be evaluated. This chapter reviews the equipment, methods and eventual output past studies have used to determine root reinforcement of soil strength. It then details the development of a device and methodology to test the root reinforcement for the mesocosm experiment in Chapter 3.

Soil shear strength is an important physical property, and determines the maximum amount of stress a body of soil can withstand before structural failure. At the landscape scale, stress can come from increased loading on the soil, such as rapid water saturation in storm events. Poor maintenance of soil strength can impact on a number of ecosystem functions; leading to reductions in plant growth (Masle and Passioura 1987), mass movement and landslips (Waldron 1977, Clark and Howell 1992) and a greater susceptibility for soils to erode (Al-Durrah and Bradford 1982a,b, Tengbeh 1989, Mouzai and Bouhadef 2011). Therefore, it is crucial for us to explore the many ways soil strength can be reinforced, and how best it can be managed for the maintenance of ecosystem services.

Plant roots can significantly increase soil shear strength, a process often referred to as root reinforcement (Waldron and Dakessian 1982, Tengbeh 1993, Mickovski et al. 2009). This comes from the ability of roots to bind soil particles together, and provide relief of local stress by transferring forces to areas of lower stress in the soil-root matrix. This capacity to reinforce is influenced by root traits: length, density, tensile strength, morphology and orientation (Coppin and Richards 1990, Stokes et al. 2009, Loades et al. 2010). Because these variations in root traits alter the strength properties of a soil, it is therefore not surprising that plant species behave differently in terms of their root reinforcement of soil shear strength. Fine rooted grasses that create dense root matting can greatly increase cohesion near the soil surface (Tobias 1994). Deeper rooting herbaceous species can form long taproots that provide deep anchorage against soil disturbance (Abdullah et al. 2011). Root systems with more complex morphological patterns have been found more efficient at soil reinforcement (Zhang et al. 2010). Given that soil strength at any vegetated site is influenced by the root properties of the plants that are present, we suggest that the variety of root traits exhibited beneath a mixed diversity plant community will be an important factor in determining the degree of root reinforcement to soil strength.

2.2 Measurement of soil shear strength: A review

Soil strength is dependent on two intrinsic soil properties: cohesion and the angle of internal friction (Gray and Sortir, 1996). Cohesion embodies the particulate binding mechanisms within soils, whereas the angle of internal friction represents the angle at which soil particles will slip and cause failure. The relationship between shear strength, cohesion and internal friction can be derived from the Mohr-Coulomb equation:

$$\tau = c + \sigma \tan\phi,$$

where τ is the shear strength, c the cohesion, σ is the applied normal stress to the failure plane, and ϕ is the angle of internal friction.

By establishing themselves in the soil, plant roots can become incorporated into a new soil-root matrix, which in turn will benefit cohesion, resulting in greater shear strength (Tobias 1994, Operstein and Fryman 2000, Huat et al. 2005, Norris 2006). In the majority of studies, the increase in soil cohesion from roots has not been associated with any change in the angle of internal friction of the soil (Norris 2006, Ali and Osman 2008, Abdullah et al. 2011). The angle of internal friction is thought not to increase because as roots grow, the force created by the roots increases greatly, whilst any gains in the root-soil contact area are very small in comparison, resulting in no significant change in the angle of internal friction (Yan et al. 2010). Because of this, the additional cohesion provided by roots has often been incorporated into the Mohr-Coulomb relationship:

$$\tau = (c + c_r) + \sigma \tan\phi,$$

where the new cohesive factor, c_r, represents the additional cohesion provided to the soil matrix by the roots, whilst internal friction parameters remain unchanged. These principles form the basis for laboratory measurement of soil shear strength.

2.2.1 *Equipment*

A number of laboratory and field apparatus are employed to directly determine soil shear strength. These methods are known as direct shear test methods, as opposed to modelling approaches, or inferring shear properties from other traits. Shear strength can be measured directly using a shearbox, which can either be used in a laboratory, or adapted to be used in situ (BS 5930:1999). A laboratory shearbox is a well suited device for geotechnical investigations, measuring the shear strength of multiple small samples of disturbed soil, either drained or under full saturation. However, a key requirement in order to study the contribution of root reinforcement to soil strength is that the soil remains undisturbed, in the natural state as found in the environment. Although conventional laboratory shearboxes have measured the impact of roots on soil strength (Genet et al. 2010, Jiao et al. 2010), reconstituting soils in order to put in the shearbox can remove the natural strengthening effect of roots (Fattet et al. 2011). In addition, the size of a conventional shearbox may limit the hoe representative a rooted soil sample may be. Because the standard laboratory method involves disturbing the soil samples, it has led many authors studying root effects on soil strength to design their own adaptations to the conventional laboratory shearbox (Norris and Greenwood 2000), designs that allow more realistic estimates of the root reinforcement to soil strength.

Aside from shearboxes, hand held devices such as shear vanes and torvanes are often used to measure the shear strength of an exposed soil surface in the field. However, these devices only determine results from a very small area of soil, and the presence of roots in these measurements can cause misleading results, often measuring the strength of the root structure alone (De Baets et al. 2008). Models of root reinforcement have also been developed in order to predict the strength of a soil based on root measurements (Waldron 1977, Wu et al. 1979). Despite the effectiveness of such models, they have sometimes been found to overestimate the additional cohesion provided by roots, and may be better suited to studying single species, rather than mixed community, impacts (Norris 2006, Comino et al. 2010). With these limitations in shear vane/torvane measurements and modelling, we decided to employ direct shear measurements, such as those of the shear box for our experiment.

2.2.2 Methodologies

The basic working principle of a shearbox involves the movement of an upper layer of soil relative to a stationary layer of soil beneath, under the action of a steadily increasing horizontal shearing force (Head 1980). The horizontal plane between the two soil layers, where the shearing force is acting upon, is known as the shear plane. The normal stress, σ, or the load acting downwards upon the soil body, remains constant throughout the test (Fig 2.3a). A conventional laboratory shearbox has dimensions of 60 x 60 mm, with field samples often disturbed and remoulded before inserting into the device in the laboratory. This remoulding process evidently creates disruption, and is therefore an unsuitable testing environment for rooted soils.

Although less conventional, shearbox studies on vegetated soil samples have been carried out on grass, shrub and tree roots (Terwilliger and Waldron 1990, Comino and Druetta 2010, Genet et al. 2010), both in the field, and in the laboratory on undisturbed samples (Lawrance et al. 1996, Ali and Osman 2008). The shearbox designs for testing rooted soil may vary, however the basic principles adopted are the same as for those of conventional laboratory shear boxes for testing non-rooted soil. Many use larger shear plane dimensions than those of conventional shearboxes, as larger samples contain whole community root systems rather than single species and will accommodate sufficient root length (Terwilliger and Waldron 1990), and have more natural ratios of root to soil area (Tobias 1994). Fully saturating the soil before shearing provides uniform pore water pressure and simulates failure conditions under heavy rainfall (Waldron 1977), however many field studies have opted to shear at in situ moisture conditions (Norris 2006, Comino et al. 2010). Some examples ex situ have involved growing plants, or inserting undisturbed field cores, into adapted pots with a pre-determined shear plane (Waldron 1977). In these cases, the entire growing container is used as a shearbox. Field studies generally require the excavation of a trench around a column of soil, and then placing and operating a field shear box around the exposed column in situ (Chandler et al. 1981). In these cases, the shear plane is that at the base of the exposed soil column. For further details on in situ shearboxes, see Norris and Greenwood (2000) who provide a comprehensive review paper on the subject.

2.2.3 Recording and reporting shear strength measurements

With conventional laboratory shearboxes on non-rooted soil, the testing process is carried out until the horizontal shearing stress exceeds the soil shear strength, causing the soil to fail. At failure, the constant normal force and the horizontal shearing force can be recorded. Multiple tests can be carried out on the same soil type, by applying different normal loads each time. If the results of these multiple tests are plotted, it can create a straight line graph, such as in figure 2.1, and the Mohr-Coulomb relationship can be used to determine values for cohesion (c) and angle of internal friction (ϕ) of the soil.

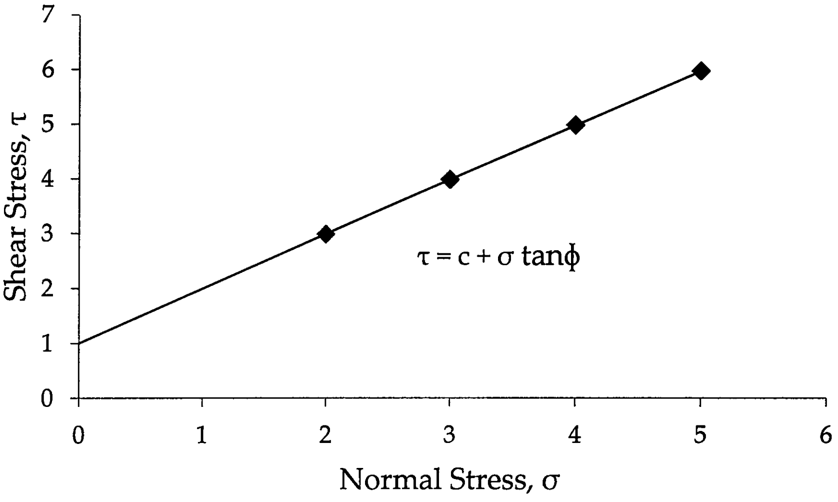


Figure2. 1 Example Mohr-Coulomb relationship given by conventional laboratory shearboxes. Repeat measures of stress at failure are plotted in order to derive cohesion (c) and angle of internal friction (ϕ) of a soil.

Unlike conventional laboratory shearboxes, which measure the point of soil failure under a series of normal loads, adapted shearboxes for rooted soil do not see a true point of failure, and instead measure the gradual displacement moved by the soil. Instead of measuring normal stress and shear stress at the point of failure, and repeating such measurements on reconstituted soils, root reinforcement studies usually plot the shear stress against the horizontal displacement moved by the soil in a continual measurement, thus creating a force-displacement graph, as in figure 2.2. Parameters from this force-displacement graph can be interpreted, such as the peak force exhibited, or the force exhibited after a given distance of displacement, which can then be used to compare treatments of rooted soils with one another. For our project on rooted soils, we will employ these force-displacement graphs (Fig 2.2) for interpretation of results, rather than determining c and ϕ from the Mohr-Coulomb graphs (Fig 2.1) that are often associated with non-rooted soils.

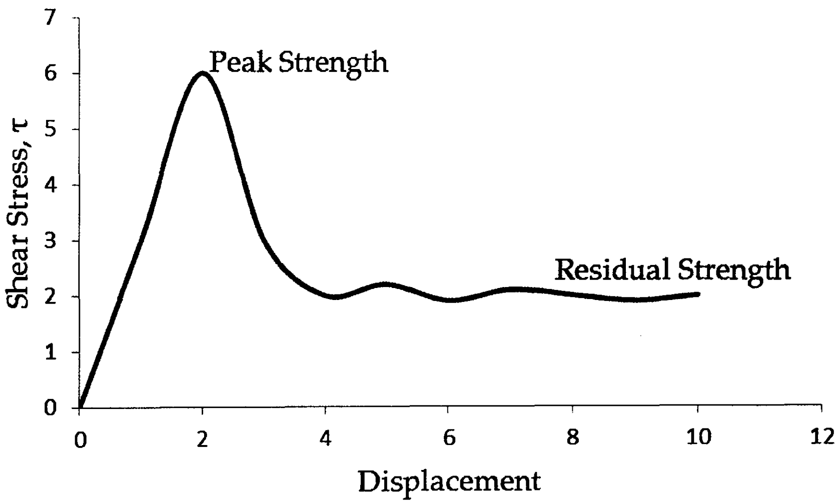


Figure 2.2: Example Force-Displacement graph from the output of measuring root reinforcement on soil strength. Often, the strength rises to a peak, followed by returning to a residual strength measurement.

2.3 Development of a custom-made device to test root reinforcement of soil strength

2.3.1 Equipment

Having reviewed both conventional, and adapted, laboratory shearboxes for measuring shear strength of root-permeated soil, I established specifications for a custom-made direct shear testing device for measuring the influence of plant diversity on the root reinforcement of soil in a mesocosm experiment. Table 2.1 shows design specifications and details for such a device, drawing insight from both field and adapted laboratory shearbox designs of previous studies. With these specifications in mind, a design for a direct shear testing rig was developed, and manufactured, by A. E. Huddleston Ltd, Quernmore (Fig 2.3).

Table 2.1: Design specifications for a custom built shearing rig

Specification	**Comments**	**See also**
Compatibility with mesocosm containers	300 mm diameter mesocosm containers will be used to house the experimental soil. This is a similar sized shear area to past studies, and will incorporate sufficient community roots.	(Chandler et al.1981)
Lightweight design	The device must be able to be lifted, and placed around 64 individual mesocosm pots.	
Circular shear plane	The experiment will also measure hydraulic conductivity, so cylindrical mesocosm pots will be used.	(Terwilliger and Waldron 1990)
Shear plane at 80 mm soil depth	As most grassland roots are active in the top 10 cm of soil, a shear plane within this region had to be selected.	(Tobias 1994)
Provide a constant horizontal force	A hydraulic pushing jack will provide the shearing force. Hand powered jacks have been used before, but fail to provide constant movement.	(Lawrance et al. 1996)
Ensure that	The top section of the shear rig will be	(Norris 2006)

movement is horizontal	attached to horizontal runners, enduring movement only in one direction.	
Measure the force applied	A STA-4 Tension/Compression load cell (LCM Systems) is fitted.	(Norris 2006)
Measure the horizontal displacement	A PD13 Linear displacement transducer (LCM Systems) is fitted.	(Norris 2006)
Graphical interpret force and displacement	Both load cell and displacement transducer are connected to a CR800 data logger (Campbell Scientific).	(Norris 2006)
No applied normal loading	The normal loading (above the shear plane) in this study will only be provided by the weight of the saturated soil above the shear plane.	(Operstein and Freeman 2000)
Potential for future use beyond this experiment	Although the apparatus will be primarily used for this mesocosm experiment, adjustable legs are attached, so that the shear plane height can be re-adjusted to be compatible with other soil set ups in the future. The adjustable legs are also removable, giving potential for the device to be used on excavated soil trenches in the field.	(Norris and Greenwood 2000)

Our shear testing rig comprised a metal frame made compatible to fit around 64pots that contain the experimental soil (Fig 2.3). The rig primarily consisted of two components: a static base that fits around the pot, and a moveable top section with the capacity to slide horizontally along two runners. A hydraulic jack was affixed to the rear of this top section, which provided the horizontal shear force to test soil strength. The jack was connected to a hydraulic pump, which gave a constant horizontal loading force throughout shearing. This horizontal movement forces the top half of soil, above the exposed shear plane, to deform for a distance of 200 mm, allowing readings of force and displacement to be logged during the shearing process. Force was recorded with an STA-4 tension/compression load cell (LCM Systems) fixed to the end of the jack, whilst horizontal displacement was recorded with a 200mm PD-13 displacement transducer (LCM systems) attached to the side of the rig. Data from both load cell and displacement transducer were collated through a CR800 data logger (Campbell Scientific). The calibration factor for the displacement transducer was: $y = 19.64 - x/298$, where x is the transducer output, and y is the total displacement in mm. The calibration factor for the load cell was: $y = 25.129x$, where x is the cell output, and y is the total force recorded in kN/m^2. The entire rig had adjustable and removable legs, so that shear plane height settings could be adjusted for any future use beyond this project.

In order to get successful force and displacement readings from the shearing process, the experimental soil had to be in a container (mesocosm) compatible with the shear testing rig. For this experiment, we used a 300 mm diameter 4 mm thick twin walled piping to create mesocosm pots. Within each pot, we made a pre-determined shear plane by cutting through the pipe at 8 cm soil depth, in effect creating two separate pipe halves to the pot, one above and one below the shear plane, which were fixed back together with reinforced adhesive tape. Just prior to shear testing, this tape could then be removed exposing a shear plane of soil. As we were carrying out the shearing on saturated soils, we also lined the inside of each pot with a watertight polyethene layer, which could be cut at the level of the shear plane just prior to testing. Once each pot was made suitable with the pre-determined shear plane and lining, 35 cm of soil was uniformly packed in at a density of 1.3 g/cm^3 at 7cm intervals above a 10 cm layer of drainage gravel, after which seedlings were planted at a uniform density in the top of the pot. After 18 months of root growth, each mesocosm pot was then ready for the shearing process.

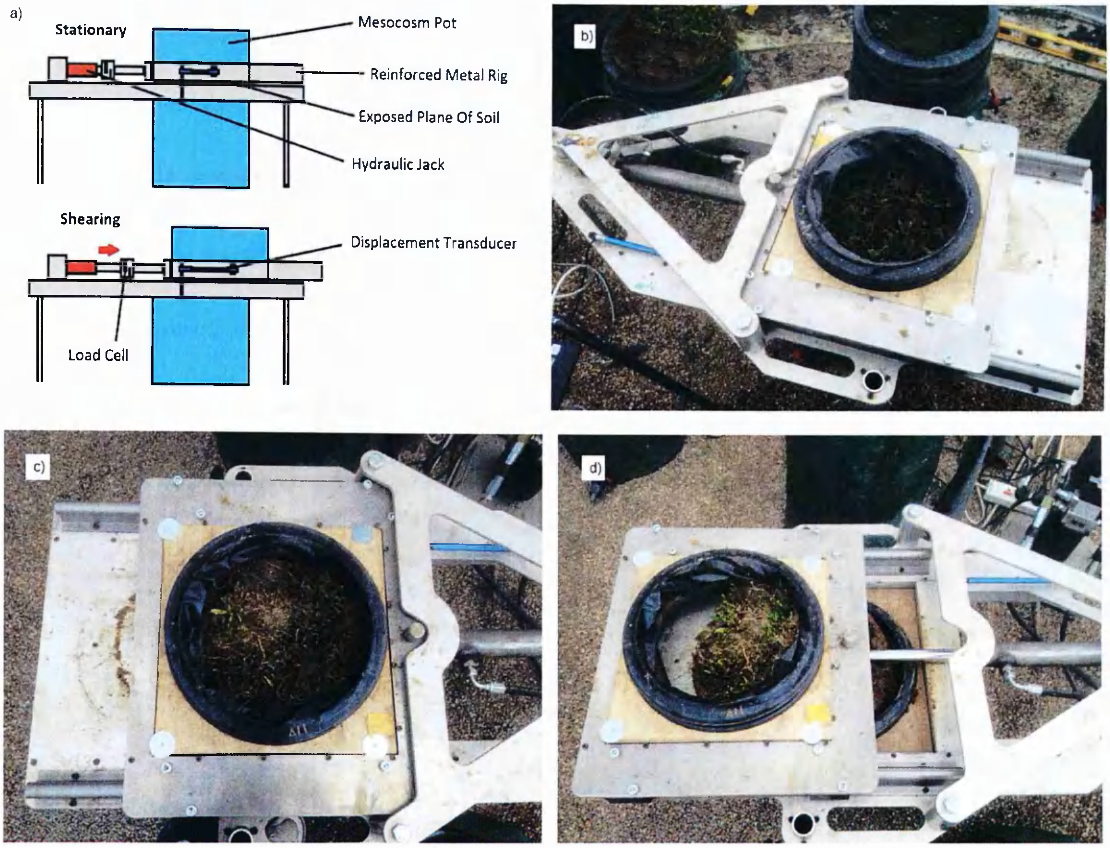


Figure 2.3: Direct shearing device. Diagram shows (a) sketch of the original design, (b) the finalised rig compatibility with mesocosm pots and (c,d) the shearing process.

2.3.2 Methodology

48 hours prior to shear testing, each pot was saturated from the bottom up, in order to expel any air pockets upwards, and create saturated conditions for strength testing. After the wetting period, the outer adhesive tape, and inner lining, around the pre-determined shear plane was cut. This exposed a plane of soil now reinforced with roots. The shear testing rig was then placed over each pot, aligning with the shear plane (Fig 2.3). Once the rig was fixed in place, the hydraulic pump was operated allowing the hydraulic jack to horizontally displace the upper layer of soil above the shear plane. Force and displacement measurements were recorded in real time in ASCII files on the CR800 data logger.

2.3.3 Output

Force and displacement ASCII files recorded from the logger were then uploaded to Microsoft Excel, where force-displacement graphs could be generated for each sample sheared. For our mesocosm experiment (See chapter 3), force-displacement graphs can be found in Appendix A1. Fig. 2.4 shows an example of the force-displacement output. For comparison between different planted soils in the mesocosm experiment, we interpreted three parameters from each graph: (i) peak shearing force (the maximum force recorded on each graph); (ii) shearing force at 10 cm displacement; and (iii) average shearing force over test (the mean value of force over the entire 20 mm shear profile). After assessing the majority of graphic outputs, we decided not to use a fourth parameter (iv) residual soil strength (strength displayed after peak force has been overcome) as many profiles reached peak force toward the end of the shear, or displayed no evident residual force. These three parameters (i-iii) were treated as relative to one another, and not absolute values, so should only used for analysis between our mesocosm treatments (See chapter 3).

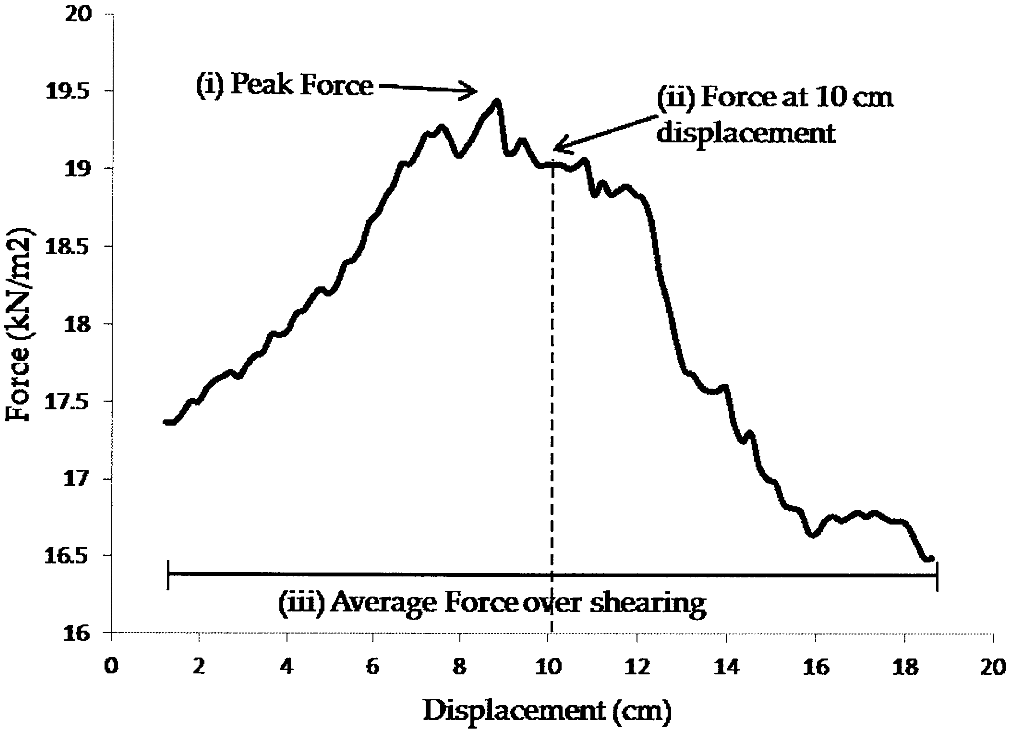


Figure 2.4: Actual output from sheared mesocosm pot from Chapter 3. Displaying the three parameters used in our analysis: (i) Peak force – the maximum strength reached; (ii) Force reading at 10 cm displacement; (iii) Average force over the entire shear process.

With a device constructed, and a methodology in place, we undertook shear testing on 8 pots of non-rooted soil, in order to ensure consistent readings. After which, the shearing rig was ready for testing on the experimental mesocosms (Chapter 3).

3. Plant diversity impacts on soil physical properties: a mesocosm study

Gould, I.J., Quinton, J.N., and Bardgett, R.D

Abstract

The last two decades have witnessed an enormous research effort exploring the consequences of species loss for the functioning of ecosystems, and the impact of this on the goods and services that ecosystems provide. This research has led to the general consensus that biodiversity loss reduces most ecosystem functions and impairs their stability through time, and that functional traits of species play a major role in determining diversity effects. Most of the research focussed on plants, however, has had an aboveground focus, exploring relationships between plant diversity and the production and stability of plant communities, and relatively few studies have explored impacts belowground. Moreover, those studies that have explored impacts of plant diversity on belowground processes have largely focused on biological and biochemical properties of soil, rather than on the soil physical environment. This is surprising, given the fundamental importance of soil structure for ecosystem functioning; not only does it provide space for soil organisms and plant roots to grow, but it also regulates the movement of water, nutrients and gases through soil. Also, the degradation of soil physical structure has drastic consequences for crop growth, water quality, carbon storage, and flood remediation.

We established an 18 month mesocosm experiment, and employed a range of methods to investigate the impact of plant diversity on a range of soil physical processes. Saturated hydraulic conductivity was measured on the planted soil columns; stability testing was conducted on soil aggregates, whilst a method of determining strength reinforcement of soil by roots was also used. After soil sampling, we measured a suite of root traits, to see how these could be influential in soil physical processes. We found a significant impact of species richness on soil aggregate stability, whilst hydraulic conductivity and soil strength associated more to the behaviour of plant functional identity, in particular legumes, within a community. Plant root properties played a critical role in these relationships. This represents the first time soil physical responses to plant biodiversity have been measured at the mesocosm scale, and reveals that, in addition to the response of biological and biochemical processes, plant diversity could also now have a significant effect on certain physical processes.

3.1 Introduction

Although very few studies have investigated the link between plant diversity and soil physical properties, recent evidence does point toward the significance of such a relationship. In experimental grassland plots, Pérès et al. (2013) showed that soil aggregate stability, a good indicator for soil physical structure (Le Bissonais 1996, Barthès et al. 2002, Six et al. 2000), increased under mixed-species communities when compared to monocultures as a result of changes to root, microbial, and carbon inputs into the soil. Such a relationship was also found in field studies in the Alps (Pohl et al. 2009), with a positive correlation between plant diversity and soil aggregate stability. With regard to other soil physical parameters essential to ecosystem functioning, such as hydraulic conductivity or soil shear strength, nothing is known of the relationship with plant diversity. Despite this gap in our knowledge of plant community impacts upon the physical environment, we do know that individual plant species vary in the way that they influence soil structure (Scott 1998). A presence of grass species can increase the extent of soil aggregation (Jastrow 1987, Rillig et al. 2002), but their expansive root networks may come at a price – blocking pore space to restrict hydraulic conductivity belowground (Barley 1954, Macleod et al. 2013). Legumes, on the other hand, with their less exploitative rooting strategies, do not have as much influence upon soil aggregate stability (Pérès et al. 2013), but can aid water flow through soils (Yaacob and Blair 1981). The fact that there is disparity between how individual species or functional groups impact on soil physical properties suggests that a plant diversity effect could be prominent.

Plant traits play a major role in defining much of the physical stability of a soil (Angers and Caron 1998), and a number of root traits have been found to show important links with soil physical behaviour. The length of roots invested by a plant, or community, belowground can govern the extent of influence over the soil physical environment. Species that exhibit greater root length can increase soil aggregate stability (Pohl et al. 2009), form cohesive topsoil matting to resist erosive forces (Tobias 1994), forage to depth and provide anchorage against slope failure (Abdullah et al. 2011), and also influence macropores and water flow (Edwards et al. 1997). In addition to root length, plants investing more root mass generate greater root-derived inputs to the soil; organic exudates and associated microbial communities that are a fundamental part of the soil aggregation process (Tisdall and Oades 1982, Oades and Waters 1991, Six et al. 2004). Root diameter influences soil hydrological properties, where thicker roots can act to aid water flow through soils (Morgan et al.

1995). Roots with greater tissue density might change tensile strength to the root-soil matrix (De Baets et al. 2007, Loades et al. 2010), whilst roots that decay easily create permeable pathways for water (Barley 1954, Macleod et al. 2013). We know that individual species within grasslands can exhibit a wide range of root characteristics (Grime et al. 2007), suggesting different species could impact soil structure in a contrasting manner. If there is any diversity impact upon soil physical behaviour, it could be an effect of a particularly influential species or trait present.

Although numerous mesocosm studies have explored biodiversity-ecosystem function feedbacks in the past, and despite our wide understanding of the role plant activity has in influencing soil structure, none have investigated plant community impacts on soil physical properties. The overarching goal of this chapter was to test how changes in plant diversity and composition impact a range of soil physical properties: aggregate stability; hydraulic conductivity; and shear strength. We tested the hypotheses that increasing plant diversity benefits soil physical properties and such responses could be driven by the root traits of influential plant species within the community. This was tested in model grassland communities exhibiting a gradient of species, and functional group, richness.

3.2 Methods

3.2.1 Experimental set up

In order to test the effects of plant community dynamics on soil physical properties, we adopted an experimental design originally devised by De Deyn et al. (2009) and based on model grasslands. This design allows us to test the effects of plant species richness, functional group richness, species identity and functional group identity on the soil processes in question. Initial planting density was kept constant, whilst plant species and functional group richness varied, from monocultures to six-species mixtures, consisting of three functional groups. The plant species used were: two grasses (G), *Lolium perenne* (Lp) and *Anthoxanthum oderatum* (Ao); two forbs (F): *Plantago lanceolata* (Pl) and *Achillea millefolium* (Am); and two legumes (L), *Trifolium repens* (Tr) and *Lotus corniculatus* (Lc). These plant species differ considerably in their rooting patterns (Thomas and Davies 1954, Cope and Gray 2009), and therefore lend themselves to investigation of community impacts on soil structure. In each block, there were 16 planted mesocosms: no plants (1), each species in monoculture (6), two-species mixtures from the same functional group (3), two-species mixtures from different functional groups (i.e. GF, GL, FL) (3), three-species mixtures from different functional groups (2) and all six species together (1), in a four block design. For treatments with two species from two functional groups, and three species mixtures, we used different combinations of species per block so that all plant species were present in an equal number of mesocosms. Two sets of parallel mesocosms were established: one set were used for root trait and aggregate analysis, whilst the other set were used to test for effects of planting treatments on hydrology and soil strength. This yielded a total of 128 (2 × 64) mesocosms, each consisting of a cylindrical pot of 50 cm depth with reinforced mesh on the underside. For the 64 strength-testing mesocosms, a pre-determined shear plane was cut in each pot at 8 cm soil depth (see Chapter 2: Measurement of biodiversity impacts on soil shear strength: developing a methodology), before taping both sides back together with reinforced tape. The shear plane was protected with a PVC inner lining in each mesocosm, to prevent disturbance from water or plant roots during the growing period. At the bottom was laid 10 cm of limestone chippings, above which was packed 35 cm depth of experimental soil at 1.3 g/cm^3 density. Plants were grown from seed in controlled environment rooms (16 hr day length, 24 $^\circ$C day temp., 16 $^\circ$C night temp.) and 8-week old seedlings were planted into mesocosm pots in glasshouses at Hazelrigg field station, Lancaster University.

The experimental soil used was a Kettering Loam, provided by Boughton Loam Ltd. (Kettering, UK). The soil was a clay loam, and had previously undergone preliminary tests to establish the capacity to measure soil aggregate stability. All mesocosms were filled with the experimental soil, packed at a uniform dry density (see Chapter 2).

Each pot was planted with 24 plant individuals, the composition of each community depended on the assigned treatment (i.e. 24 individuals for each monoculture, 2 sets of 12 individuals per 2-species mixture, 3 sets of 8 individuals per 3-species mixture, 6 sets of 4 individuals per 6-species mixture). The experiment was a random block design, using 4 separate glasshouses, each glasshouse acting as a block. Within each block, there were 16 treatment combinations, and two duplicate mesocosms for each: one for aggregate/root analysis, the other for hydrology/strength, totalling 32 mesocosms per glasshouse. The position of each treatment combination per block was randomly allocated. Throughout the growing period, mesocosms were watered via an automated sprinkler system, and aboveground biomass was cut twice yearly in June and August. Plant communities were allowed to grow for 18 months before the final destructive harvest.

3.2.2 Sampling

3.2.2.1 Soil properties

Aggregate stability

Le Bissonais (1996) developed a framework for the assessment of aggregate stability of soils that considers the impact of different environmental factors. The ability of soils to resist: (i) slaking under rapid wetting; (ii) microcracking under gentle or slow wetting; and (iii) mechanical breakdown as a result of raindrop impact; are all examined, and hence, this methodology was suitable for our experiment. Using a trowel, soil clods in the upper 10cm of soil were removed from each of the 64 aggregate stability mesocosms. The Le Bissonais protocol subsequently calls for the 3-5 mm fraction of aggregates from these soil clods to be separated by passing through a 5 mm sieve and retaining the required fraction on a 3 mm sieve. Preliminary analysis found that inclusion of aggregates > 3 mm ignored a large portion of aggregates we found adhering to roots of soil. With rooting activity an essential detail of this study, it was decided to include aggregates of > 2 mm instead. Tisdall and Oades (1982) also comment that aggregates > 2 mm are those most susceptible to change from shifts in land use or plant community. After the 2–5 mm fraction was separated, aggregates were dried at 40 °C for 24 hours to achieve a constant matric potential across all soils. At this stage, the samples were ready for subsequent analysis of each of the three environmental breakdowns, following the Le Bissonais protocol:

Aggregate stability (i): Slaking

5 g of aggregates were gently immersed in 50 cm^3 of deionised water, inducing slaking. After 10 minutes, the water was sucked off and the soil material was transferred to a 50 µm sieve. Using the 50 µm sieve, the soil sample was gently wet sieved in a non-polar liquid, in this case methylated spirits, and then allowed to air dry in a fume hood overnight, after which the sample was further dried at 105 °C for 24 hours. The final stage of aggregate stability analysis involves gently dry sieving the soil sample through a stack of six sieves (2000 µm, 1000 µm, 500 µm, 200 µm, 100 µm, 50 µm). The fraction remaining on each sieve was weighed, and the mass recorded multiplied by the mean aperture of the sieve. The sum of each of these sieve fraction calculations determines the Mean Weight Diameter (MWD) of each treatment, the value used to represent aggregate stability under this environmental breakdown.

Aggregate stability (ii): Microcracking

5 g of aggregates were placed on pre-wetted filter paper on a sand tension table of matric potential of -0.3kPa. The original protocol calls for the aggregates to be left at this state for a recommended 30 minutes, however due to the clay content of our soils, it was decided to gently saturate on the tension table for 60 minutes. After gently wetting on the sand table, the aggregate samples were removed, wet sieved and subsequently dry sieved in the same manner as the slaking samples.

Aggregate stability (iii): Mechanical breakdown

5 g of aggregates were immersed in 50 cm^3 of methylated spirits for 10 minutes. The liquid was then sucked off and the aggregates transferred gently into a conical flask

with 250 cm^3 of deionised water. The flask was corked and agitated end-over-end 20 times to simulate mechanical disturbance, before allowing settling for 30 minutes. After this time, the water was removed and the aggregates were transferred to a 50 μm sieve, for subsequent wet sieving and dry sieving as before.

Saturated hydraulic conductivity

Measurement of saturated hydraulic conductivity involved the second set of duplicate mesocosms (n=64). For saturation, mesocosms were placed in flexible polyethene outer containers. The containers were then filled with water, above the level of the soil, in order to saturate the mesocosms from bottom up, allowing the release of air bubbles upwards, creating minimal disturbance to the soil structure. After water was seen ponding on the soil surface, the pots were left for 48 hours before conductivity measurements were taken. The falling head method of measuring saturated hydraulic conductivity was employed, in essence using the entire mesocosm pot as a permeameter (Klute and Dirksen 1986, Smith and Mullins 1991). Additional narrow piping was attached and sealed to the top of the mesocosm pot so that any fall in head could be read accurately in a narrow-diameter duct. An outflow was created by lowering the flexible polyethene container, on the outside of the mesocosm pot, to a set height below the soil surface height. Because the column has a permeable base, this creates a differential gradient that induces water flow through a saturated soil column and out of the outflow (Fig 3.1). Using Darcy's principles of saturated hydraulic conductivity, we could then calculate the rate of flow of water based on the area and length of the soil column, and the fall in head over time (Klute and Dirksen 1986, Smith and Mullins 1991).

Figure 3.1 shows an example of the hydraulic conductivity set up, saturated hydraulic conductivity (K_{sat}) was calculated with the following equation:

$$Ksat = \frac{A1\,L}{A2\,t}\ln(\frac{h0}{h1})$$

Where A1 is the cross sectional area of the standpipe and *A2* is the cross sectional area of the soil column. *L* represents the length of the soil column, *t* is the time period and *h0* and *h1* are head heights at *t*=0 and *t*=*t* (Figure 3.1). For each mesocosm, *t* was recorded after a drop in head height of 10 mm, and repeat measures were taken on each mesocosm until two concurrent readings were evident.

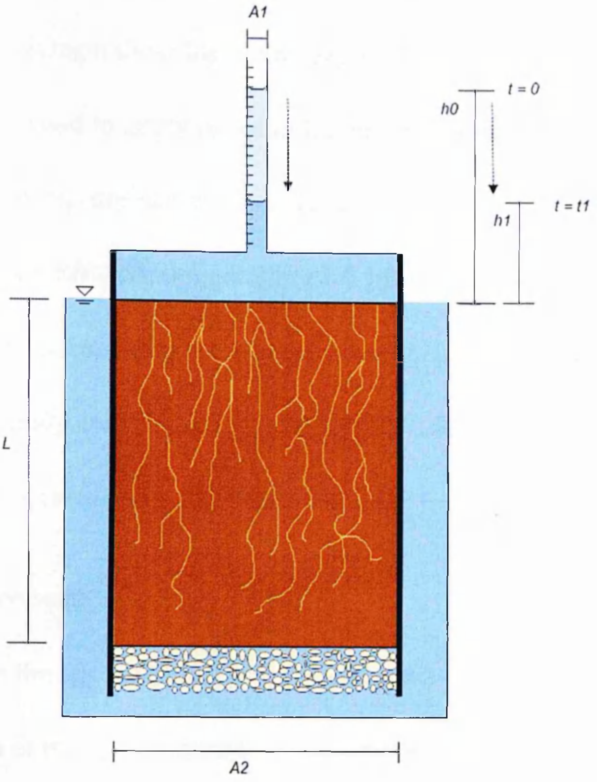


Figure 3.1: **Diagram of mesocosm set up for measurement of hydraulic conductivity. See Ksat equation for details.**

Root reinforcement of soil shear strength

After hydraulic conductivity measurements were taken, the polyethene outer containers were re-adjusted to submerge the entire mesocosm, once again allowing full saturation. The pots were left to re-saturate for a further 24 hours before strength testing was undertaken. Immediately prior to strength testing, the polyethene container was removed, and the pre-determined shear plane was exposed. A blade was used to cut the inner PVC lining of the mesocosm, resulting in an entire plane of exposed soil. We developed a shearing rig that fitted the mesocosm pot (see Chapter

2: Measurement of biodiversity impacts on soil shear strength: developing a methodology), applying a shear load to an exposed shear plane at 8 cm soil depth. A hydraulic ram was used to apply constant loading to the soil column, whilst force and displacement of the top half of the soil column were constantly recorded with a Campbell Scientific CR800 datalogger. For each individual mesocosm, we created a force-displacement graph. From these graphs, we derived three strength parameters to be used in our analysis: (i) Peak shearing force, (ii) Shearing force at 10 cm displacement, (iii) Average shearing force over test.

Moisture content

Soil samples from the aggregate stability mesocosms were taken and bulked together for determination of moisture content, organic matter content and microbial C. Fresh soils were weighed, dried at 105 °C for 48 hours to remove all water, and then weighed again to determine soil moisture content.

Organic matter content

We measured organic matter content of the soil using the loss on ignition method, which combusts any organic material at high temperatures. Dried soil samples were placed in crucibles and the mass was recorded. Crucibles were placed in a furnace set to 560 °C for 6 hours, after which the samples were re-weighed. Organic matter content refers to the mass lost on ignition, leaving behind only the mineral fraction of the soil.

3.2.2.2 Plant properties

At final harvest, the aboveground biomass for each species per community was cut at 2 cm height, dried at 70 °C for 48 hours and dry biomass recorded. In the aggregate stability mesocosms (n=64), three 30 cm soil cores of 3.4 cm diameter were taken, separated into 10 cm depths, and each depth bulked for root trait analysis. We, separated into 10 cm depths to assist root scanning, all results presented represent root traits measured for the entire 30 cm depth unless otherwise stated. Roots were removed from soil by gently washing through a 710 μm sieve. Roots were then stored in 20 % ethanol solution until scanning was undertaken. Root sample solutions were spread out over several transparent plastic trays and scanned with an Epson V700 Photo scanner, after which the images were analysed using WinRhizo 2009 (Regent Instruments Canada Inc.) for root properties of length, average diameter and volume. After scanning, the root samples were patted dry with paper towels and fresh mass was recorded. The samples were then dried at 70 °C for 48 hours after which dry mass was recorded. Using the root measures obtained, the following six root traits were calculated (Pohl et al. 2009, Hummel et al. 2006):

Root Length Density (RLD) = Length of roots per unit of soil volume (m/dm^3)

Root Density (RD) = Dry mass of roots per unit of soil volume (g/dm^3)

Average Root Diameter (RDIAM) = Average diameter of roots per community sample (mm)

Specific Root Length (SRL) = Length of root per dry mass of root (g/m)

Dry Matter Content (DMC) = Dry mass of roots per fresh mass (fraction)

Tissue Mass Density (TMD) = Mass of roots per root volume (g/cm^3)

In order to see if changes in community composition affect the allocation of root length and mass at different depths of soil, the relative proportions of length and mass in each 10 cm depth layer were also calculated. This was done by dividing the length or mass at each layer by the total length or mass throughout the soil, giving a fraction to represent root allocation.

For root samples from the second batch of mesocosms subjected to saturation and shearing (see *Root reinforcement of soil shear strength*)(n=64), the two sheared soil layers were positioned back on top one another and two 30 cm cores were taken. Soil was washed from the roots, whilst roots mass, and subsequent RD, were determined after drying at 70 °C for 48 hours. Because of the destructive nature of the shearing, only RD was used, and no other measures of root traits were derived from these pots.

3.2.2.3 Microbial properties

A fumigation process was employed to determine the amount of carbon bound in soil microbial biomass. Soil subsamples fumigated with chloroform, releasing C for extraction, were compared to non-fumigated subsamples of soil, in order to derive how much C was bound in microbial cells. 5 grams of sieved soil per sample was placed in a dessicator with chloroform. Air was evacuated using a vacuum pump, and the samples with chloroform were left under vacuum for 24 hours. After evacuation, samples were extracted with potassium sulphate (K_2SO_4), along with an additional cohort of samples that did not undergo fumigation. K_2SO_4 was added to each sample before 30 minutes of mixing on an orbital shaker, after which the solution was filtered through Whatman No. 1 filter paper. Potassium persulphate was added to each sample before using a total organic carbon (TOC) analyser to determine C. The total amount of C contained in the microbial community was calculated:

$$\text{Microbial C} = \frac{\text{Total Carbon in fumigated sample}}{\text{Dry soil (g)}} - \frac{\text{Total Carbon in unfumigated sample}}{\text{Dry soil (g)}}$$

3.2.3 *Data analysis*

Data for aboveground biomass, root traits, slaking aggregate stability, hydraulic conductivity, and shear properties were transformed in order to meet the assumptions of normality. Correlations between aboveground biomass and root traits were found with Pearson correlations on the 64 aggregate stability mesocosms. We used general linear mixed effects models, with block as a random effect, to test the response of plant community properties on all plant properties and all soil physical processes. Species richness effects were investigated using all mesocosms, whilst individual species effects were identified by using monoculture data alone. The effects of functional group identity on root traits and all physical responses were tested using only the pots displaying one functional group. Functional group richness effects were explored using just the 2-species mixtures, where species richness is kept constant yet functional group richness can increase. Differences between treatments were determined with Tukey post hoc tests.

To investigate how certain species or functional groups could play a dominant role over physical properties in the mixed communities, the effect of the presence/absence of the species *L. perenne* on aggregate stability and *L. corniculatus* on strength parameters was explored using t-testing of the 1-, 2- and 3-species mixtures. We also used t-testing to find any effect of the presence/absence of legumes on saturated hydraulic conductivity in the 1- and 2-functional group mixtures.

In order to test whether diversity effects could be explained by the traits of a community we used further ANCOVA analysis investigating the influence of root traits and aboveground biomass, alongside species richness, over soil physical properties. All root traits, aboveground biomass and species richness were used as predictors for aggregate stability, whilst only root mass density, aboveground biomass and species richness were used for hydraulic conductivity and strength properties. For their relationship with aggregate stability, we only used the root traits measured in the top 10 cm of the soil column, as aggregates were only measured from this upper depth of soil. Species richness was fitted last in all ANCOVAs in order to separate its effects from those of the plant properties. Relationships between the root traits, aboveground biomass, organic matter and microbial C content, and each physical property were investigated with linear regression. We tested the impact of *L. perenne* on the root effect by repeating models with root length density as a factor and aggregate stability as a response, however this time only on communities with, and then, without *L. perenne*. All analyses were carried out in R 3.0.1 (R Development Core Team).

3.3 Results

3.3.1 Community effects on soil physical properties

Increasing species richness had a positive effect over all three aggregate stability measures (**Figure 3.2a-c**) (Slaking:$F_{1,55}=15.65$, $p<0.01$, Microcracking: $F_{1,55}= 4.09$, $p<0.05$, Mechanical Breakdown: $F_{1,55}=6.69$. $p<0.05$). Greater plant species richness also showed a tendency to benefit saturated hydraulic conductivity, but this response was not significant to 95 % confidence ($F_{1,55}=3.75$, $p=0.06$), whilst species richness had no impact on soil strength properties (Average force: $F_{1,49}=1.5$, $p=0.22$; Force at 10 cm: $F_{1,49}=1.16$, $p=0.29$; Peak force $F_{1,49}=0.73$,$p=0.40$). When species richness was held constant, there was no effect of increasing functional group richness, from one to two groups, on any of the aggregate stability measures or saturated hydraulic conductivity of the soil. However, unlike the aggregate and hydrology measures, increasing the number of functional groups present in a community had a significant effect on soil strength properties. Average strength, strength at 10 cm, and peak strength all increased when the number of functional groups present in a community rose from 1 to 2 (Average force: $F_{1,16}=8.51$, $p<0.05$; Force at 10 cm: $F_{1,16}=9.68$, $p<0.01$; Peak force $F_{1,16}=9.67$,$p<0.01$), all three strength parameters are strongly correlated, so for graphical representation of the data we just display peak force data (**Figure 3.2d**).

Grass species displayed significant positive impacts for soil aggregate and strength properties. Soils under the grass, *L. perenne,* showed the greatest aggregate stability resistance to slaking and mechanical breakdown (**Table 1**); whilst the effects of root-reinforcement of the other grass species, *A. oderatum,* showed a positive increase on soil strength in monocultures. Grasses did however tend to have lower rates of saturated hydraulic conductivity in comparison to legumes (Mean K_{sat}: Grasses: 8.24±4.49 mm/hr; Forbs: 8.52±3.52 mm/hr; Legumes: 19.77±3.46 mm/hr; $F_{2,30} = 5.29$ $P<0.01$). The positive effect on aggregate stability of *L. perenne* was not only seen in monocultures: slaking stability in 3-species mixtures increased by 21 % in the presence of this species, whilst mechanical breakdown stability increased by 7 % when 2-species mixtures contained *L. perenne*. In many cases, the effect of legumes on soil physical properties contrasted to that of the grasses. Aggregates sampled under *T. repens* displayed the lowest aggregate resistance to slaking, and along with *L. corniculatus*, lowest aggregate resistance to mechanical breakdown also (**Table 3.1**). Despite poorer aggregate stability, the legumes did exhibit the highest rates of hydraulic conductivity, not only increasing conductivity in monocultures, they also contributed to greater hydraulic conductivity with their presence in mixtures (t test 2-FG mixtures: mean K_{sat} in presence of legumes: 30.62 ± 9.97 mm/hr; mean K_{sat} in absence of legumes: 1.38±0.42mm/hr; $P <0.001$). The roots of *L. corniculatus* also demonstrated the greatest strength reinforcing effect of the soil columns (Table 3.1). Despite having a strong positive impact on soil strength in monocultures, the presence of *L. corniculatus* in the 2- and 3-species mixtures had no impact on soil strength properties. In all measures, slaking and mechanical aggregate stability were greater in planted mesocosms than under bare soil (Table 3.1), whilst only the two grass species, *L. perenne* and *A. oderatum*, and the forb, *P. lanceolata*, displayed better aggregate stability to microcracking than bare soil. Although bare soil was found to have consistently low soil strength properties, it was not significantly different from the strength properties of soils planted with *T. repens* and *P. lanceolata* (Table 3.1).

Table 3.1: Species effects on soil physical properties.

Soil Physical Properties	Species Identity						
	Grasses		Legumes		Forbs		Bare Soil
	Lp	Ao	Tr	Lc	Am	Pl	
Aggregate Stability (mm)							
Slaking	1.61 ± 0.1^{a}	1.47 ± 0.09^{ab}	1.11 ± 0.15^{b}	1.14 ± 0.09^{ab}	1.35 ± 0.13^{ab}	1.50 ± 0.11^{ab}	0.71 ± 0.04^{c}
Microcracking	2.77 ± 0.11^{a}	2.92 ± 0.09^{a}	2.69 ± 0.14^{ab}	2.64 ± 0.2^{ab}	2.66 ± 0.14^{ab}	3.01 ± 0.03^{a}	1.99 ± 0.35^{b}
Mechanical Breakdown	2.67 ± 0.11^{c}	2.52 ± 0.03^{a}	2.17 ± 0.13^{ab}	2.15 ± 0.13^{bc}	2.32 ± 0.04^{bc}	2.59 ± 0.18^{abc}	1.90 ± 0.15^{ab}
Hydrology (mm/hr)							
Saturated hydraulic conductivity	1.82 ± 0.74^{ns}	8.69 ± 8.18^{ns}	6.54 ± 2.63^{ns}	23.05 ± 3.53^{ns}	8.9 ± 6.1^{ns}	3.97 ± 1.99^{ns}	10.92 ± 6.53^{ns}
Strength (kN/m^2)							
Peak force	12.55 ± 3.66^{ab}	19.19 ± 7.16^{a}	6.25 ± 2.24^{bc}	30.33 ± 5.39^{a}	11.99 ± 3.45^{bc}	3.3 ± 1.38^{ab}	1.77 ± 0.46^{c}

Mean values ± SE for the physical properties of soils from monocultures of Lolium perenne (Lp), Anthoxanthum oderatum (Ao), Trifolium repens (Tr), Lotus corniculatus (Lc), Achilliea millefolium (Am), Plantago lanceolata (Pl) and bare soil. Letters indicate significant differences between planted treatments P>0.05

3.3.2 Plant trait effects on soil physical properties

Root length density and specific root length were both positively related to all aggregate stability measures (Table 3.2), whilst average root diameter showed a significant negative relationship with aggregate stability (Table 3.2). Increased root mass in soil showed no significant relationship with any form of aggregate stability, however, it did show a positive correlation with both hydraulic conductivity and soil strength (Table 3.2). Root dry matter content showed slight negative correlations with two of the three aggregate measures - slaking and mechanical breakdown resistance, whilst root tissue density displayed a negative relationship to aggregate resistance to microcracking (Table 3.2). Aboveground biomass was not related to aggregate stability, but did however display positive links with both hydraulic conductivity and soil strength (Table 3.2). Linear regression found a significant positive relationship between microbial carbon and soil aggregate stability (Slaking: $F_{1,59} = 7.01$, $P<0.05$; Microcracking: $F_{1,59} = 5.33$, $P<0.05$; Mechanical Breakdown: $F_{1,59} = 5.37$, $P<0.05$), but no significant relationship between organic matter and aggregate stability (Slaking: $F_{1,59} = 3.05$, $P=0.09$; Microcracking: $F_{1,59} = 3.23$, $P=0.08$; Mechanical Breakdown: $F_{1,59} = 0.74$, $P=0.39$).To investigate whether the root traits of a community could explain the plant diversity effect, we fitted species richness after traits in the ANCOVA model, and found that aggregate stability to slaking breakdown – species richness effects were still significant after fitting traits (Table 3.2). For the other aggregate stability measures, microcracking and mechanical breakdown, species richness effects could be fully explained by the traits, and there was also no effect of species richness on hydraulic conductivity and shear strength found with ANCOVA analysis (Table 3.2). We used further analysis to investigate how key species could be impacting on the trait-physical property dynamics. When the data for root regression analysis was separated into two - mesocosms in the presence, and absence, of *L. perenne* - we found that this species had a governing role over how root length related to soil aggregate stability: when *L. perenne* was present in a community, there was no relationship between root length and aggregate stability, however when the species was absent, the relationship was positive (Figure 3.3).

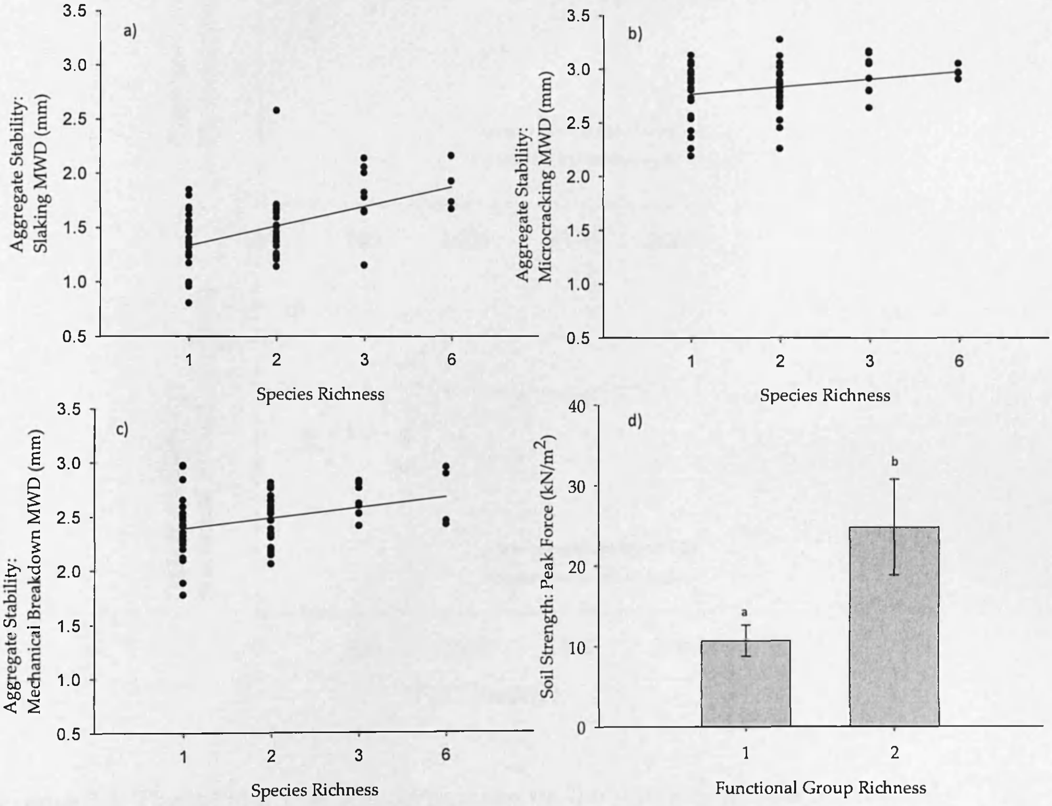


Figure 3.2: Plant diversity effects over soil physical properties. Displaying the significant positive impact of species richness upon all three aggregate stability measures: slaking (a); microcracking (b); and mechanical breakdown (c); and the significant positive impact of increasing functional group richness upon soil strength (d). Letters denote significant difference between treatments P<0.05.

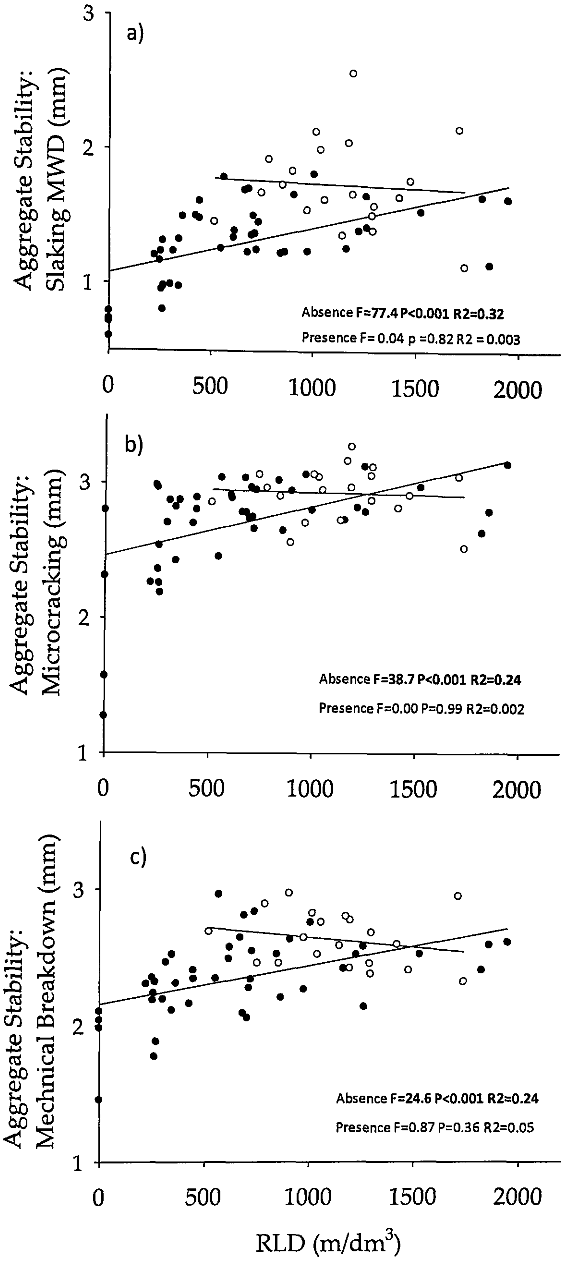


Figure 3.3: The influence of Lolium perenne on the root length effects over soil aggregates. Relationship between root length density (RLD) on three aggregate stability measures: slaking (a); microcracking (b); and mechanical breakdown (c) in the presence and absence of *Lolium perenne*. Black dots indicate *L. perenne* absent in a community, white dots indicate *L. perenne* present.

Table3.2: ANCOVA results investigate whether traits explain any species richness effect.

	Soil Physical Properties									
	Aggregate Stability						Hydrology		Strength	
	Slaking		Microcracking		Mechanical Breakdown		Saturated hydraulic conductivity		Peak shear force	
Covariates	$F_{1,48}$	p	$F_{1,48}$	p	$F_{1,48}$	p	$F_{1,53}$	p	$F_{1,47}$	p
RLD (m/dm3)	**27.34**	**<0.0001 +**	**20.79**	**<0.0001 +**	**17.03**	**<0.0001 +**	NA	NA	NA	NA
RD (g/dm3)	3.04	<0.1	1.76	0.19	1.56	0.22	3.72	<0.1	**10.08**	**<0.01 +**
RDIAM (mm)	**22.13**	**<0.0001 -**	**15.3**	**<0.001 -**	**14.13**	**<0.001 -**	NA	NA	NA	NA
SRL (g/m)	**17.12**	**<0.0001 +**	**15.95**	**<0.001 +**	**9.48**	**<0.01 +**	NA	NA	NA	NA
DMC	4.01	<0.1	2.91	<0.1	3.48	<0.1	NA	NA	NA	NA
TMD (g/cm3)	1.9	0.17	**5.31**	**<0.05**	0.39	0.54	NA	NA	NA	NA
Aboveground biomass (g)	0.25	0.62	0.01	0.92	1.33	0.25	**4.26**	**<0.05 +**	**12.73**	**<0.001 +**
Factors										
Species Richness	**8.21**	**<0.01 +**	0.66	0.42	1.74	0.19	1.62	0.21	0.15	0.6993

Displaying F and p values from two ANCOVA analysis for: the effects of root length density (RLD), root mass density (RD), average root diameter (RDIAM), specific root length (SRL), root dry matter content (DMC), root tissue mass (TMD), aboveground biomass and the factor species richness on aggregate stability, andthe effects of: RD, aboveground biomass and species richness on hydrology and strength. F values are those were respective covariate was fitted first, the factor species richness was always fitted last. + indicates a positive relationship between covariate/factor and physical property

3.3.3 Community effects on plant properties

The individual species *L. corniculatus* produced the most aboveground biomass (13.44 ± 2.72 g) (Figure 3.3a), significantly more than *A. oderatum* (5.38 ± 1.17 g), *A. millefolium* (5.06 ± 0.24 g) and *P. lanceolata* (5.93 ± 0.58 g) (F5,15 = 4.67, $p < 0.01$). Aboveground plant biomass increased significantly with increasing plant species richness (F1,55 = 6.17 $p < 0.05$) (**Figure 3.4**). Also, increasing functional group richness from 1 to 2 groups, whilst keeping species richness constant, increased aboveground biomass (F1,19 = 10.58, $p < 0.01$) (**Figure 3.4**).

As expected, root traits varied among plant species (**Table 3.3**). The grasses, *L. perenne* and *A.oderatum,* displayed the greatest root length, specific root length and narrowest average diameter. *T. repens* had the lowest root length, root mass and tissue density, and the highest dry matter content, whilst the other legume *L. corniculatus* had the greatest root mass, diameter and tissue density, and consequently the lowest specific root length. The two forb species *A. millefolium* and *P. lanceolata* displayed no extremes of root traits, but tended to occupy the intermediate range for all characteristics.

Plant diversity influenced a number of community root traits (**Figure 3.5**). Increased species richness caused greater root length (F1,55 = 7.19, $p < 0.01$) and lower average diameter (F1, 55 = 5.21, $p<0.05$) and dry matter content (F1,55 = 4.81, $p<0.05$). Nevertheless, root mass density (F1,55 = 1.6, $p = 0.21$), specific root length (F1,55 =2.5, $p=0.11$) and tissue mass (F1,55= 0.06,$p=0.81$) did not change as a consequence of species richness. Increasing plant functional group richness from one to two functional groups increased community root mass (F1,19 = 9.11, $p<0.01$) and tissue density (F1,19=14.65, p<0.01), but reduced specific root length (F1,19 =5.02,p<0.05) (Figure 3.3). Increasing functional group richness from one to two groups had no impact on root length (F1,19 =0.42,p=0.52), diameter (F1,19=0.37,p=0.55) or dry matter content (F1,19 = 0.92, p=0.35).Aboveground biomass correlated with root mass (Pearsons = 0.38, p<0.01) and tissue density (Pearsons = 0.26, p<0.05), whilst no significant pattern was found with the other belowground traits.

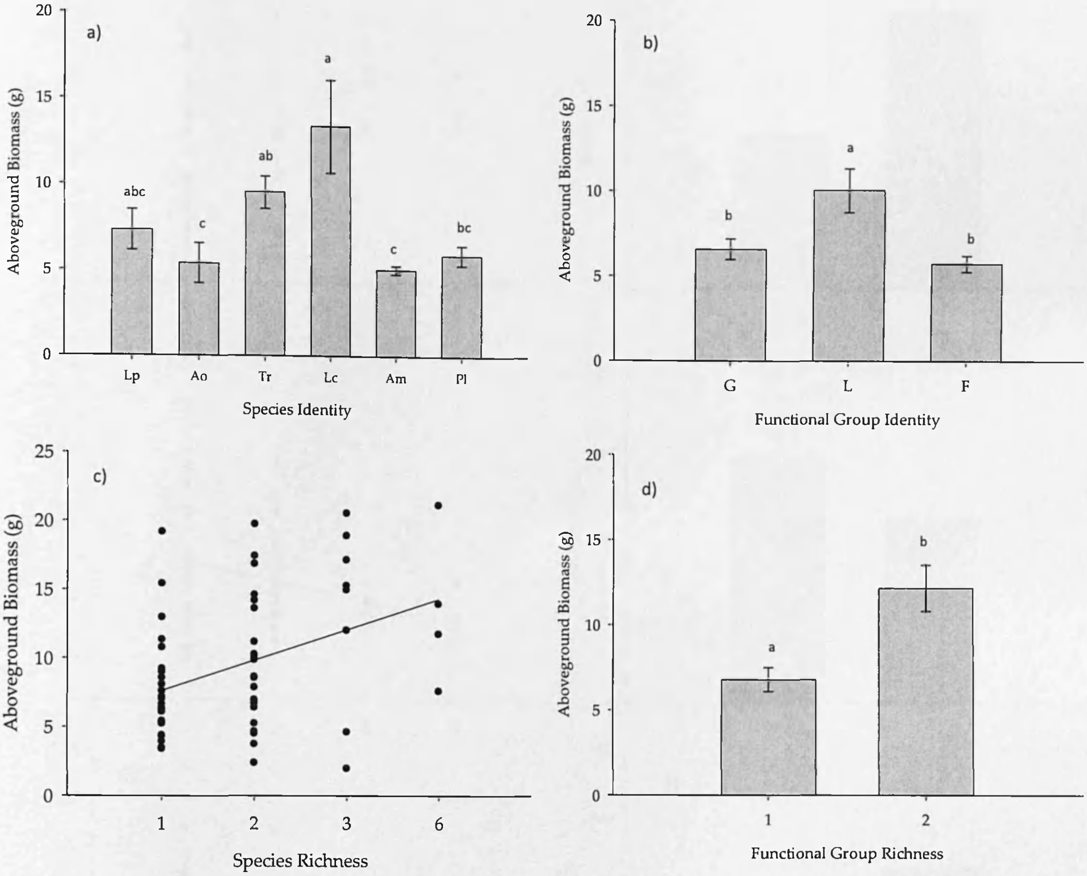


Figure 3.4: Plant community effects on plant productivity. Displaying the influence of a) species identity (*Lolium perenne,* Lp, *Anthoxanthum oderatum,* Ao, *Trifolium repens,* Tr, *Lotus corniculatus,* Lc, *Achilliea millefolium,* Am, *Plantago Lanceolata,* Pl), b) functional group identity (Grass, Legume, Forb), c) species richness, and d) functional group richness on aboveground biomass. Letters denote significant difference between treatments P <0.05.

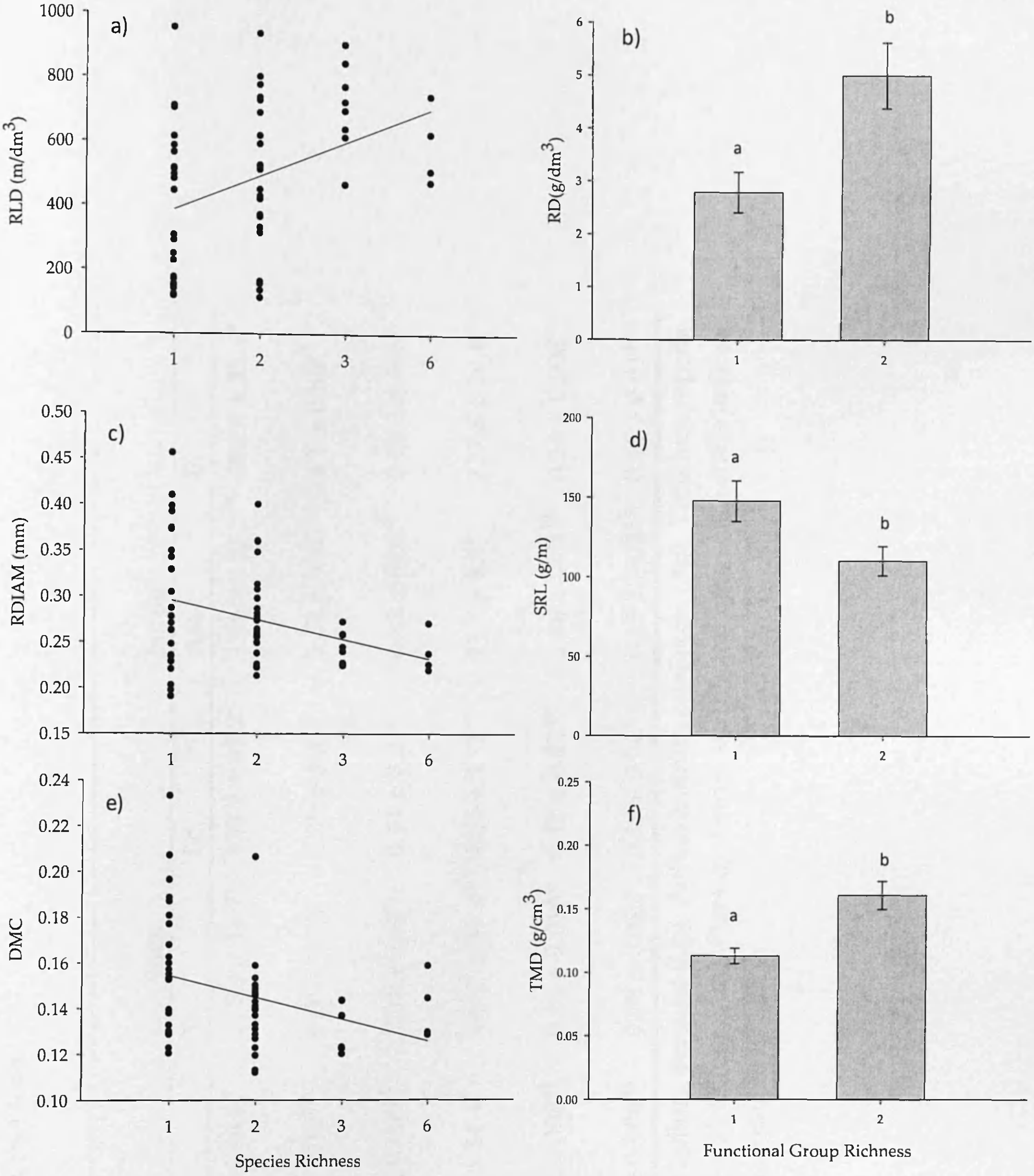


Figure 3.5: Plant diversity impacts on root traits. Displaying the significant influence of species richness on: root length density (RLD) (a); average root diameter (RDIAM) (c); and root dry matter content (DMC) (e); and the significant influence of raising functional group richness from one to two groups upon: root mass density (RD) (b); specific root length (SRL) (d); and root tissue mass density (TMD) (f). Letters denote significant difference between treatments $P<0.05$.

Table 3.3: Individual species variation in root traits.

Root Traits	Species Identity					
	Grasses		Legumes		Forbs	
	Lp	Ao	Tr	Lc	Am	Pl
RLD (m/dm^3)	689.8 ± 99.6^{a}	568.6 ± 46.8^{a}	135.9 ± 11.7^{c}	230.9 ± 39.2^{b}	193.5 ± 25.5^{bc}	496.5 ± 33.5^{a}
RD (g/dm^3)	4.03 ± 0.84^{ab}	2.53 ± 0.19^{bc}	1.05 ± 0.23^{c}	5.79 ± 0.86^{a}	2.51 ± 0.36^{bc}	4.1 ± 0.56^{ab}
RDIAM (mm)	0.22 ± 0.009^{d}	0.21 ± 0.009^{d}	0.33 ± 0.02^{b}	0.41 ± 0.01^{a}	0.33 ± 0.02^{b}	0.26 ± 0.005^{c}
SRL (m/g)	177.0 ± 9.6^{a}	231.7 ± 34.4^{a}	149.7 ± 35.2^{ab}	40.5 ± 5.7^{c}	79.3 ± 8.7^{b}	127.6 ± 14.8^{ab}
DMC	0.13 ± 0.003^{b}	0.16 ± 0.008^{ab}	0.19 ± 0.016^{a}	0.17 ± 0.02^{ab}	0.16 ± 0.01^{ab}	0.14 ± 0.008^{b}
TMD (g/cm^3)	0.15 ± 0.01^{ab}	0.12 ± 0.006^{bc}	0.08 ± 0.012^{c}	0.19 ± 0.02^{a}	0.15 ± 0.02^{ab}	0.15 ± 0.01^{ab}

Mean values ± SE for the root traits of Lolium perenne (Lp), Anthoxanthum oderatum (Ao), Trifolium repens (Tr), Lotus corniculatus (Lc), Achilliea millefolium (Am), Plantago lanceolata (Pl) and bare soil. Letters indicate significant differences between plant treatments $P>0.05$

3.4 Discussion

In this experiment we found significant effects of species richness, functional group richness, species identity and functional group identity on an array of soil physical properties. The response of soil structure to plant community dynamics varied widely, illustrating the broad range and complexity of processes involved in the stabilisation of soils by plants. Increased species richness provided greater root length in the soil and benefitted aggregate stability. For microcracking and mechanical breakdown of aggregates, we found that all of the impact of species richness could be explained by root traits of the community, whilst for aggregate slaking there was an additional richness effect unexplained by roots. Functional group richness, on the other hand, did not affect root length or aggregates but showed a positive increase in root mass and subsequent root-soil strength properties. The role of single species in plant community-soil physics dynamics varied widely. For the aggregate processes, the prominent monoculture grass species, *L. perenne*, provided a significant benefit to aggregate properties under mixed plant communities. In the case of soil strength, the greatest contributor in monoculture, the legume *L. corniculatus*, did not however provide significant benefits to strength in the mixed communities. Throughout the experiment, legumes and grasses consistently showed notable impacts over all physical properties. Soils under legumes exhibited poorer aggregate stability, yet increased hydraulic conductivity in monocultures, whilst the legume *L. corniculatus* also provided considerable gains to soil strength. Conversely, grasses provided greater aggregate stability, but slower hydraulic conductivities through the soil.

Throughout the experiment plant community impacts were less pronounced on soil hydraulic conductivity and shear strength responses, than they were on the responses of aggregate stability. Although shear strength did respond to greater numbers of functional groups in a community, and hydraulic conductivity did show a relationship with legumes, these two physical properties were mainly affected by plant function and root mass within the soil. In contrast, aggregate stability was influenced by species richness, an effect which can be attributed to both the role of greater root length, and the presence of certain plant identities, within the mixed communities.

3.4.1 Plant species interactions and soil aggregate stability

Greater species richness had a positive impact over all breakdown mechanisms of aggregate stability. This relationship has received little attention from the biodiversity-ecosystem function literature (Pohl et al. 2009, Pérès et al. 2013), and this is the first time a study has investigated the process in manipulated mesocosm communities. This experimental set up allowed us to explore some of the mechanisms behind the relationship. Roots play a major role in soil aggregation (Angers and Caron 1998), and it is not surprising that we found significant links between finer root length and greater aggregate stability. Finer roots exhibit high decomposability within the soil (Goebel et al. 2011), and this greater organic input belowground is a central part of aggregate formation and stabilization (Oades and Water 1991). In addition, with increased root length, the root hairs, organic exudates and microbial stimulation associated with roots (Tisdall and Oades 1982, Six et al. 2004) are provided with a more expansive platform to influence aggregate binding processes. What is interesting is that increasing species richness also increased fine root length, and reduced the average diameter of roots in a community. This suggests that higher diversity is increasing aggregate stability by influencing the root traits belowground. By testing this idea in ANCOVA analysis, we found that root properties did indeed explain the species richness effect for aggregate resistance to microcracking and mechanical breakdown. However, for the third aggregate stability measure, slaking, root traits did not account for all the variation caused by species richness. For slaking, the most aggressive soil aggregate breakdown process (Saygin et al. 2011), the diversity effect is not solely caused by the increase in rooting structures belowground, suggesting a diversity effect based on species-specific interactions.

Root length is thought to increase under higher diversity due to vertical niche separation, where roots are driven to explore less-populated areas of the soil in the presence of competition (Mueller et al. 2013). However, it is not the case here, as percentage root allocation at different depths in the soil did not vary between diversity treatments. The increases in root length may be a result of the presence of certain species dominating belowground, and although we did not separate out individual species' contributions to mixed rooting communities, we do know that exploitative grasses will become superior belowground competitors, greatly increasing community root length (Mommer et al 2010). As a function of their exploitation of belowground space, and the fact that they exhibit the finer, narrower root traits most influential to aggregation, grass species had a particularly notable effect on soil aggregate stability. In monocultures, and more importantly in mixtures, the presence of the grass L *perenne* contributed to greater aggregate stability. Further analysis showed us that this contribution is a result of the exploitative root properties of this species. When we separated the samples into those with, and those without, the presence of L. *perenne* in the aboveground community, we found that the inclusion of L. *perenne* significantly altered the relationship between roots and aggregate stability. With L. *perenne* in a community, root length contributions to aggregate stability exceeded a threshold, and all aggregates were sufficiently stabilized by roots. The presence of this species provides sufficient root length to a soil to stabilize aggregates, whereas in its absence, any other plant species would have to substantially increase their root length in the soil to achieve the same levels of aggregation. Much like other biodiversity-ecosystem function studies which have found the importance of single plant species for driving certain soil biochemical processes (De Deyn et al. 2010), here we find the importance of one species in controlling soil physical processes.

3.4.2 Plant function, soil hydraulic conductivity and strength

Whilst aggregate stability responded to plant species and root length dynamics, the other physical properties we investigated were influenced by different biological processes. Increasing the number of functional groups within a community showed a significant rise in the strength properties of the soil. Much like the diversity effect over aggregates, again we can attribute the influence of functional group richness on soil strength to the contribution of roots. Soil strength benefitted from increased root mass belowground, which provides anchorage and cohesion against disturbance (Waldron and Dakession 1982, Loades et al. 2010). Interestingly, we found these desirable root reinforcing properties to be more prominent in communities of greater functional group richness. Contrasting with the species richness effect on root length and aggregates, raising functional group richness encourages a separate suite of root traits, thicker and denser, more often associated with conservative plant resource strategies. It is these root traits that are strengthening the soil, and causing the diversity effect. Although monocultures showed that the most densely rooting species, *L. corniculatus*, which proliferated thick tap roots throughout the soil, reinforced the soil much more than other species, we did not find this species to be making an additional contribution to the soil strength of diversity mixtures. Species-specificity does not impact on community-shear strength dynamics in the same was as it does on community-aggregate stability dynamics.

The saturated hydraulic conductivity of the soil did not respond significantly to any changes in diversity properties of the plant community. Although species richness and functional group richness had no impact on hydraulic conductivity, we did find that plant functional group identity was playing a role in the soil hydrology dynamics. Legumes have been used in farming systems as cover crops to benefit infiltration (Yaacob and Blair 1981, McVay et al. 1989), and we found the inclusion of legumes in our two-species mixtures dramatically increased the hydraulic conductivity of the soil. The stouter tap rooted systems of legumes provides this greater conductivity (Morgan et al. 1995), by creating hydraulically effective pathways for water to flow down the soil profile. These root characteristics are a contrast to the densely matting roots of the grass species, which can act to obstruct pore space and hinder water flow (Edwards et al. 1997), and they provide significant benefits to soil water flow when included in mixed grassland communities. In fact, throughout the experiment legumes showed stark contrasts to the grasses in the manner they influence soil physical properties. This is not surprising, given the different nature of the rooting properties of both functional groups, and the significance of these root traits in governing soil physical properties. Whilst the expansive rooting grasses contributed to soil aggregation, legumes played a much more significant role in benefitting soil water flow and strength properties, with poor soil aggregation properties.

3.4.3 Aboveground properties and canopy effects

As a result of increased species richness, we also found aboveground over yielding, which agrees with the general trend of many previous studies (Hooper et al. 2005, Cardinale et al. 2012). Although we know of the considerable benefits of increased aboveground cover to soil stability (Casermeiro et al. 2004, Puigdefabregas et al. 2005, Bautista et al. 2007), canopy effects were not considered here. By only assessing the intrinsic soil structural properties, we thus found no effect of aboveground productivity on the binding mechanisms of aggregate stability. This does not go to say that the aboveground effects are not of great importance; however they contribute more to protective factors against soil erosion, as opposed to the stabilising factors studied here. The correlative relationship we found between aboveground productivity and both hydraulic conductivity and soil strength was an artefact of the increased growth, and subsequent increased root mass belowground, which in turn benefitted both hydraulic conductivity and shear strength. It is also worth noting that as legumes produced considerably more aboveground biomass, they would evidently contribute some canopy benefits that may not be provided by grasses or forbs.

3.5 Conclusion

Our results show that plant species richness can have strong effects over soil aggregate stability, whilst plant functional groups can hold some influence over the soil hydraulic conductivity and strength properties. For two of the three measured aggregate stability breakdowns, the species richness effect was caused by diversity contributions to root length. For a third aggregate breakdown, slaking, rooting contributions did not explain all of the diversity effects, and we suggest the presence of certain species may play a major role in contributing to aggregate stability under grassland communities. Until now, most plant diversity research has focused on the response to biological and biochemical processes (Cardinale et al. 2012), and the very little that is known of the response to physical structure has been investigated at correlative field studies (Pohl et al. 2009), or small scale plot experiments (Pérès et al. 2013). This is the first time the relationship has been explored in a mesocosm experiment, representing a significant contribution to our knowledge of biodiversity-ecosystem function, and allowing us to advance our understanding of the mechanisms behind these patterns.

Acknowledgements

The authors would like to thank John Huddleston, James Paul Stride, and members of the soil and ecosystem ecology team at Lancaster University, for their contributions to the study. The research was funded by a BBSRC studentship.

4. Plant species richness and functional identity impacts on soil aggregate stability: an experimental plot study

Gould, I.J., Quinton, J.N., Weigelt, A., De Deyn, G.B., and Bardgett, R.D

Chapter 3 revealed that plant community composition does have strong effects over soil physical properties in a tightly controlled mesocosm study. Of particular note was the impact of species richness and root traits over soil aggregate stability. This chapter aims to expand our understanding of such processes, by investigating which mechanisms are driving the relationships, studied in a wider environmental context.

Abstract

To date, most research on the effects of plant diversity on the functioning of ecosystems has focussed on impacts on plant production and soil processes of carbon and nutrient cycling. Consequently, virtually nothing is known about how plant diversity might impact on soil physical properties and what mechanisms might be involved. This represents a serious gap in knowledge, given that maintaining soils with good structural integrity can reduce soil erosion and water pollution, and can lead to improved plant yield. Therefore, there is a need for a greater understanding of how plant communities and ecological interactions between plant roots and soils can play a role in regulating soil physical structure.

One soil physical attribute that has strong links to soil degradation is aggregate stability, which represents the ability of soil aggregates to bind together and withstand erosive forces. Here, we report on results of a long term grassland biodiversity experiment, where we tested for the effects of plant diversity on soil aggregate stability. The Le Bissonnais method of aggregate stability analysis was used to measure the potential for soils from different diversity treatments to break down under three different environmental conditions: (i) slaking; (ii) slow wetting; and (iii) mechanical breakdown. Root trait measurements, and glomalin analysis, were also made to evaluate the role that they play in affecting soil aggregate stability. It was revealed that increasing plant species richness benefitted soil aggregate stability, and that plant community composition, root traits, glomalin, and organic matter all played a significant role in these dynamics.

4.1 Introduction

Soil aggregate stability is a fundamental component of soil physical structure, representing the capacity for soil aggregates to resist breakdown from the effects of erosive agents, such as water (Le Bissonais 1996, Barthès et al. 2002). In the coming century, a predicted increase in the intensity of severe hydrological events (Gordon et al.1992, Fowler et al. 2009, Diffenbaugh et al. 2013) poses a significant threat to soil aggregate structure, with the potential to intensify the breakdown of aggregates by processes such as slaking; microcracking; and mechanical disturbance. It is well established that plants influence soil aggregate stability, through the capacity of plant roots and arbuscular mycorrhizal (AM) fungal hyphae to physically enmesh particles, the soil-binding nature of root and microbial exudates, and the cohesive capabilities of plant-derived organic matter (Tisdall and Oades 1982, Degens et al. 1994, Angers and Caron 1998, Six et al. 2004). Plant characteristics relevant to soil stability mechanisms are those that invest large root mass belowground (Mamo and Bubenzer 2001, Gyssels et al. 2005, Pérès et al. 2013) leading to more organic input into the soil. Furthermore, greater root length also benefits aggregation (Pohl et al. 2009), by increasing the spatial extent that plants can exert these soil-binding capabilities. Plant functional groups that display expansive rooting strategies to acquire belowground resources, such as grasses, therefore increase soil aggregate stability, whilst the presence of functional groups that need not root so extensively, for example the N-fixing legumes, could result in less stable aggregation. Also, traits that stimulate growth of soil fungal communities play a crucial role in soil aggregation regimes (Oades and Waters 1991, Ritz and Young 2004); not only do their expansive hyphae act to enmesh microaggregates together, but the build-up of glomalin in the hyphal walls has been postulated to have a robust cementing effect upon soils too (Rillig et al. 2002).

Glomalin is a glycoprotein produced by AM funghi. Since the discovery of the presence of glomalin in fungi and soils in the mid-1990s (Wright and Upadhyaya 1998), evidence has revealed good correlation between glomalin and soil binding mechanisms. Because of the highly recalcitrant properties of this substance, positive correlations have been found between glomalin related soil protein (GRSP) content of a soil, soil aggregate stability, and subsequent soil carbon sequestration (Wright and Uphyaya 1998, Rillig et al. 2002, Treseder and Turner 2007). Although plant diversity has been found to influence AM fungal communities in the soil (De Deyn 2009), the effect of increasing plant diversity on glomalin levels in the soil is unknown, and therefore the consequences for soil stability remain unexplored.

If individual plant species are able to impact on soil aggregates, through the actions of their belowground traits, it is likely that diversity can impact on soil structure too.

We know that a loss of plant diversity within a community can lead to a reduction in primary productivity; changes to root properties; and shifts in nutrient cycling regimes (Hector et al. 1999, Steinbess et al. 2008, Bessler et al. 2009, Eisenhauer et al. 2010). These processes all have the potential to influence the aforementioned mechanisms by which plants impact on soil aggregates. Although receiving very little attention in the biodiversity-ecosystem function literature, a very small number of studies have examined this relationship within grassland communities. In an alpine survey, Pohl et al. (2009) found a positive correlation between plant diversity and soil aggregate stability. Further work was conducted by Pérès et al. (2013) at a subset of plots on the biodiversity manipulation experiment in Jena, Germany, finding an increase in aggregate stability between monocultures and mixed species communities. A pattern begins to emerge from these studies, suggesting higher species richness within a community could have strong effects over soil aggregates. However, this relationship lacks sufficient experimental evidence, having never been studied on a large diversity gradient.

The overall goal of this chapter was to investigate the impact of increased plant species richness on soil aggregate stability across a large scale plant diversity gradient. We suggest that a community of higher species richness will not only comprise a wider variety of soil-binding traits, beneficial to aggregate stability, but will also influence aggregate stability through species-richness induced effects on the biological and biochemical properties of the soil. To investigate this, we aimed to test how diversity impacts on aggregate stability, via changes in root behaviour, organic matter, and glomalin content of the soil, and through the contribution of different plant functional groups within a community.

4.2 Methods

4.2.1 Experimental design

The study was undertaken at the Jena Experiment, a large scale plant manipulation experiment on the outskirts of Jena, Germany (50°55′N, 11°35′E). The site is built upon the floodplain by the Saale River, and the soil is a Eutric Fluvisol. Mean annual temperature is 9.3 °C with annual precipitation of 587 mm (Kluge and Müller-Westermeier 2000). Soil texture ranges from silty clay to sandy loam with increased distance from the river. This change in soil composition is incorporated into the parallel block design of the experiment, with each block encompassing a slight change in soil texture.

The experiment was established in May 2002. From a pool of 60 species, a gradient of plant species (1, 2, 4, 8, 16, and 60) and functional group richness (1, 2, 3, and 4) was sown in a total of 82 plots of 20 × 20 m (For further experimental design, see Roscher et al. 2004). The species chosen were representative of Central European mesophilic grassland, pooled into four functional groups: grasses, legumes, small herbs, and tall herbs. Species and functional group richness is replicated over four blocks, whilst mixtures within blocks are fully randomised. Plots are mown twice a year, in June and September, and weeded regularly to maintain original species composition.

Sampling

Sampling was undertaken in June 2012, ten years after establishment of the experiment, and just after the biannual harvest of aboveground plant biomass. Within a 0.3 × 1.6 m allocated strip per plot, two topsoil (~ 10 cm depth) clod samples were extracted with a trowel, and three 3.2 cm diameter cores were taken to 10 cm depth. To ensure minimal disruption of the aggregate structure, the two soil clods were carefully wrapped in protective lining before transportation to Lancaster University, UK, for analysis of soil aggregate stability. The three soil cores per plot were bulked and remained in Jena for root trait analysis.

4.2.2 Soil Analysis

The soil samples for aggregate analysis were air dried as a clod, before being passed through a 5 mm sieve, after which the 2-5 mm aggregate size fraction was selected for testing. Aggregates were then placed in an oven at 40 ^{0}C for 24 hours whereafter moisture loss ceased and a constant matric potential resulted, before undergoing stability testing. The Le Bissonais method (1996) was used to determine aggregate stability, which subjects the aggregates to three breakdown mechanisms representing different environmental pressures: slaking (rapid immersion in water for 10 minutes), microcracking (slow wetting on a -0.3KPa tension table for 1 hour), and mechanical breakdown (placing in a 250ml conical flask and agitating end over end 10 times). After the breakdown mechanisms, each aggregate sample was wet sieved in ethanol before oven drying and measuring the eventual aggregate size distribution (Le Bissonais 1996). The Mean Weight Diameter (MWD) of each post-breakdown aggregate sample was used as the measure of aggregate stability. This methodology yields three MWD's, one for each breakdown mechanism. In addition, aggregates were also used for determination of organic matter content using the loss on ignition method, which involved placing the soil in a furnace at 560°C for 6 hours to ignite all organic matter. Soil aggregate samples were also sent to the Department of Soil Quality, Wageningen University, Netherlands, for determination of glomalin-related protein (GRP) (see Rosier et al. 2006, Koide and Peoples 2013).

4.2.3 Root Analysis

Soil cores for root measurement were washed of all soil, and placed in a water-filled clear tray and scanned. Root images were analysed with WinRhizo (Regent instruments, Quebec, Canada) software to determine total root length of a sample. Root Length density (RLD) was calculated as total length per unit of soil and Root Mass Density (RD) was determined by dry mass of roots per unit of soil.

4.2.4 Data Analysis

Data for species richness and root length and mass density were log transformed in order to reach assumptions for normality. ANOVA as part of a general linear model (GLM, type 1 sum of squares) was used to analyse the effects of block (BL), plant species richness (SR), plant functional group richness (FR), the presence of grasses (G), legumes (L), small herbs (SH), and tall herbs (TH), on each of our measured responses. This resulted in seven models, one for community effects on each of the four biological responses: root length (RLD), root mass (RD), glomalin-related protein (GRP) and organic matter (OM); and then one for community effects on each of the aggregate stability responses: slaking (MWDf), microcracking (MWDs), mechanical breakdown (MWDm). In all ANOVAs, block effects were fitted first, followed by the diversity effects of species and functional group richness. In order to conclude which diversity measure – species or functional group richness – was a more powerful predictor, we ran each model twice, alternating the order of these two diversity measures. Therefore, we present values for the result of fitting species richness before, and then after, functional group richness effects. After fitting the diversity measures, the presence/absence of each functional group was included. Because we alternated the order of functional group presence in the model, the *F* values given in the text and tables refer to those where the respective factor was fitted first (Schmid et al. 2002).

We used path analysis to see if plant community dynamics were directly related to soil aggregate stability, or whether it is an effect caused by plant community contributions to the root, glomalin, and organic matter characteristics of the soil. We designed the path analysis based on the ANOVA results, and further linear regression between the root, glomalin, and organic matter properties and the aggregate stability properties at each plot. Three path analyses were performed, one for each aggregate breakdown measure. In each model, we used species richness, the presence of grasses and the presence of legumes as exogenous variables (chosen from prior ANOVAs), with RLD, RD, GRP and OM as endogenous variables. In the full model, all potential pathways were supported by previous ANOVA or linear regression. After the full model was fitted, we performed stepwise model selection based on non-significant x^2 tests and Akaike's information criterion (AIC) values, removing non-significant pathways to improve the model fit. Plant species richness and the presence of grasses and legumes were correlated in the models. Path analysis were performed using Amos (v20, Amos Development Corporation), all other analyses were carried out in R.3.0.1 (R Development Core Team, 2013).

4.3 Results

4.3.1 Soil aggregate stability

There was a significant positive relationship between higher plant species richness and all three measures of aggregate stability (slaking: $F_{1,70}$=75.89, P<0.0001; microcracking: $F_{1,70}$=27.68, P<0.0001; and mechanical breakdown: $F_{1,70}$=53.65, P<0.0001) (Table 4.1, Figure 4.1a-c). This relationship was so pronounced, it even remained significant after the effects of species richness were fitted behind functional group richness in the model (Table 4.1). All three measures of aggregate stability were also positively affected by increased plant functional group richness, but only when fitted in the model prior to species richness; including species richness beforehand nullified any functional group richness effect (Table 4.1, Figure 4.1d-f). After accounting for variance attributed to the richness properties, we investigated the impact of the presence of grasses, legumes, tall herbs and short herbs upon soil aggregate stability. The presence of grasses significantly increased aggregate stability (slaking: $F_{1,70}$=30.09, P<0.0001; microcracking: $F_{1,70}$=5.10, P<0.05; mechanical: $F_{1,70}$=12.39, P<0.001), whilst the presence of legumes significantly decreased all three of these stability measures (slaking: $F_{1,70}$=29.96, P<0.0001; microcracking: $F_{1,70}$=7.73, P<0.01; mechanical: $F_{1,70}$=12.94, P<0.001). Block effects also showed a significant relationship with all three measures of aggregate stability (Table 4.1).

We detected significant positive relationships between root mass, root length, aggregate organic matter, and glomalin-related protein content upon all three measures of aggregate stability (Fig. 4.2). Subsequent path analysis was employed in order to disentangle some of the potential mechanisms underlying plant community impacts on soil aggregate stability (Fig. 3, Supplementary Table 1). Not only did species richness have a direct positive effect upon soil aggregate stability, it also influenced aggregate stability indirectly, by increasing root mass and organic matter content of the soil, mechanisms which subsequently benefitted all three aggregate stability measures (Fig.4.3). Grasses positively influenced two of the aggregate stability measures (slaking, and mechanical stability) directly, and influenced aggregate stability indirectly by providing greater root mass (for the case of slaking, microcracking, and mechanical breakdown) and greater root length (for slaking alone) in the soil. Legumes only showed significant negative effects on one of the aggregate stability measures, slaking, both directly, and indirectly through reduced root length in the soil. Our path analysis explained 27% of variation in root mass, 27% of variation in root length, 20% of variation in organic matter, 59% of variation in glomalin-related protein. For the three aggregate stability measures, it explained 72% of variation in slaking, 40% of variation in microcracking, and 66% of variation in mechanical breakdown.

Table 4.1: Summary of ANOVA analysis of plant community properties on soil biological and physical properties.

		Soil biological properties				Soil aggregate stability		
Factor	df	RLD	RD	GRP	OM	Slaking	Microcracking	Mechanical
Block	3,70	2.04	0.84	__37.43***__	__21.18***__	__8.15***__	__4.06*__	__12.83***__
SR first	1,70	0.91	__14.64***↑__	__17.36***↑__	__32.35***↑__	__75.89***↑__	__27.68***↑__	__53.65***↑__
SR second		0.57	__6.31*↑__	__10.90**↑__	__16.99***↑__	__37.42***↑__	__8.29**↑__	__26.08***↑__
FR First	1,70	0.34	__8.81**↑__	__6.47*↑__	__15.54***↑__	__39.39***↑__	__22.86***↑__	__28.31***↑__
FR second		0	0.49	0.02	0.18	0.92	3.47·↑	0.75
Grass	1,70	__34.98***↑__	__12.36***↑__	0.74	0.67	__30.09***↑__	__5.10*↑__	__12.39***↑__
Legume	1,70	__14.36***↓__	2.22	0.97	2.5	__29.96***↓__	__7.73**↓__	__12.96***↓__
S. Herb	1,70	0.17	0.97	0.15	3.81·↑	0.01	1.12	1.75
T. Herb	1,70	2.17	0.87	0.05	1.22	0.10	0.17	1.14

Displaying *F* values from ANOVAs for effects of block, species richness (SR), functional group richness (FR), and the presence of Grasses, Legumes, Short herbs and Tall herbs on root length density (RLD), root mass density (RD), glomalin-related protein (GRP), organic matter (OM), and aggregate stability against slaking, microcracking and mechanical breakdown. SR and FR are indicated as to whether fitted before, or after each other. Text in bold indicates a significant effect to $p>0.05$. Arrows indicate an increase or decrease in the response as a result of the relevant factor.

· $p <0.01$; * $p <0.05$; ** $p<0.01$; *** $p<0.001$

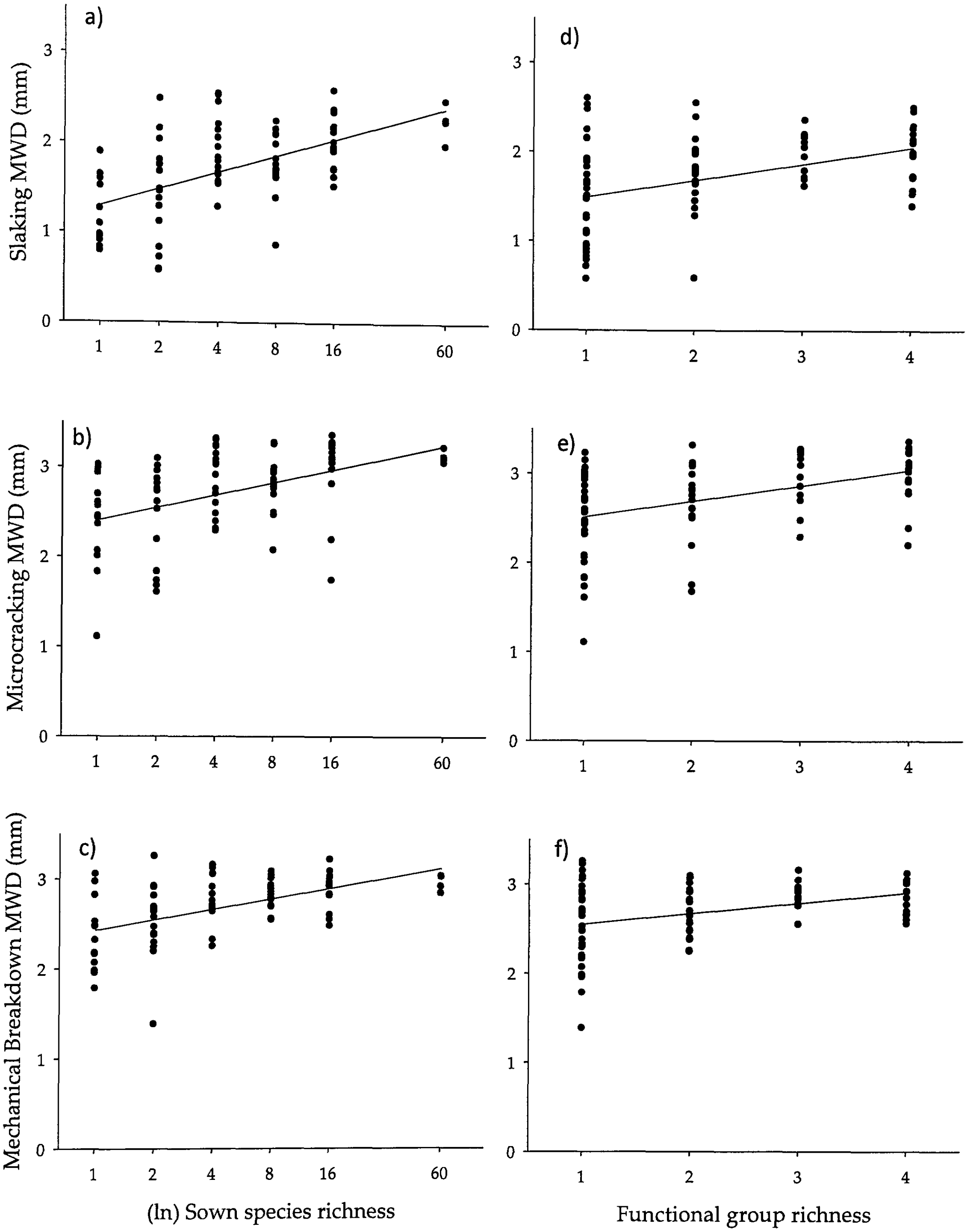


Figure 4.1: Relationship between sown species richness (log transformed) (a-c) and functional group richness (d-f) and the mean weight diameter (MWD) of each of the aggregate breakdown measures (slaking, microcracking, mechanical breakdown). Trendlines indicate significant effect of richness on aggregate stability ($P < 0.05$).

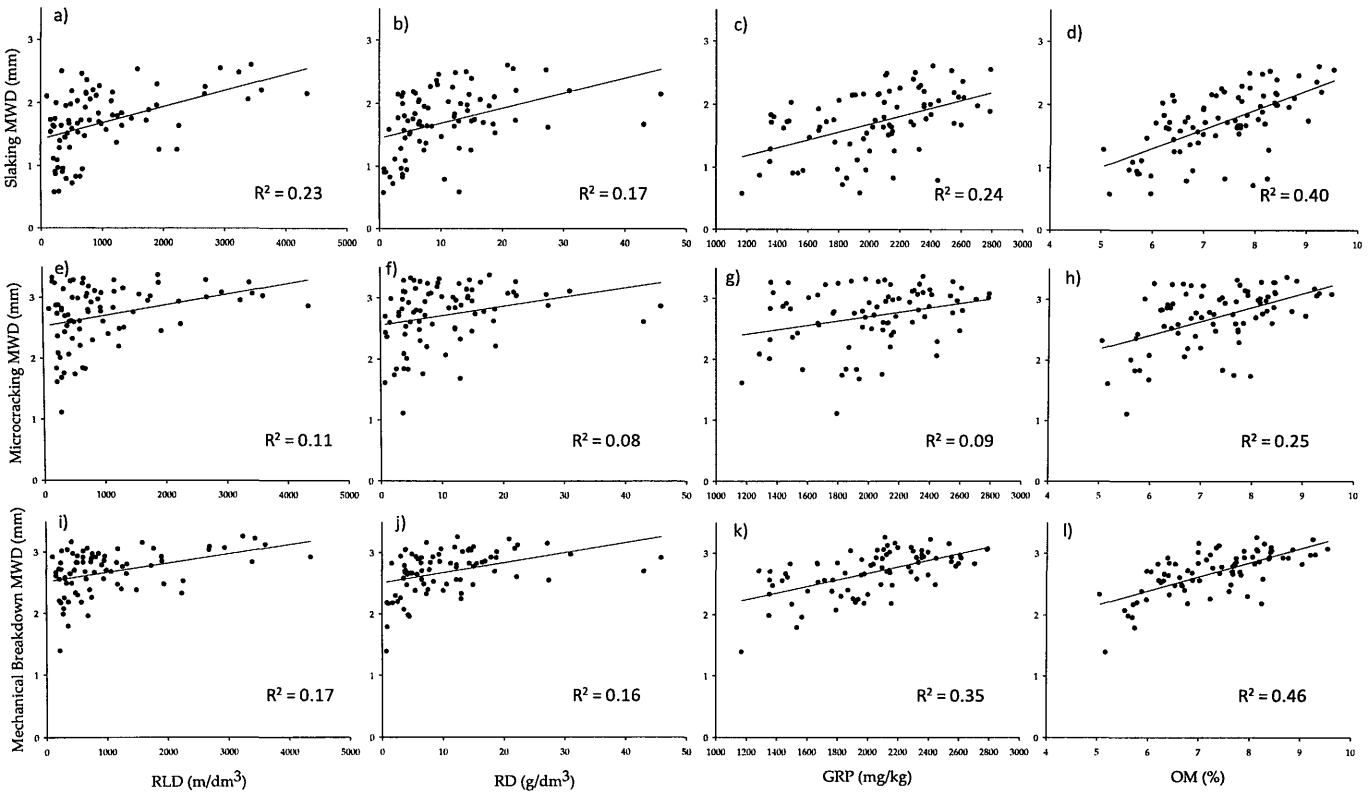


Figure 4.2: Regressions between aggregate stability (as represented by mean weight diameter, MWD) resistance to slaking (a-d), microcracking (e-h) , and mechanical breakdown (i-l) with root length density (RLD) (a,e,i), root mass density (RD) (b,f,j), glomalin-related protein (GRP) (c,g,k) and organic matter content of aggregates (OM) (d,h,l).

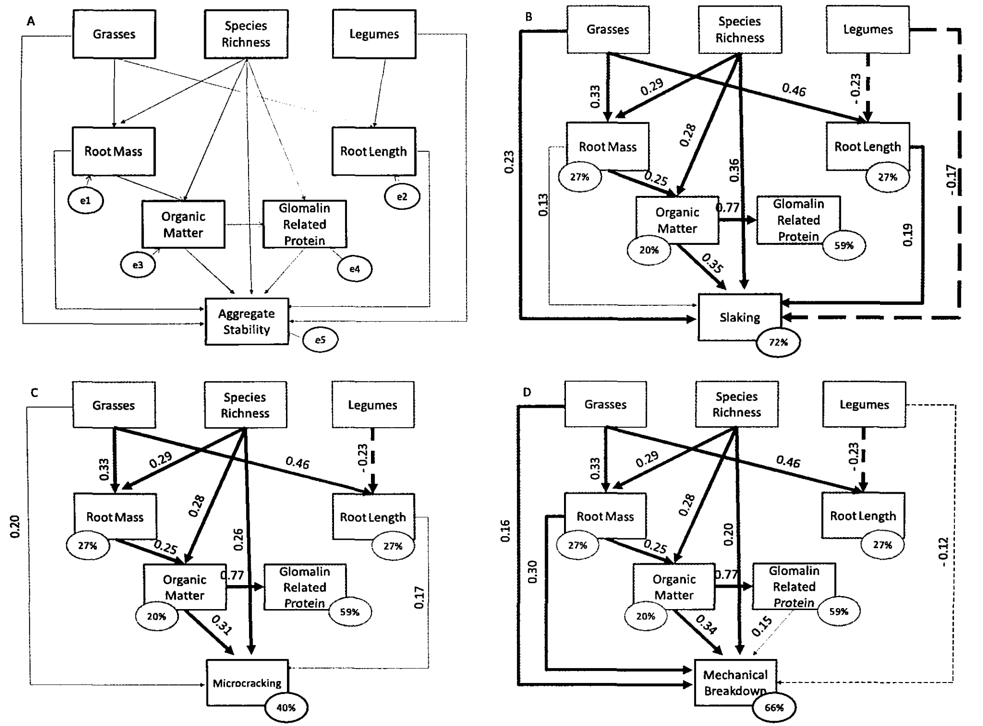


Figure 4.3: Path analysis of plant community impacts on slaking (B), microcracking (C) and mechanical breakdown (D), whilst (A) shows the initial model before selection of optimal pathways for each aggregate stability measure. Exogenous variables used were presence of grasses and legumes, and species richness. Endogenous variables were root mass, organic matter, root length, and glomalin-related protein. Solid (positive relationship) and dashed (negative relationship) arrows represent those that remained in optimal model, those in bold represent a significant relationship between variables ($P<0.05$). Numbers on arrows are path coefficients, whilst percentage values in circles represent variance explained of relevant variable by model. Circles in initial model (A) represent error terms.

4.3.2 Soil biotic activity: root properties, organic matter, and glomalin

Plant species richness significantly increased root mass ($F_{1,70}$=14.64, P<0.001), glomalin-related protein ($F_{1,70}$=17.36, P<0.0001), and organic matter content ($F_{1,70}$=32.35, P<0.0001) of soil aggregates (Table 4.1), and this relationship remained significant even after accounting for effects of functional group richness in the model (Table 4.1). Functional group richness also increased root mass ($F_{1,70}$=8.81, P<0.01), glomalin-related protein ($F_{1,70}$=6.47, P<0.05) and organic matter content ($F_{1,70}$=15.54, P<0.001) of aggregates, but only when fitted in the model prior to species richness (Table 4.1); including species richness beforehand nullified any functional group richness effects. Neither plant species richness nor functional group richness had any significant impact on root length (SR: $F_{1,70}$=0.91, P=0.34; FR: $F_{1,70}$=0.34, P<0.56), although the presence of grasses significantly increased both root length and root mass, and the presence of legumes significantly reduced this measure. No relationship was found between the presence of grass or legumes on aggregate organic matter content or glomalin-related protein (Table 4.1). There was a block effect on glomalin-related protein and organic matter content of the soil, but no effects on root length or mass were detected.

4.4 Discussion

This chapter revealed a significant positive impact of higher plant diversity upon soil aggregate stability. The study also highlights the potential mechanisms of this relationship: greater species richness increased root mass, organic matter, and glomalin content within the soil, all of which subsequently benefitted soil stability. In addition, we found that the presence of grasses allowed greater aggregate stability, by increasing community root mass and length. Contrastingly, the presence of legumes had negative effects on aggregate stability, by contributing less root length within the soil.

4.4.1 Impact of plant diversity on soil aggregate stability

Higher plant diversity, represented both by species and functional group richness, had a significant positive impact on all measures of aggregate stability, with species richness rather than functional group richness being the dominant driver. Although this experimental set up did not allow us to investigate individual contributions from each species within a community, we do know from Chapter 3 that individual species differ in the extent that they control soil physical properties (Scott 1998, Rillig et al. 2002, also Chapter 3). These findings not only confirm the hypothesis that plant diversity enhances soil aggregate stability, a key measure of soil structure, but also suggests that contributions toward aggregation from several different species within a plant community could act in contrasting ways alongside one another.

The positive impact of species richness on soil aggregates was not only explained by direct species interactions; diversity also determined aggregate dynamics through the contributions of increased species richness to soil biotic properties. Root mass and organic content of the soil were increased by higher plant diversity, which had cascading effects on all three types of soil aggregate stability. Organic inputs to the soil, such as decaying plant matter and root exudates, are an integral part of the aggregate forming process (Oades and Waters 1991), acting like glues as a fundamental part of aggregation. They bind microaggregates into macroaggregates, contributing toward a more stable soil structure. Roots not only provide a spatial platform for the release of organic exudates into the soil, but also form part of the aggregation process themselves, enmeshing soil particles and organic matter together (Miller and Jastrow 1990, Gyssels et al. 2005). Plant diversity increases the intensity of these processes, by altering root properties in such a competitive environment.

We found significant increases to glomalin content of the soil as a result of higher species richness. Glomalin-related protein displayed a positive relationship with soil aggregate stability. It has been postulated that the recalcitrant properties of glomalin contribute to persistent aggregate binding; both our linear regression, and past studies (Wright and Upadhyaya 1998, Rillig et al. 2002, Driver et al. 2005), support this process. Glomalin is deposited in soil mainly through the degradation of mycorrhizal hyphae, and, as well as binding soil, it is a hydrophobin and hence alters the wetting behaviour of soils; by impeding soil wetting, aggregate stability is enhanced due to reduced slaking (Guggenberger et al. 1999). Nonetheless, any influence of glomalin is intertwined with the effects of organic matter on soil stability, which path analysis proved to be a better predictor of soil stability than glomalin itself.

4.4.2 Impact of functional group identity on soil aggregate stability

The results show that the presence of different plant functional groups played an important role in promoting soil aggregate stability. In all cases, the presence of grasses lead to increased aggregate stability, which is consistent with previous results that show grasses to benefit soil aggregation (Chapter 3, Rillig et al. 2002; Jastrow 1987). Grasses produces large amounts of fine roots within the topsoil, an exploitative strategy to maximise resource uptake (Hodge 2003). This results in an extensive area of root coverage belowground (Bessler et al. 2009), providing a platform for microbial activity and organic exudation into the soil. As previously discussed, these mechanisms will act to bind soil micro aggregates (μm scale) together into the stable aggregates (mm scale) we have seen here. Although the benefits of grasses to root length only directly influenced stability against slaking, and not the other two measures of aggregate stability, all three forms of breakdown benefitted from the increased root biomass in the soil.

In contrast to grasses, the presence of legumes in mixtures led to poorer aggregate stability. This disparity between grasses' and legume's contribution toward aggregate stability is a consequence of the contrasting belowground strategies of each functional group. Through their association with nitrogen fixing bacteria, legumes increase soil nutrient availability, and as a consequence need not root so expansively to acquire resources. As root length has a positive relationship with all forms of aggregate breakdown, this will reduce the area of influence over aggregation, and lead to a diminished contribution of legumes toward soil structure. Legumes often contribute a substantial role in ecosystem function studies, influencing key nutrient cycling processes (Spehn et al. 2002, Milcu et al. 2008). To our knowledge, very few studies have investigated the role of legumes upon soil physical structure before, aside from some anecdotal evidence noting the contribution of legumes toward increased soil friability (Mytton and Cresswell 1992)

Our findings, of a negative impact on aggregate stability, do concur with this; although planting legumes will provide less stable soil aggregation, this will no doubt help to break up the soil and increase friability. Much like their impact on biochemical processes, in terms of contributions to physical properties we now find them to be a functional group of major importance.

4.4.3 Disparity between aggregate breakdown measures

We measured three forms of soil aggregate breakdown, and although the general trend of results showed similarities between all three mechanisms – i.e. a positive effect of species richness, and the significance of grasses, legumes, roots and organic matter in soil binding – our study highlights several differences between aggregate measures too. The two most aggressive breakdowns: slaking and mechanical disruption, were positively affected by root properties, whilst microcracking, governed more so by the internal shrink-swell capacity of the aggregate, displayed no such dependency on root mass or length. Stability against slaking is dependent upon the hydrophobicity of aggregates; often an artefact of the fine root and fungal hyphae exudates of a belowground community (Abiven et al. 2007), explaining why our path analysis showed slaking to be the only breakdown directly relating to root length. It is not surprising that slaking breakdown was thus the most responsive to plant community dynamics, with over 70 % of its variation explained by our path analysis, because of its inherent reliance on soil root and fungal behaviour. Interpreting these results into consequences for soil degradation could therefore vary depending on the relevant breakdown mechanism. Soils subjected to intense rainfall events, that would cause rapid flooding and heightened raindrop impact at the soil surface, would benefit from the presence of grasses, but breakdown more rapidly under legumes. Microcracking of soils, which would result from gentle increases in the wetting of soils over time, was less sensitive to plant community dynamics compared to the other two breakdowns. Nonetheless, all three stability measures clearly benefitted from increases in biodiversity.

4.5 Conclusion

The major finding of this study was that an increase in plant diversity resulted in a positive impact to soil aggregate stability. The response of aggregates related not only to diversity-induced changes in the root, organic matter and glomalin structure belowground, but also to the presence of particular functional groups; relationships that have been rarely studied before. Although studies on the biodiversity-ecosystem function relationship have provided us with a wealth of information on how reductions in diversity could lead to shifts in productivity, nutrient cycling, and microbial activity, we reveal that the impact of species loss could have farther reaching implications than first thought; declines in biodiversity could have detrimental effects on soil physical quality too. Our results are highly significant for situations where the management of soil structure is important, for example: flooding, erosion control, water movement through soils, and transport of contaminants; and may have practical applications in buffer strips and pasture management.

Table 4.S1: **Supplementary path analysis model selection information**

model	Chi Square	Df	p-value	Δ AIC initial model - final model
Slaking	12.63	12	0.396	11.4
Microcracking	14.75	14	0.395	13.25
Mechanical Breakdown	13.99	12	0.302	10

Table S1: Supplementary model selection information for the path analysis. Non-significant (P>0.05) Chi-square, Df and p-values allow us to accept the models. Δ AIC (Akaike information criterion) displayed represents the difference in AIC values between the selected model and the original model where all possible pathways have been included.

Acknowledgements

The authors would like to thank the gardeners of the Jena experiment, and members of the soil and ecosystem ecology team at Lancaster University, for their contributions to the study. The research was funded by a BBSRC studentship.

5. The influence of management intensity and plant community composition on soil physical properties of temperate grassland: A field study

Gould, I.J., Quinton, J.N., Ward, S.E., Quirk, H, and Bardgett, R.D

Chapters 3 and 4 have revealed that plant diversity can have a strong influence over soil physical properties, in both highly-controlled model communities, and larger manipulated experimental plots. However, these two chapters cannot determine the importance of this relationship when regarding real environmental context of managed grasslands. Across the globe, grasslands are managed for agricultural production, and therefore this chapter will explore the impact of grassland management on the biodiversity-physical property relationship.

Abstract

Grasslands cover a large proportion of the UK land surface, generally in areas of higher rainfall and steeper topography, making them important systems for studying the processes of soil stability. Research has found many significant effects of grassland management and plant community composition on ecological responses, such as productivity, nutrient cycling and microbial communities. However, very little is known of how grassland plant community composition could impact on the soil hydrological environment.

We conducted a survey across a range of grassland sites of Northern England in order to investigate relationships between grassland management, plant diversity, and soil physical properties. We tested soils, from fields of improved and unimproved land management, for three measures of aggregate stability (slaking,

microcracking, and mechanical breakdown) and saturated hydraulic conductivity. Despite a few site-specific effects, overall, management had no significant effect on soil physical properties, but did influence plant rooting activity. Although a positive correlation was determined between plant diversity and one of the aggregate stability measures, most other physical properties showed no relationship with plant diversity in the managed field, instead showing closer associations with soil texture, carbon, and root properties.

5.1 Introduction

Grasslands form an essential component of our agricultural landscape, and constitute an estimated 40% of the global land area. Since the advent of cost-effective and easily available fertilisers, the intensity of management, and consequent levels of production, of most grassland pastures has increased dramatically (FAO 2014). Throughout much of Europe, for example, the last half-century has witnessed a major shift in the management of grasslands from traditional practices of farmyard manure application and light grazing, to intensive, high N-input systems with high yields and grazing pressures (Hopkins and Wilkins 2006, Dungait et al. 2012, Eisler et al. 2014). Although this shift has led to economic gain, it has also had significant negative impacts on the wider environment, causing declines in biodiversity (Donnison et al. 2000, Klimek et al. 2007, Kirkham et al 2014) and increased pollution due to leaching and runoff of nutrients, leading to eutrophication of adjacent waterways (Hooda et al. 2000, Bilotta et al. 2010).

Changes in grassland management intensity can significantly impact soil physical structure both directly, via increased trampling and machinery usage, which increases soil compaction, disrupts aggregation, and impedes hydraulic conductivity (Greenwood and Mckenzie 2001, Zhou et al. 2010), and indirectly, through shifts in vegetation composition and reductions in diversity brought about by changes to management intensity (Pohl et al. 2009). Shifts in vegetation diversity and community composition have been linked to changes in a range of biological and biogeochemical ecosystem processes (Hooper & Vitousek 1998, Fornara & Tilman 2008, Eisenhauer et al. 2010). Very little is known, however, about how changes to plant communities resulting from management intensity impact the soil physical environment. Different species and functional identity of plants within a community, such as grasses, legumes and forbs, exhibit contrasting root traits belowground (McLaren et al. 2004, Pohl et al. 2011). Shifts in aboveground composition, leading to gains or losses in the abundance of any particular functional group, result in changes in the overall root properties of a plant community (Mueller et al. 2013). These root traits hold a governing role over many soil physical properties: not only do they act to bind soil particles together to form stable aggregates, thus reducing particulate losses, but they also affect soil water flow, and subsequent mobility of entrained particles (Mamo & Bubenzer 2001, Gyssels et al. 2005, De Baets et al. 2007, Durán Zuazo & Rodríguez Pleguezuelo 2008). Shifts in plant community composition therefore have the potential to impact on soil physical processes, with consequences for soil erosion and water pollution.

Here, we investigated how differences in the intensity of management, and consequent shifts in the diversity and composition of plant communities, impact soil physical properties of grasslands. In doing so, we first aimed to test how intensification (i.e. the direct impacts of fertilisation, machinery and grazing intensity) affects soil physical properties. Our second aim was then to see whether any changes in soil physical properties are related to changes in vegetation composition and the variation in root characteristics belowground. This experiment measured soil aggregate stability, bulk density and soil hydraulic conductivity. These properties act as indicators of soil breakdown and water flow dynamics, which are soil processes of concern in grassland erosion dynamics (Barthès and Roose 2002).We hypothesised that an increase in management intensity leads to declines in the quality of soil structure, which is, aside from the substantial impact of physical pressures such as trampling and machinery compaction, in part related to reductions in plant diversity, and subsequent changes to the overall community rooting structure belowground. This relationship was tested on a range of sites in northern England, of both intensive and extensive management.

5.2 Methods

5.2.1 Study sites and sampling

Sampling was undertaken in June 2011 at five grassland sites within a 30 km radius of each other in northern England: Askrigg Bottoms (ASK) (National Grid Reference-SD9590), Yockenthwaite (YT) (NGR- SD9178), Waldendale Meadows (WLD) (NGR-

SE0083), and Thornton Rust (TR) (NGR-SD9789) in the Yorkshire Dales, and Ravenstonedale (RD) (NGR- NY7204) in Cumbria. Each site consisted of two adjacent commercial fields of contrasting levels of management intensity: one improved, intensively managed field, receiving > 50kg N ha^{-1} per year, and one unimproved, extensively managed field under traditional management (de Vries et al. 2012). Each paired field had the same soil type, climate and topography, so the main difference between the sites was long-term management history. See Table 5.1 for a summary of the soils. Within each field, four 1m *x* 1m quadrats were randomly allocated, and we recorded the presence of all plant species and percentage cover of grasses, legumes and forbs. For each plot, vegetation data were used to calculate species richness, the percentage cover of the main plant functional groups (grasses, legumes, and forbs), and the Shannon-index of diversity ($H= -\Sigma P_i \ln P_i$ where P_i is proportional abundance of species i). Within each quadrat, two soil cores were extracted to 100 mm depth, one 50 mm diameter core for soil and root analysis and one 64 mm diameter core for hydraulic conductivity analysis, and taken back to Lancaster University for analysis of root and soil properties.

Table 5.2: Summary of site soil properties

Site		Management	Soil Texture	FAO Classification
Askrigg	Intensive	Fertilised (>100 kg N ha^{-1} y^{-1}), Grazed	Sandy Silt Loam	Eutric Gleysol
	Extensive	Unfertilised, Extensively grazed, Annual Cut	Sandy Silt Loam	Eutric Gleysol
Waldendale	Intensive	Fertilised (>100 kg N ha^{-1} y^{-1}), Grazed	Sandy Loam	Eutric Gleysol
	Extensive	Unfertilised, Extensively grazed, Annual Cut	Sandy Silt Loam	Eutric Gleysol
Ravenstonedale	Intensive	Fertilised (>100 kg N ha^{-1} y^{-1}), Grazed	Sandy Silt Loam	Orthic Podzol
	Extensive	Unfertilised, Extensively grazed, Annual Cut	Sandy Silt Loam	Orthic Podzol
Tornton Rust	Intensive	Fertilised (>100 kg N ha^{-1} y^{-1}), Grazed	Sandy Silt Loam	Eutric Gleysol
	Extensive	Unfertilised, Extensively grazed, Annual Cut	Sandy Silt Loam	Eutric Gleysol
Yockenthwaite	Intensive	Fertilised (>100 kg N ha^{-1} y^{-1}), Grazed	Sandy Silt Loam	Eutric Gleysol
	Extensive	Unfertilised, Extensively grazed, Annual Cut	Sandy Silt Loam	Eutric Gleysol

5.2.2 Root analysis

Soil and root samples from the 50 mm diameter cores were deposited onto trays, where soil aggregates > 3 mm were removed for measurement of aggregate stability (see *Soil analysis*). The remainder of the sample was used for root trait analysis. Visible roots were removed by hand, whilst wet sieving of the remaining soil sample allowed the extraction of finer roots. All roots extracted were combined for each core and stored in 15 % ethanol at 4°C until root measurements were undertaken. Total root length and average root diameter (Rdiam) were determined using WinRhizo software (Regent Instrument, Quebec, Canada). Roots were then dried at 70°C for 48 hours to determine root dry mass. Root length density (RLD) and root mass density (RD) was determined by dividing the root length and dry mass, respectively, by the volume of soil in the sampling core. Specific root length (SRL) was calculated in metres per gram from the length and mass measurements of each sample.

5.2.3 Soil analysis

The aggregates extracted from the 50 mm diameter cores were air dried before being passed through a 5 mm sieve, after which 2-5 mm aggregates were selected for aggregate stability analysis. 15 grams of these aggregates were placed in an oven at 40 ^{0}C for 24 hours to ensure constant matric potential, before undergoing stability testing. The Le Bissonais method (1996) was used to determine aggregate stability, which subjects the aggregates to three breakdown mechanisms: (i) slaking, under fast wetting; (ii) microcracking, under slow wetting; and (iii) mechanical breakdown. After these breakdown mechanisms, each sample was wet sieved in methylated spirits before drying and measuring the eventual aggregate size distribution. The Mean Weight Diameter (MWD) in mm of each post-breakdown aggregate sample was used as the measure of aggregate stability, resulting in three MWD values for each sample point: slaking MWD; microcracking MWD; and mechanical breakdown MWD.

The 64 mm diameter cores were saturated in deionised water for 48 hours before undergoing testing for saturated hydraulic conductivity. We used a constant head permeameter method to determine hydraulic conductivity. A constant head of water was maintained above the soil sample, creating a steady hydraulic gradient (ΔH) though the soil and into an outflow. Over time, discharge is measured (Q), and along with the length (L) and cross-sectional area (A) of the soil column, we were able to calculate a value for the saturated hydraulic conductivity (K_{sat}):

$$K_{sat} = \frac{Q \; x \; L}{\Delta H \; x \; A}$$

Where K_{sat} indicates saturated hydraulic conductivity, Q is the discharge (volume per unit time), L is the length of the sample, ΔH is the change in head, and A is the sample cross sectional area. Readings were taken until steady state flow was reached, whereby the average discharge over two concurrent readings was used for each sample.

After saturated hydraulic conductivity was determined, we measured the bulk density of each sample by drying at 105°C for 48 hours and dividing the dry mass of soil by the volume of core extracted. We measured organic matter of each sample by subjecting a subsample of dry soil to 6 hours in a 560°C furnace in order to burn off all organic content. Carbon content of soil was determined using an Elementar Vario EL elemental analyser (Hanau, Germany). We also determined the sand, silt and clay content by dispersing each sample in sodium hexametaphosphase solution and analysing particle size distribution with a Malvern Mastersizer 2000MU, running each sample three times (Armstrong et al. 2011).

5.2.4 Statistical Analysis

To meet the assumptions of normality, we transformed the variables: grass, forb, legume cover and clay content using arcsine square root transformation; mechanical breakdown MWD with square root transformation; and hydraulic conductivity and root density with log transformation. All field data were analysed using linear mixed effects models. Firstly, differences resulting from field management on soil physical properties, community root traits, and diversity properties, were first tested using ANOVA analysis, with site included as a random effect. For each site, differences in measured physical properties (aggregates, hydraulic conductivity) were further assessed using t-tests. We then investigated the correlative relationship of both aggregate stability and hydraulic conductivity with all other measured root, soil and plant community parameters using linear regression. To account for land management intensity, we this time included field as a random effect. All calculations were performed with the software R 3.0.1 (R Development Core Team).

5.3 Results

When data were pooled across all sites, slaking aggregate stability and microcracking aggregate stability were only marginally lower, found to be non-significant, in the intensive than the extensive grasslands. Likewise, we detected no significant difference in mechanical aggregate stability, hydraulic conductivity, or bulk density between intensive and extensive grasslands when data were pooled across all fields. When data were analysed at the individual site level, however, t-tests found aggregate stability to be lower under intensive than extensive management at 2 of the 5 sites (Figure 5.1). At the Waldendale site, two of the three aggregate stability measures were reduced under intensive management (Slaking MWD by 13 %, $P<0.05$; and mechanical breakdown MWD by 16 %, $P<0.001$); at Thorntun Rust, slaking MWD was 55 % lower ($P<0.05$), and at the Yockenthwaite site, microcracking MWD was 5 % ($P<0.05$) lower respectively under intensive management. Across all sites, root length density ($F_{1,34}=7.83$, $p<0.01$) and root mass density ($F_{1,34}=17.22$, $p<0.001$) were reduced by intensive management (Table 5.2), but had no significant impact on root diameter or specific root length (Table 5.2). Intensive management also reduced plant diversity across all sites (Richness: $F_{1,34} = 14.00$, $p<0.001$; Shannon: $F_{1,34} = 14.10$,$p<0.001$).

The use of linear regressions highlighted relationships between plant community, soil, and root properties and the aggregate and hydrological responses (Table 5.3). Across all sites and both land use intensities, we found a weak positive relationship between Shannon index and aggregate stability against slaking ($F_{1,29} = 4.56$, $P<0.05$, $R^2 = 0.18$). However, microcracking and mechanical breakdown aggregate stability, and saturated hydraulic conductivity, showed no relationship with any of the measured plant community properties (Table 5.3). Soil properties showed no significant relationship with slaking, however, microcracking stability was weakly positively related to soil C content, although not significant ($F_{1,29} = 3.27$, $P<0.1$, $R^2 = 0.06$), and mechanical breakdown stability was positively and negatively correlated with soil silt ($F_{1,29} = 7.20$, $P<0.05$, $R^2 = 0.16$) and sand ($F_{1,29} = 4.49$, $P<0.05$, $R^2 = 0.09$) respectively. The bulk density and organic matter content of soil did not show a significant relationship with saturated hydraulic conductivity. These two soil properties had no correlation with any aggregate stability measures either. Of the plant root properties, the only significant relationship detected was a weak negative relationship between root density and saturated hydraulic conductivity ($F_{1,29} = 5.38$, $P<0.05$, $R^2 = 0.15$). Root properties showed no link to aggregate stability across the sites (Table 5.3).

Table 5.2: ANOVA results for the effects of grassland management on soil and root properties

Parameter	Management		*Significance*		
	Intensive	Extensive	*Df*	*F*	*P*
Soil					
Slaking MWD (mm)	**2.67 ± 0.10**	**2.9 ± 0.10**	**1,34**	**3.19**	**<0.1**
Microcracking MWD (mm)	**3.33 ± 0.02**	**3.37 ± 0.01**	**1,34**	**3.46**	**<0.1**
Mechanical MWD (mm)	3.07 ± 0.06	3.14 ± 0.03	1,34	1.46	0.23
Sat. Hydraulic Conductivity (m/day)	3.79 ± 1.42	1.01 ± 0.53	1,34	0.79	0.38
Bulk Density (g/cm^3)	0.92 ± 0.02	0.89 ± 0.03	1,34	0.68	0.42
Roots					
Root Length Density (m/dm^3)	**138.7 ± 14.2**	**197.3 ± 17.1**	**1,34**	**7.83**	**<0.01**
Root Density (g/dm^3)	**2.5 ± 0.3**	**5.2 ± 1.0**	**1,34**	**17.22**	**<0.001**
Avg. Root Diameter (mm)	0.28 ± 0.01	0.28 ± 0.01	1,34	0.05	0.82
Specific Root Length (m/g)	64.6 ± 5.59	53.67 ± 5.49	1,34	2.40	0.13
Community properties					
Richness	**12.05 ± 1.43**	**17.35 ± 0.79**	**1,34**	**14.00**	**<0.001**
Shannon Index	**1.71 ± 0.20**	**2.34 ± 0.06**	**1,34**	**14.10**	**<0.001**

Values under management for each parameter are means ± 1 standard error. F and P values are those given from GLM mixed effects models using Site as a random effect.

Table 5.3: Relationship between plant community, soil, and root properties and soil aggregate stability and hydraulic conductivity

Parameter		Aggregate Stability			Hydrology
	Df	Slaking	Microcracking	Mechanical Breakdown	Saturated Hydraulic Conductivity
Plant Community					
Species Richness	1,29	0.89 ns	1.28 ns	0.14 ns	1.87 ns
Shannon Index	1,29	**4.56** * +	1.92 ns	0.00 ns	0.00 ns
Grass %	1,29	0.16 ns	0.84 ns	0.96 ns	1.10 ns
Legume %	1,29	0.00 ns	0.61 ns	1.06 ns	2.66 ns
Forb %	1,29	0.01 ns	0.39 ns	0.38 ns	0.45 ns
Soil	1,29				
Bulk Density	1,29	1.15 ns	0.61 ns	0.23 ns	**3.92** $^{(*)}$ -
Organic Matter	1,29	2.60 ns	0.85 ns	0.77 ns	**3.32** $^{(*)}$ +
Clay %	1,29	0.59 ns	0.03 ns	0.68 ns	0.04 ns
Silt %	1,29	0.84 ns	0.43 ns	**7.40** * +	1.19 ns
Sand %	1,29	0.12 ns	0.24 ns	**4.49** * -	0.51 ns
C content	1,29	1.93 ns	**3.27** $^{(*)}$ +	0.01 ns	1.25 ns
Roots	1,29				
Root Length Density	1,29	0.23 ns	1.80 ns	0.59 ns	2.55 ns
Root Density	1,29	0.11 ns	0.25 ns	0.52 ns	**5.38** * -
Avg. Root Diameter	1,29	1.82 ns	0.01 ns	0.39 ns	0.14 ns
Specific Root Length	1,29	0.00 ns	0.00 ns	1.04 ns	1.17 ns

The relationship between each measured parameter and aggregate stability/hydrology from linear mixed effects models, field field fixed as a random effect. Numbers in table indicate F values, whilst significance is denoted by (*) $P<0.1$, * $P<0.05$, ns = non-significant. Positive relationships between variables are shown by +, negative relationships by -.

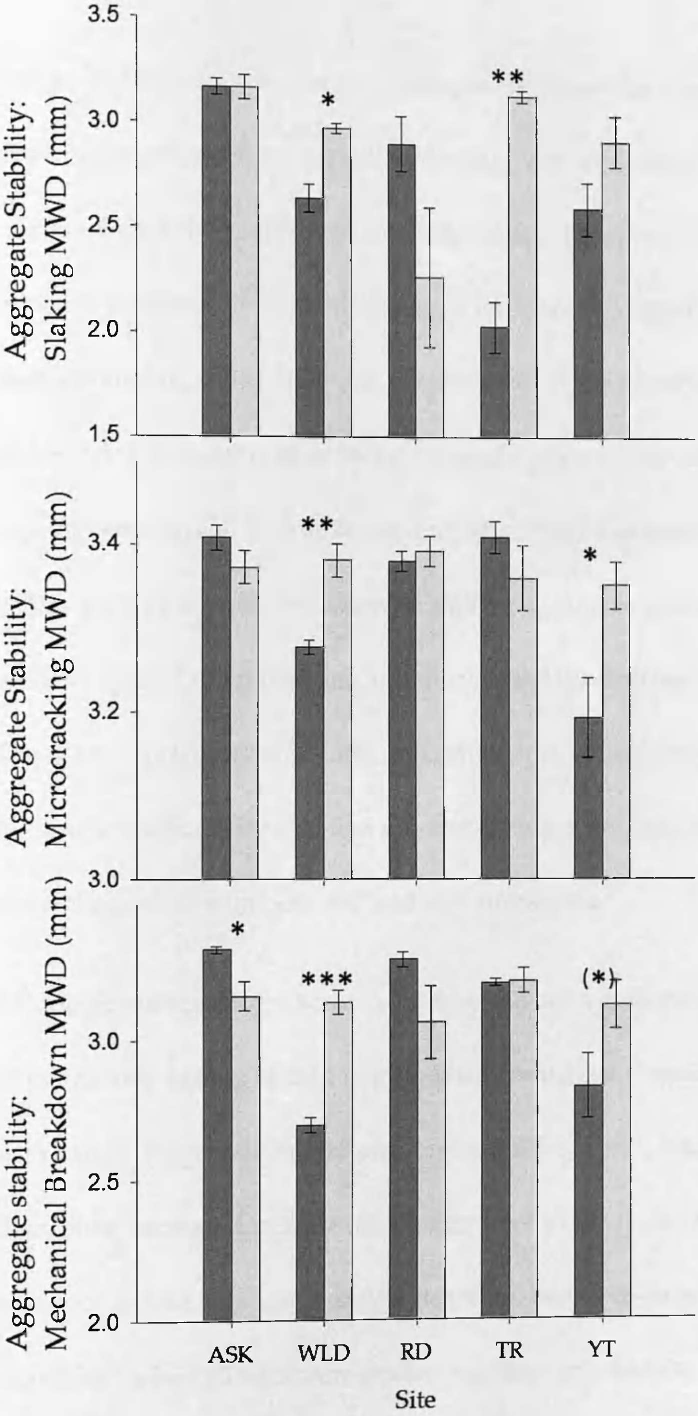


Figure5.1: Impact of grassland management on the three measures of soil aggregate stability (slaking, microcracking, and mechanical breakdown) at each of the five sites studied. Dark shading represent intensive management, light shading bars represent extensive management. Bars signify ± 1 S.E. Significant differences between managements are denoted by $^{(*)}$ $P<0.1$, * $P<0.05$, ** $P<0.01$, *** $P<0.001$. ASK – Askrigg, WLD – Waldendale, RD – Ravenstonedale, TR – Thornton Rust, YT – Yockenthwaite.

5.4 Discussion

The goal of this chapter was to investigate the influence of grassland management upon aggregate stability and soil hydrology, aiming to highlight any significant relationships between plant community composition, root properties, and these physical processes. In general, although there was no significant effect of management on soil stability, there were several site specific differences where intensifying management reduced soil aggregate stability. We also found no effect of management on soil hydraulic conductivity. Plant diversity, represented by Shannon index, showed significant links with slaking aggregate stability. However, aggregate stability against microcracking and mechanical breakdown had no relationship with any plant community measures, and only related to soil properties. Saturated hydraulic conductivity also had no relationship with plant community composition, but did correlate with both soil and root properties.

Although not significant across all fields, soil aggregate stability was in general lower in intensively managed than in extensively managed grasslands in two of the five sites tested. Aggregate breakdown under slaking, microcracking, and mechanical disruption increased in these sites under intensified systems, suggesting negative effects of intensive farming on soil structure, however these differences were not significant when all sites were pooled together. In addition, there was no effect of management on saturated hydraulic conductivity, or bulk density of the soil, which is surprising, given the compacting nature of increased grazing and machinery use, and the contrasts in root density between managements; two factors known to have profound effects on soil water processes (Greenwood and Mckenzie 2001, Powis et al. 2003, Macleod et al. 2013). Furthermore, extensively managed grassland have been shown to reduce nutrient leaching, an ecosystem process controlled by soil hydraulic conductivity, more effectively than their intensively managed counterparts (de Vries et al. 2012), our work now suggests this is likely due to greater nutrient retention within the system, and not a result of changes to the rate of water loss through the soil.

One potential explanation of the tendency for lower aggregate stability in some of the grassland sites, is through reductions in plant diversity. The increased nutrient loading, associated with intensive land use, reduces plant diversity in grasslands (Stevens et al. 2004, Klimek et al. 2007), as we found in this study. Greater nutrient availability gives fast-growing species, more adept at easily-available resource acquisition, a competitive advantage (Haddad et al. 2000), resulting in the dominance of a select group of plants in the system, which were found in our surveys, and a reduction in overall species richness. Despite no significant effects of management on soil structural properties, across all sites, this study did find that some measures of soil aggregate stability responded to plant community dynamics. In particular, we found a significant positive relationship between measured Shannon Index and aggregate stability against slaking breakdown. Although this relationship might not be causative, it suggests that removal of a number of species from a community could therefore not only have the well-documented negative consequences for biological and biochemical ecosystem processes (Hooper & Vitousek 1998, Fornara & Tilman 2008, Eisenhauer et al. 2010), but also for some physical processes too. However, although there were positive links between diversity and slaking aggregate stability, we found no such relationship with the other aggregate stability measures: microcracking and mechanical breakdown.

Regardless of management regime, all soils exhibited very high aggregate stability, which could explain the contrasts in aggregate breakdown responses. In highly aggregated systems, the positive effect of soil biota, a very influential component in soil binding processes (Tisdall and Oades 1982), can be less apparent (Barto et al. 2010). Only in the most aggressive breakdown, slaking, did we find a relationship between diversity and soil stability. Slaking is caused by rapid air release, which can result from the internal hydrophobicity and pore structure of the aggregate (Hallett 2007). These aggregate properties are maintained by the biological activity of fungi and fine roots, two factors known to be greater in high diversity grasslands (De Deyn et al. 2010, de Kroon et al. 2012) and more extensive management systems (Yeates et al. 1997, Bardgett & McAlister 1999), which could explain why slaking was the only aggregate stability breakdown to show a significant response.

Intensive grassland management also reduced root mass and density, which often affect soil physical properties. The result of greater fertiliser input to grasslands can lead to reduced root activity through two mechanisms. The first mechanism is a consequence of N addition to grasslands changing the plant community composition, encouraging the survival of less conservative species. Plants of slower-growing life strategies tend to invest in woodier, higher density roots (Craine et al. 2001), and create the greater root mass detected in the extensively managed fields of this study, but are less prominent in the intensively managed systems. Root foraging strategies in low nutrient environments explain the second pathway. Plants can invest in greater root length to exploit a limited resource (Hodge 2003), meaning that species in unimproved grasslands may need to root extensively to uptake enough nutrients (Głąb and Kacrorzyk 2011).

Although root traits are known to influence soil physical properties (Angers and Caron 1998, Gyssels et al. 2005), this experiment found few significant relationships between root measures and soil structural properties, aside from a negative relationship between soil hydraulic conductivity and root mass. High root activity is often regarded as having a positive effect on soil water flow (Angers and Caron 1998), but our results prove contrary to this expectation. This might be explained by the fact that, in some cases, increased root mass can block voids that are potential hydraulic pathways (Edwards et al. 1997), particularly if there is not enough die-back of old root networks (Barley 1954). We suggest that the impact of root die back forming effective channels could be seasonal, and as we sampled in summer, there would be substantially more root growth to block pores. However, we only measured root content and hydraulic conductivity in the top 10 cm of soil, where the majority of roots are situated, so there is potential for these relationships to change at greater depth in the soil. Unlike hydraulic conductivity, soil aggregate stability appeared to have no relationship with any root traits properties the sites. Numerous studies have shown the considerable influence that root traits exert over aggregate dynamics (Miller and Jastrow 1990, Gyssels et al. 2005). Along with roots come organic exudate releases into the soil, stimulating microbial communities and contributing to aggregate structure (Tisdall and Oades 1982. Oades and Waters 1991). It is possible that the overriding influence of land management negates any significant effects of rooting properties on soil aggregate stability (Cambardella and Elliot 1992, Six et al. 1998).

Although plant diversity was related to slaking stability, the other two aggregate breakdown measures were related more to soil, and not plant, properties. The only measured variable to show a positive relationship with microcracking aggregate stability was soil carbon content. Soil carbon is well known to promote the formation of more stable aggregates, and incorporation of carbon into aggregate structure not only acts to increase the stability and binding mechanisms of the aggregate (Tisdall and Oades 1982), but also contributes to carbon sequestration (Six et al. 2000, Kong et al. 2005). Mechanical breakdown aggregate stability, on the other hand, was related more to soil textural properties, correlating with both silt and sand content of the soil. Mechanical breakdown is reliant on the cohesion of the aggregate, which in turn often depends on clay content (Le Bissonais 1996). It is therefore surprising that clay was the only soil size fraction not to relate to mechanical breakdown. Nevertheless, the largest soil fraction, sand, was negatively related to mechanical breakdown across sites, whereas the silt faction was positively related to this measure, suggesting a reliance on particle size distribution for the stability of aggregates against mechanical stress (Elliot et al. 1986, Six et al. 2000). Soil bulk density and organic matter were weakly related to soil hydraulic conductivity. Increased organic input to soil is often witnessed alongside a reduction in bulk density, as the mineral bulk soil is diluted with a less dense organic fraction. This lighter medium provides less resistance for hydraulic conductivity, which may explain the positive association with soil hydraulic conductivity.

5.5 Conclusion

Although not significant across all fields, aggregate stability, a key measure of soil structural integrity, tended to be greater in extensively than intensively managed grassland at two sites, suggesting the benefits of extensive management for soil physical integrity. Also, stability against the most aggressive of aggregate breakdowns, slaking, increased alongside higher plant diversity, which itself was promoted by extensive management. Our results advance the understanding of the complex relationship between biodiversity-ecosystem function and soil physics. Moreover, this is one of the first studies to suggest the promotion of biodiversity in grassland management systems may have positive impacts on soil physical stability. However, only one of three aggregate stability measures responded to plant diversity, and the effect of intensive management on soil stability was not significant across all sites, proving that such relationships may be affected more by environmental controls, such as geological and climatic factors, beyond the influence of land management. In addition, our results are only correlative, and further detailed experiments are needed to separate cause from effect..

Acknowledgements

The authors would like to thank the members of the soil and ecosystem ecology team at Lancaster University, for their contributions to the study. The research was funded by a BBSRC studentship.

6. General Discussion

Growing interest in biodiversity-ecosystem function (BEF) has generated a wealth of studies into the response of soil biological, and biochemical, processes to shifts in grassland biodiversity. These investigations have led to significant progress in biodiversity research, however, evidence of impacts on the soil physical environment is limited (Allen et al. 2011, Cardinale et al. 2012). The primary goal of this thesis was to address this gap in our knowledge and advance our understanding of the impact of biodiversity change on soil physical properties. Specifically, this project investigated the impact that a shift in plant community diversity and composition has over an array of soil physical processes at a range of spatial scales, ranging from a pot experiment, to sampling from experimental plots, to field observations. In doing so, several key findings emerged at either one, two, or all three different spatial scales within the study.

6.1 The impact of plant diversity on soil processes

6.1.1 Aggregate Stability

A major finding of this project was the strong effect that higher plant diversity had over soil aggregate stability. Greater aggregate stability was found in the mesocosms of higher species richness in chapter 3. Sampling from the longer term experimental plots, in chapter 4, revealed that soils under higher plant diversity exhibited greater soil aggregate stability. In chapter 5, even after accounting for the dominant effects of land management across contrasting grasslands, there was still a positive association seen between plant diversity and aggregate resistance to slaking. Until now, the positive impact of species richness over soil aggregates has only been found on a small plot experiment (Pérès et al. 2013), or in correlative studies (Pohl et al. 2009). This thesis represents the first time such substantial evidence, across a range of spatial scales, has revealed the positive effect of plant diversity on a physical property of the soil. This demonstrates potential for a new direction in BEF studies, to begin to explore the consequences of species loss on a wider range of ecosystem services.

The experimental chapters (3, 4, and 5) brought to light some of the mechanisms behind this relationship. Species richness was the dominant diversity measure that impacted on aggregate stability in both chapters 3 and 4, whilst functional group richness had no effect in the mesocosm study, and was not as powerful a predictor of soil aggregate stability in the Jena experimental plot sampling. The mesocosm experiment also revealed that the presence of certain plant species, in this case *Lolium perenne,* had a prominent effect over the diversity impact on aggregates. Particular individuals, such as *Anthoxanthum oderatum,* can drive community effects on root length or fungal communities (Mommer et al. 2010, De Deyn et al. 2010), whilst certain legume species may greatly influence soil biochemical properties under plant communities (De Deyn et al. 2009), in much the same way *L. peren*ne affected soil physical dynamics here. Their species traits have significant impacts on soil processes, and the loss of such influential species is likely to change the dynamics of the soil environment. To address this, it is important that we begin to identify and understand the effect that promoting the establishment of these key species has in ecosystem dynamics. In this instance, the influential species, *L. perenne,* is not a species whose abundance in natural ecosystems is of concern, and, in fact, in diversity restoration many efforts have been implemented to reduce the abundance of *L. perenne*. Conflicting with ecological management advice, it is somewhat ironic that this species, often associated with high intensive management, actually has a benefit for soil structural properties.

The underlying role that plants roots had in the diversity-aggregate relationship was evident throughout the thesis. In chapters 3 and 4, strong associations were found between increases in rooting activity, and more stable aggregation. The relationship between roots and aggregates was not, however, evident in the field sampling of chapter 5, suggesting that the confounding effects of other environmental factors have a stronger impact on soil aggregate stability than the diversity-root impacts found in the other chapters. Fine rooting strategies are an essential part of the aggregate forming process (Miller and Jastrow 1990, Gyssels et al. 2005) and form the cohesive basis by which aggregates resist breakdown (Le Bissonais 1996, Barthes et al. 2002, Six et al. 2004). The association between more extensive fine rooting strategies and greater aggregate stability, found in chapters 3 and 4, is consistent with many past studies (Mamo and Bubenzer 2001, Gyssels et al. 2005, Pérès et al. 2013). This project revealed that higher species richness induced more expansive rooting, and this capacity allowed greater diversity mixtures to have profound effects on soil stabilising processes.

Although the findings identified the substantial effects that diversity had on aggregate stability, there were differences detected in the responses of the three different forms of aggregate breakdown. Aggregate resistance to microcracking, caused by the gradual wetting of aggregates over time, tended to have the least pronounced association with plant diversity, and biological factors in general. The benefits of diversity to microcracking resistance were fully explained by increases in root length in chapter 3, microcracking showed the weakest regressions with other soil biological factors in chapter 4, and only correlated with soil carbon, not root or diversity measures, in chapter 5. Aggregate disruption through microcracking relies on the shrink-swell properties of the aggregate (Le Bissonais 1996), which are influenced more by physical factors such as clay content, rather than biological factors (Boivin et al. 2004). Hence, it is not surprising that aggregate microcracking resistance responded to plant diversity in a less pronounced manner than other breakdowns. As such, soils subjected to gradual wetting over time, common in UK winters, would perhaps be less susceptible to structural restoration strategies involving planting regimes, and more affected by physical maintenance strategies.

In contrast to microcracking, the diversity effect on aggregate resistance to mechanical breakdown had slightly more dependency on biological factors. In chapter 3, the diversity effect on mechanical breakdown was fully explained by community increases in root length. Chapter 4 revealed that resistance to mechanical breakdown increased through the actions of root mass, organic matter, and glomalin inputs to soil. However, the field study of chapter 5 did not show positive correlations with biological parameters, and mechanical breakdown related to soil textural properties only. The presence of roots enforces mechanical resistance in soils (Mamo and Bubenzer 2001). Any factors that promote root presence, such as increasing plant diversity (Mueller et al. 2013, Pérès et al. 2013) , will influence mechanical breakdown of soil aggregates. However, despite being reliant on soil biological properties, aggregate mechanical resistance was not as profoundly affected by diversity in comparison to slaking breakdown.

The third aggregate stability measure, resistance to slaking, exhibited the most interesting responses to changes in plant diversity. It was not fully explained by rooting contributions alone in chapter 3, suggesting factors other than roots offer a contribution. Slaking resistance showed some of the strongest associations with biological soil properties in chapter 4, and even displayed a significant correlation with a diversity index in the field study of chapter 5. Slaking is often the most aggressive breakdown measure; and it may be that even some of the very small contributions to aggregation from biological inputs are easier to detect with this breakdown. Slaking also relies on internal aggregate properties less associated with microcracking and mechanical breakdown, such as hydrophobicity and internal porosity. Hydrophobic compounds, released by plant roots and microbial associations, have the capacity to influence soil water repellancy, and as such, can affect the wetting rates of soil aggregates (Czarnes et al. 2000, Hallett 2007). As mentioned, the mesocosm experiment suggests that factors other than the root structural properties measured could be influencing slaking resistance. Further work on the effect of plant diversity on aggregate hydrophobicity would prove interesting, as this property can play a pivotal role in aggregate disruption to slaking.

The impact of species richness on soil aggregate stability, and in particular slaking, suggest that diversity will have a part to play in soil management against physical degradation. Instances where slaking is most evident, for example under rapid flooding, are predicted to increase in coming years (Fowler et al. 2009, Diffenbaugh et al. 2013). This will exacerbate the problem, leading to dry summers and flash flooding – ideal conditions for slaking to occur. In the UK, agri-environment schemes have been implemented in order to promote plant diversity, associated with the consequential benefits to invertebrate and animal diversity, and soil biological status. The evidence presented here provides scope for additional positive effects of biodiversity on ecosystem processes under threat – this time for soil structural stability.

6.1.2 Soil Hydraulic Conductivity

The impacts of plant community composition on saturated hydraulic conductivity were less pronounced than for aggregate stability. There was a tendency for greater plant species richness to increase hydraulic conductivity in the mesocosms of chapter 3, although this proved not to be significant to the 95% confidence limit. The presence of legumes within mixed communities did increase hydraulic conductivity, which will be discussed later. In the two chapters investigating soil hydraulic responses, it was evident that root mass in the soil had a significant effect on saturated hydraulic conductivity. Sparser rooting legumes benefitted saturated hydraulic conductivity in chapter 3, whilst chapter 5 found a negative association between root density and saturated hydraulic conductivity. In the long term, rooting activity increases soil water flow, as growth and die-back form hydraulically effective channels (Macleod et al. 2013). However, this process relies on the balance between root growth and root senescence (Barley et al. 1954), and as such, will experience temporal fluctuation. The negative effect of increased rooting on hydraulic conductivity detected here is possibly a response to the time frames of both experiments. The mesocosm experiment of chapter 3 may have been on too short a timeframe to incorporate enough root dieback, whilst the field sampling of chapter 5 was conducted in summer, when root growth is at an optimum in the soil, potentially obstructing pore space.

6.1.3 Root reinforcement of soil strength

Unlike soil aggregate stability or saturated hydraulic conductivity, plant species richness had no relationship with the soil strength properties measured. Root mass and plant identity in monocultures did improve strength properties, however, these were not through a contribution of any plant diversity factors. It is not surprising that thick roots, and thicker rooting species, provided more root reinforcement to soil. The increased anchorage allows the soil structure to withstand stress (Coppin and Richards 1990, De Baets et al. 2008, Loades et al. 2010), and as such, we found the grassland plant with the thickest roots, *Lotus corniculatus,* to have the greatest effect on soil strength. Although roots from grassland species such as this may only have an influence on the top 10 to 30 cm of soil (Comino et al. 2010), they may still hold relevance in a wider environmental context. In recent years, increased water saturaton in the UK has resulted in an ever present threat of shallow landslips to disrupt roads and erode riverbanks (British Geological Society 2013). Finding a British grassland species with strengthening properties, like *Lotus corniculatus,* may play a role in increasing root reinforcement in vulnerable grassland areas. In fact, recent work by Macleod et al. (2010) has begun to explore the role of single grassland species, such as this, in grassland hydrology

6.1.4 Diversity impacts on soil biological properties

Although the principal aim of this study concerned soil physical properties, several of the biological parameters measured throughout the experiments also responded to changes in plant community composition.

Until recently, most biodiversity-ecosystem function studies have focused on the response of aboveground plant properties to shifts in diversity. This thesis, therefore, contributed to the growing evidence on the influence of plant diversity to root properties (de Kroon et al. 2012, Mueller et al. 2013). In chapter 3, the mesocosm experiment, species richness increased plant community root length, whilst functional group richness increased root mass. Plots of higher species richness in reported in the Jena sampling of chapter 4, displayed greater root mass. Non-intensively managed, botanically diverse, grassland fields in chapter 5 had more extensive root networks in the soil. As discussed previously, these increases in root properties of the soil explained much of the plant community composition influence over soil physical properties. Although the intensification of grassland management has long been associated with declines in grassland botanical diversity (Klimek et al. 2007, Kirkham et al 2014), few studies have investigated root responses to grassland management (Głąb and Kacrorzyk 2011), such as in chapter 5. With less nutrient input to soils, plants have to invest more in rooting structures, in order to maximise nutrient uptake (Hodge 2003), resulting in this extensive root network in lower nutrient environments.

Few studies have investigated the impact of higher plant diversity on the levels of glomalin-related protein in the soil. In chapter 4, greater species, and functional group, richness improved the levels of glomalin-related protein in the soil. Glomalin levels in soil are a consequences of fungal wall degradation, from mycorrhizal communities (Wright and Upadhyaya 1998, Driver et al. 2005) At the Jena experiment where glomalin was measured, increased diversity has driven changes in these microbial communities (Lange et al. 2014). This may have lead to cascading effects on the soil aggregate stability, as this often correlates with the strong, and persistent, aggregate-binding capacities of glomalin. A relatively recent discovery, glomalin is gaining ever more attention as a potentially crucial soil property for carbon storage and structural stability (Rillig et al. 2002, Driver et al. 2005) reinforced by the findings here.

6.2 Plant Identity and Function

In comparison to forbs and legumes, grass species were influential in the stabilisation of soil aggregates throughout the project. In mesocosm monocultures, grass species exhibited the highest aggregate stability, and their presence increased aggregate stability in the Jena experimental plots. Their fine and expansive rooting networks allowed for this effect on aggregation, increasing the area of influence that plants and biological factors have over aggregates. In the mesocosm experiment of chapter 3, the prominent grass species, *Lolium perenne,* showed very pronounced effects on aggregation because it rooted far more exploitatively than the other species used.

Legumes exhibited contrasting behaviour to grasses. In chapter 3, their presence benefited hydraulic conductivity in 2-species mixtures, but showed poor aggregate stability in monocultures. The mesocosm experiment also found legumes to contribute the least root length to soil. In chapter 4, their presence in plots had a negative effect on soil aggregate stability, which was found to be a result of a decreased rooting input to the soil. One legume species, *Lotus corniculatus,* had a strong impact on soil strength, due to its thick anchoring roots, but this effect was only evident in monocultures and not mixtures. This disparity between legumes and grasses, as a function of their contrasting root strategies, proved an interesting aspect of the project. Much as clover seeding is employed in grasslands to benefit nitrogen input to soils, and increase the nutrient content of swards, these results reveal that there may be effects of clover seeding for other ecosystem processes. Although anecdotal evidence indicates that clover should be planted to increase soil friability, in effect 'breaking up' clay soils to improve infiltration (Mytton and Creswell 1992), there is very little experimental evidence to reinforce this. This thesis, however, revealed that legume species can benefit water flow into soils, reinforcing this anecdotal evidence. The reductions in aggregation under legume species found in this study may also be explained by these management practices – poor aggregate stability will be displayed in instances of greater soil friability.

6.3 Conclusion

This thesis contributes new insight into the study of biodiversity and ecosystem functioning, by investigating the effects that shifts in plant community composition can have on a range of soil physical properties in temperate grassland communities. Plant species richness was found to have strong effects over soil aggregate stability. This represents the first time such a relationship has been revealed at a range of scales, and provides valuable insight into a new direction for biodiversity-ecosystem function studies. Plant functional group and species identity also impacted on soil strength and hydraulic regimes, with legumes and grasses displaying contrasting behaviour. The impact of changes in rooting structure, and their associated inputs to the soil, played a significant role throughout all of these relationships. This study suggests potential implications for grassland management, whereby promotion of more diverse plant communities could benefit soil resistance to breakdown during flood events, whilst the seeding of legumes or grasses may have important consequences for soil aggregation and water dynamics.

6.4 Future Work and Challenges

This thesis advances our understanding of the role of biodiversity in maintaining soil physical structure. However, it also highlighted several questions and challenges that would provide interesting future work.

The influence of *Lolium perenne* on soil aggregate stability

The most influential soil-aggregating species was *Lolium perenne*. *Lolium perenne* is regarded as an exploitative species, responding to high nutrient levels in soils and out-competing many other graminoid species. The presence of this species in grasslands is often associated with less botanically diverse plant communities, which are often regarded to be detrimental to a number of ecosystem biochemical processes (Allan et al. 2013). It is therefore interesting to find it having distinct benefits for soil aggregation, whereas the plant community investigations of this thesis found that higher diversity communities provide better environments for soil aggregation.

It would be interesting for further work to weigh up the trade-off between the presence of this species, as an indicator of less diverse communities, on soil stability, against the benefits that higher diversity communities have on aggregate stability.

The different effects on soil physical properties under grass and legume communities

The contrasting aggregate, hydraulic, and strength properties displayed between grasses and legumes were evident throughout the thesis. Whilst densely rooting grasses exploited soil space and increased soil aggregate stability, legumes showed less influence over aggregates, but increased soil water flow and strength properties.

Legumes are widely regarded for enhancing soil biogeochemical processes, due to their role in nitrogen-fixation (Spehn et al. 2002, D Deyn et al. 2009). These characteristics have lead them to often be incorporated into agricultural management regimes, for example in the instance of clover seeding of grazed pasture (Mytton et al. 1992). As such, the widespread growing of legumes, such as clover, across the UK and Europe lends itself as a platform for the measurement of soil physical processes under these plant types.

In this thesis, plant surveys were used in conjunction with an array of soil physical measures to investigate links between community composition and physical properties in 10 sites across Northern England. If the detail of the survey was scaled down to percentage legume/percentage grass cover, there is potential to enlarge the experiment to incorporate a larger number of study sites investigating grass/legume dominance and aggregation and hydrological patterns.

Further investigation into the role of community composition on root dynamics

As discussed in Chapter 1, the response of belowground processes to Biodiversity-Ecosystem Function dynamics has been much underlooked in comparison to the response of aboveground properties (Isbell et al. 2011, Cardinale et al.2012). We know far more about plant community composition effects on aboveground biomass over yielding, nutrient composition and food webs, than we do about the dynamics below the soil surface. As this project had revealed, many soil physical properties respond significantly to differences in belowground plant structures.

The spotlight of BEF research is beginning to focus on the response of root traits (Monmer et al. 2010, Mommer et al. 2011) to biodiversity shifts. However, analysis of root properties is more labour intensive and requires greater accuracy of species identity in order to determine sufficient results. As such, the development of technologies to assist root identity within mixed plant community assemblages, such as spectroscopy (Rewald and Meinen 2013), may play a role in this process.

The next scale down

In this study, the smallest scale of analysis was at the mesocosm level. This incorporated mixtures of 32 plant individuals, which was a scale deemed suitable in order to incorporate enough representation of plant community dynamics within the soil.

However, even at this relatively small scale, there were some trade-offs that were made. The large number of roots that occupied the soil matrix made single species identity of roots an impractical task. As such, relative growth of root traits per species could only be inferred by using monocultures to extrapolate.

It is possible that whole root networks of single species within a community could be investigated at a smaller scale – perhaps the microcosm scale. By growing 2-species assemblages in small containers (e.g. ~ 5cm diameter), it would still be feasible to analyse the entire root structures beneath a community, and in large part separate out root structures to each individual species (Baxendale et al. 2014). Although it may reduce 'community' effects, scaling down to an experiment of this size may allow us to investigate the dynamics between species interactions, roots, and physical properties with greater accuracy.

Why is slaking the aggregate measure that is most influenced by plant community dynamics?

Aggregate stability under slaking was the most pronounced stability measure to be affected by plant diversity. As such, it would be interesting to develop a study to investigate the effects of greater species richness on some of the mechanisms behind slaking. As slaking is reliant on the internal pore space and wetting capabilities of aggregates, perhaps looking at aggregate wetting rates and hydrophobicity could provide a valuable insight. It may be possible that altering the species composition of a community aboveground could be affecting the hydrophobic nature of a soil, as a result of changes in the exudation regimes and behaviour of rooting structures (Inderjit and Weston, 2003). This would, in turn, be impacting on the nature of aggregate disruption via slaking. In effect, hydrophobicity could be another 'soil physical response' added to the range of parameters looked at in this thesis. A hydrophobicity investigation could even be set up using the same experimental designs as those in this thesis.

7. References

Abdullah MN, Osman N, Ali FH (2011) Soil-root shear strength properties of some slope plants. Sains Malaysiana, 40, 1065-1073.

Abiven S, Menasseri S, Angers DA, Leterme P (2007) Dynamics of aggregate stability and biological binding agents during decomposition of organic materials. European Journal of Soil Science, 58, 239-247.

Al-Durrah MM, Bradford JM (1982a) The mechanism of raindrop splash on soil surfaces. Soil Science Society of America Journal, 46, 1086-1090.

Al-Durrah MM, Bradford JM (1982b) Parameters for describing soil detachment due to single water-drop impact. Soil Science Society of America Journal, 46,836-840.

Ali FH, Osman N (2008) Shear strength of a soil containing vegetation roots. Soils and Foundations, 48,587-596.

Allan E, Weisser WW, Fischer M, Schulze E-D, Weigelt A, Roscher C, Baade J, Barnard RL, Bessler H, Buchmann N, Ebeling A, Eisenhauer N, Engels C, Fergus AJF, Gleixner G, Gubsch M, Halle S, Klein AM, Kertscher I, Kuu A, Lange M, Le Roux Z, Meyer ST, Migunova VD, Milcu A, Niklaus PA, Oelmann Y, Pasalic E, Petermann JS, Poly F, Rottstock T, Sanais ACW, Scherber C, Scherer-Lorenzen M, Scheu S, Steinbess S, Schwichtenberg G, Temperton V, Tscharntke, Voigt W, Wilcke W, Wirth C, Schmid B (2013) A comparison of the strength of biodiversity effects across multiple functions. Oecologia, 173, 223-237.

Angers DA, Caron J (1998) Plant-induced changes in soil structure: processes and feedbacks. Biogeochemistry, 42, 44-72.

Armstrong A, Quinton JN, Heng BCP, Chandler JH (2011) Variability of interill erosion at low slopes. Earth Surface Processes ad Landforms, 36, 97-106.

Bardgett RD (2005) The biology of soil: A community and ecosystem approach. Oxford University Press, Oxford.

Bardgett RD, Hobbs PJ, Frostegard A (1996) Changes in soil fungal:bacterial biomass ratios following reductions in the intensity of management of an upland grassland. Biology and Fertility of Soils 22 ,261-264.

Bardgett RD, Mawdsley JL, Edwards S, Hobbs PJ, Rodwell JS, Davies WJ (1999) Plant species and nitrogen effects on soil biological properties of temperate upland grasslands. Functional Ecology, 13, 650-660.

Bardgett RD, McAlister E (1999) The measurement of soil fungal:bacterial biomass ratios as an indicator of ecosystem self-regulation in temperate meadow grasslands. Biology and Fertility of Soils 29, 282-290.

Bardgett RD, Wardle DA (2010) Aboveground-belowground linkages: biotic interactions, ecosystem processes, and global change. Oxford University Press, Oxford.

Barley KP (1954) Effects of root growth and decay on the permeability of a synthetic sandy loam. Soil Science, 78, 205-210.

Barthès B, Roose E (2002) Aggregate stability as an indicator of soil susceptibility to runoff and erosion; validation at several levels. Catena, 47, 133-149.

Barto EK, Alt F, Oelmann Y, Wilcke W, Rillig MC (2010) Contributions of biotic and abiotic factors to soil aggregation across a land use gradient. Soil Biology and Biochemistry, 42, 2316-2324.

Bautista S, Mayor AG, Bourakhouadar J, Bellot J (2007) Plant spatial pattern predicts hillslope semiarid runoff and erosion in a Mediterranean landscape. Ecosystems 10,987-998.

Baxendale C, Orwin K, Poly F, Pommier T, Bargdett RD (2014) Are plant-soil feedback responses explained by plant traits? New Phytologist. 204, 408-423.

Baveye PC, Rangel D, Jacobson AR, Laba M, Darnault C, Otten W, Radulovich R, Camargo FAO (2011) From Dust Bowl to Dust Bowl: Soils are still very much a frontier of science. Soil Science Society of America Journal. 73, 2037-2048.

Berendse F (1979) Competition between plant populations with different rooting depths. 1: Theoretical considerations. Oecologia, 43,19-26.

Berendse F (1982) Competition between plant populations with different rooting depths. 3: Field Experiments. Oecologia, 53,50-55.

Bessler H, Temperton VM, Roscher C, Buchmann, N, Schmid, B, Schulze ED, Weisser WW, Engels C (2009) Aboveground overyielding in grassland mixtures is associated with reduced biomass portioning to belowground organs. Ecology, 90, 1520-1530.

Beven K, Germann P (1982) Macropores and water-flow in soils. Water Resources Research. 18, 1311-1325.

Bilotta G S, Brazier RE, Haygarth PM, Macleod CJA, Butler R, Granger S, Krueger T, Freer J, Quinton JN, (2008) Rethinking the contribution of drained and undrained grasslands to sediment-related water quality problems. Journal of Environmental Quality, 37,906-914.

Bilotta GS, Grove M, Mudd SM (2012) Assessing the significance of soil erosion. Transactions of the Institute of British Geographers, 37, 342-345.

Bilotta GS, Krueger T, Brazier RE, Butler P, Freer J, Hawkins JMB, Haygarth PM, Macleod CJA, Quinton JN (2010) Assessing catchment-scale erosion and yields of suspended solids from improved temperate grassland. Journal of Environmental Monitoring, 12, 731-739.

Brisson J, Reynolds JF (1994) The effect of neighbours on root distribution in a creosotebush (*Larrea tridentata*) population. Ecology, 75, 1693-1702.

Bronick CJ, Lal R (2005) Soil structure and management: a review. Geoderma, 124, 3-22.

British Standards Institute (1999) BS 5930:1999 Code of practice for site investigations.

Cambardella CA, Elliott ET (1992) Carbon and nitrogen distribution in aggregates from cultivated and native grassland soils. Soil Science society of America Journal, 57, 1071-1076.

Cardinale BJ, Duffy JE, Gonzalez A, Hooper DU, Perrings C, Venail, P, Narwani A, Mace GM, Tilman D, Wardle DA, Kinzig AP, Daily GC, Loreau M, Grace JB, Larigauderie A, Srivatava DS, Naeem S (2012) Biodiversity loss and its impact on humanity. Nature, 486, 59-67.

Cardinale BJ, Wright JP, Cadotte MW, Carroll IT, Hector A, Srivastava DS, Loreau M, Weis JJ (2007) Impacts of plant diversity on biomass production increase through time because of species complementarity. Proceedings of the National Academy of Sciences, 104, 18123-18128.

Caron J, Epsindola CR, Angers DA (1996) Soil structural stability during rapid wetting: influence of land use on some aggregate properties. Soil Science Society of Americ Journal, 60, 901-908.

Carpenter SR, Caraco NF, Correll DL, Howarth RW, Sharpley AN, Smith VH (1998) Nonpoint pollution of surface waters with phosphorus and nitrogen. Ecological Applications, 8, 559-568.

Clark JE, Howell JH (1992) Development of bioengineering strategies in rural mountain areas.in Erosion, debris flows and environment in mountain regions (Proceedings of the Chengdu Symposium, July 1992)

Casermeiro MA, Molina JA, de la Cruz Caravaca MT, Hernando Costa J, Hernando Massanet MI, Moreno PS (2004) Influence of scrubs on runoff and sediment loss in soils of Mediterranean climate. CATENA 57,91-107.

Chandler MP, Parker DC, Selby MJ (1981) An open-sided field direct shear box with applications in geomorphology. British Geomorphological Research Group Abstracts Ltd., Norwich.

Clark JE, Howell JH (1992) Development of bioengineering strategies in rural mountain areas.in Erosion, debris flows and environment in mountain regions , Proceedings of the Chengdu Symposium.

Conti G, Diaz S (2013) Plant functional diversity and carbon storage – an empirical test in semi-arid forest ecosystems. Journal of Ecology, 101, 18-28.

Comino E, Druetta A (2010) The effect of Poaceae roots on the shear strength of soils in the Italian alpine environment. Soil & Tillage Research, 106,194-201.

Comino E, Marengo P, Rolli V (2010) Root reinforcement effect of different grass species: A comparison between experimental and models results. Soil & Tillage Research, 110, 60-68.

Cope T, Gray A (2009) Grasses of the British Isles. BSBI Handbook 13, Botanical Society of the British Isles with Royal Botanic Gardens, Kew.

Coppin NJ, Richards IG (1990) Use of vegetation in civil engineering. Butterworths.

Craine JM., Froehle J, Tilman GD, Wedin DA, Chapin FS (2001) The relationships among root and leaf traits of 76 grassland species and relative abundance along fertility and disturbance gradients. Oikos, 93, 274-285.

Czarnes S, Hallett PD, Bengough AG, Young IM (2000) Root- and microbial-derived mucilages affect soil structure and water transport. European Journal of Soil Science, 51, 435-443.

De Baets S, Torri D, Poesen J, Salvador MP, Meersmans J (2008a) Modelling increased soil cohesion due to roots with EUROSEM. Earth Surface Processes and Landforms, 33, 1948-1963.

De Baets S, Poesen J, Knapen A, Galindo P (2007) Impact of root architecture on the erosion-reducing potential of roots during concentrated flow. Earth Surface Processes and Landforms, 32, 1323-1345.

De Baets S, Poesen J, Reubens B, Wemans K, De Baerdemaeker J, Muys B (2008b) Root tensile strength and root distribution of typical Mediterranean plant species and their contribution to soil shear strength. Plant and Soil, 305, 207-226.

De Deyn GB, Quirk H, Yi Z, Oakley S, Ostle NJ, Bardgett RD (2009) Vegetation composition promotes carbon and nitrogen storage in model grassland communities of contrasting fertility (2009) Journal of Ecology, 97, 864-875.

De Deyn GB, Quirk H, Bardgett RD (2010) Plant species richness, identity and productivity differentially influence key groups of microbes in grassland soils of contrasting fertility. Biology Letters, 2010, 7, 75-78.

Degens BP (1997) Macro-aggregation of soils by biological bonding and binding mechanisms and the factors affecting these: A review. Australian Journal of Soil Research, 35,431-459.

Degens BP, Sparling GP, Abbott LK (1994) The contribution from hyphae, roots and organic-carbon constituents to the aggregation of a sandy loam under long-term clover-based and grass pastures. European Journal of Soil Science, 45, 459-468.

de Kroon H, Hendriks M, van Ruijven J, Ravenek J, Padilla FM, Jongejans E, Visser EJW, Mommer L (2012) Root responses to nutrients and soil biota: drivers of species coexistence and ecosystem productivity. Journal of Ecology, 100, 6-15.

Delahaye CH, Alonso EE (2002) Soil heterogeneity and preferential paths for gas migration. Engineering Geology, 64, 251-271.

de Vries FT, Bloem J, Quirk H, Stevens CJ, Bol R, Bardgett RD (2012) Extensive management promotes plant and microbial nitrogen retention in temperate grassland. PLoS ONE, 7, e51201.

Diffenbaugh NS, Scherer M, Trapp RJ (2013) Robust increases in severe thunderstorm environments in response to greenhouse forcing. Proceedings of the National Academy of Sciences, 110, 16361-16366.

Donnison LM, Griffith GS, Hedger J, Hobbs PJ, Bardgett RD (2000) Management influences on soil microbial communities and their function in botanically diverse haymeadows of northern England and Wales. Soil Biology & Biochemistry, 32, 253-263.

Driver JD, Holben WE, Rillig MC (2005) Characterization of glomalin as a hyphal wall component of arbuscular mycorrhizal fungi. Soil Biology and Biochemistry, 37, 101-106.

Dungait JAJ. Cardena LM, Blackwell MSA, Wu L, Withers PJA, Cadwick DR, Bol R, Murray PJ, Macdonals AJ, Whitmore AP, Goulding KWT (2012) Advances in the understanding of nutrient dynamics and management in UK agriculture. Science of the Total Environment, 434, 39-50.

Durán Zuazo VH, Rodríguez Pleguezuelo CR (2008) soil-erosion and runoff prevention by plant covers. A review. Agronomy for Sustainable Development, 28, 65-86.

Edgerton DL, Harris JA, Birch P, Bullock P (1995) Linear relationship between aggregate stability and microbial biomass in 3 restored soils. Soil Biology & Biochemistry 27,1499-1501.

Edwards GM, Green A, Quinton JN, Morgan RPC (1997) The influence of rooting density and root morphology on saturated hydraulic conductivity. In Proceedings of the international conference on soil degradation, Lisbon, Portugal.

Eisenhauer N, Bessler H, Engels C, Gleixner G, Habekost M, Milcu A, Partsch S, Sabais ACW, Scherber C, Steinbess S, Weigelt A, Weisser WW, Scheu S (2010) Plant diversity effects on soil microorganisms support the singular hypothesis. Ecology, 91, 485-496.

Eisler MC, Lee MRF, Tarlton JF, Martin GB, Beddington J, Dungait JAJ, Greathead H, Liu J, Mathew S, Miller H, Misselbrook T, Murray P, Vinod V, Van Saun R, Winter M (2014) Agriculture: Steps to sustainable livestock. Nature, 507, 32-34.

Ekholm P, Lehtoranta J (2012) Does control of soil erosion inhibit aquatic eutrophication? Journal of Environmental Management, 93, 140-146.

Emery SM, Gross KL (2007) Dominant species identity, not community evenness, regulates invasion in experimental grassland communities. Ecology, 88, 954-964.

Falchini L, Sparvoli E, Tomaselli L (1996) Effect of Nostoc (Cyanobacteria) inoculation on the structure and stability of clay soils. Biology and Fertility of Soils, 23, 346-352.

Fargione J, Tilman D, Dybzinski R, Lambers JHR, Clark C, Harpole WS, Knops JMH, Reich PB, Loreau M (2007) From selection to complementarity: shifts in the causes of biodiversity-productivity relationships in a long-term biodiversity experiment. Proceeding of the Royal Society of Biological sciences, 274, 871-876.

Fattet M, Fu Y, Ghestem M, Ma W, Foulonneau F, Nespoulous J, Le Bissonnais Y, Stokes A (2011) Effects of vegetation type on soil resistance to erosion: Relationship between aggregate stability and shear strength. CATENA, 87,60-69.

FAO (2014) Food and Agricultural Organisation of the United Nations.

Fornara DA, Tilman D (2008) Plant functional composition influences rates of soil carbon and nitrogen accumulation. Journal of Ecology, 96, 314-322.

Fowler HJ, Ekström M, Kilsby CG, Jones PD (2005) New estimates of future changes in extreme rainfall acorss the UK using regional climate model integrations. 1. Assessment of control climate. Journal of Hydrology, 300, 212-233.

Fransen B, de Kroon H, Berendse F (1998) Root morphological plasticity and nutrient acquisition of perennial grass species from habitats of different nutrient availability. Oecologia 115:351-358.

Gastine A, Scherer-Lorenzen M, Leadley PW (2003) No consistent effects of plant diversity on root biomass, soil biota and soil abiotic conditions in temperate grassland communities. Applied Soil Ecology, 24, 101-111.

Genet M, Stokes A, Fourcaud T, Norris JE (2010) The influence of plant diversity on slope stability in a moist evergreen deciduous forest. Ecological Engineering, 36,265-275.

Głąb T, Kacorzyk P (2011) Root distribution and herbage production under different management regimes of mountain grassland. Soil and Tillage Research. 113, 99-104.

Goebel M, Hobbie SE, Bulaj B, Zadworny M, Archibald D, Oleksyn J, Reich PB, Eissenstat DM (2011) Decomposition of the finest root branching orders: linking belowground dynamics to fine-root function and structure. Ecological Monographs, 81, 89-102.

Goodman AM, Ennos AR (1998) The effects of soil bulk density on the morphology and anchorage mechanics of root systems in sunflower and maize. Annals of Botany, 83, 293-302.

Gordon HB, Whetton PH, Pittock AB, Fowler AM, Haylock MR (1992) Simulated changes in daily rainfall intensity due to to enhanced greenhouse effect: implications for extreme rainfall events. Climate Dynamics, 8, 83-102.

Gray DH, Sotir RB (1996) Biotechnical and soil bioengineering slope stabilization: a practical guide for erosion control. John Wiley & Sons.

Greenwood KL, McKenzie BM (2001) Grazing effects on soil physical properties and the consequences for pastures: a review. Australian Journal of Experimental Agriculture, 41, 1231-1250.

Guggenberger G, Frey SD, Six J, Paustian K, Elliot ET (1999) Bacterial and fungal cell-wall residues in conventional and no-tillage agroecosystems. Soil Science Society of America Journal, 63, 1188-1198.

Gyssels G, Poesen J, Bochet E, Li Y (2005) Impact of plant roots on the resistance of soils to erosion by water: a review. Progress in Physical Geography, 29,189-217.

Haddad NM, Tilman D, Haarstad J, Ritchie M, Knops JMH (2001) Contrasting effects of plant richness and composition on insect communities: A field experiment. American Naturalist, 158, 17-35.

Hallet PD (2007) An introduction to soil water repellancy. International Society for Agrochemical Adjuvants (ISAA), *taken from* Proceedings of the 8th International Symposium on Adjuvants for Agrochemicals. Columbus, Ohio, 6-9 August 2007.

Harrison KA, Bardgett RD (2010) Influence of plant species and soil conditions on plant-soil feedback in mixed grassland communities. Journal of Ecology, 98, 384-395.

Haygarth P M, Bilotta GS, Bol R, Brazier RE, Butler PJ, Freer J, Gimbert LJ, Granger SJ, Krueger T, Macleod CJA, Naden P, Old G, Quinton JN, Smith B, Worsfold P (2006) Processes affecting transfer of sediment and colloids, with associated phosphorus, from intensively farmed grasslands: an overview of key issues. Hydrological Processes, 20,4407-4413.

Head KH (1980) Manual of Soil Laboratory Testing: Soil classification and compaction tests. Wiley, US.

Hector A, Beale AJ, Minns A, Otway SJ, Lawton JH (2000) Consequences of reduction of plant diversity for litter decomposition: effects through litter quality and microenvironment. Oikos, 90, 357-371.

Hector A, Schmid B, Beierkuhlein C, Caldeira MC, Diemer M, Dimitrakopolous PG, Finn JA, Freitas H, Giller PS, Good J, Harris R, Högberg P, Huss-Danell K, Joshi J, Jumpponen A, Körner C, Leadley PW, Loreau M, Minns A, Mulder CPH, O'Donovan G, Otway SJ, Pereira JS, Prinz A, Read DJ, Scherer-Lorenzen M, Schulze ED, Siamantziourous ASD, Spehn EM, Terry AC, Troumbis AY, Woddward FI, Yachi S, Lawton JH (1999) Plant diversity and productivity experiments in European grasslands. Science, 286, 1123-1127.

Hedlund K, Santa-Regina I, Van der Putten WH, Leps J, Diaz T, Korthals GW, Lavorel S, Brown VK, Gormsen D, Mortimer SR, Barrueco CR, Roy J, Smilauer P, Smilauerova M, Van Dijk C (2003) Plant species diversity, plant biomass and responses of the soil community on abandoned land across Europe: idiosyncracy or above-belowground time lags. Oikos, 103, 45-58.

Hillel D (1998) Environmental Soil Physics. CA Academic Press, New York.

Hodge A (2003) The plastic plant: root responses to heterogenous supplies of nutrients. New Phytologist, 162, 9-24.

Hooda PS, Edwards AC, Anderson HA, Miller A (2000) A review of water quality concerns in livestock farming areas. Science of the Total Environment, 250, 143-167.

Hooper DU, Chapin, III FS, Ewel JJ, Hector A, Inchausti P, Lavorel S, Lawton JH, Lodge DM, Loreau M, Naeem S, Schmid B, Setälä H, Symstad AJ, Vandermeer J, Wardle DA (2005) Effects of biodiversity on ecosystem functioning: a consensus of current knowledge. Ecological Monographs, 75, 3-35.

Hooper DU, Vitousek PM (1998) Effects of plant composition and diversity on nutrient cycling. Ecological Monographs, 68, 121-149.

Hopkins A, Wilkins RJ (2006) Temperate grassland: key developments in the last century and future perspectives. Journal of Agricultural Science, 144, 503-523.

Huat BBK, Ali FH, Maail S (2005) The effect of natural fibre on the shear strength of soil. American Journal of Applied Sciences (Special Issue) 9-13.

Hummel I, Vile D, Violle C, Devaux J, Ricci B, Blanchard A, Garnier E, Roumet C (2007) Relating root structure and anatomy to whole-plant functioning in 14 herbaceous Mediterranean species. New Phytologist, 173, 313-321.

Humphreys MW, Yadav RS, Cairns AJ, Turner LB, Humphreys J, Skøt L (2006) A changing climate for grassland research. New Phytologist 169,9-26.

Inderjit, Weston LA (2003) Root exudates: an overview. In: de Kroon, H. and Visser E.J.W (eds) Root Ecology. Ecological Studies, Vol.168. Springer-Verlag Heidelberg.

Isbell F, Calcagno V, Hector A, Connolly J, Harpole WS, Reich PB, Scherer-Lorenzen M, Schmid B, Tilman D, van Ruijven J, Weigelt a, Wilsey BJ, Zavaleta ES, Loreau M (2011) High plant diversity is needed to maintain ecosystem services. Nature, 477, 199-202.

Jastrow JD (1987) Changes in soil aggregation associated with tallgrass prairie restoration. American Journal of Botany, 74, 1656-1664.

Jacinthe PA, Lal R, Owens LB, Hothem DL (2004) Transport of labile carbon in runoff as affected by land use and rainfall characteristics. Soil and Tillage Resaearch, 77, 111-123.

Jeffery S, Harris JA, Rickson RJ, Ritz K (2010) Effects of soil-surface microbial community phenotype upon physical and hydrological properties of an arable soil: a microcosm study. European Journal of Soil Science, 61, 493-503.

Jiao Z, Wang DJ, Xie H, Zhang JH, Guo LH (2010) Experimental analysis of shear strength of undisturbed soil in leucaena forest in Jiangjia Ravine, Yunnan, China. Journal of Mountain Science, 7, 386-395.

Kay BD (1990) Rates of change of soil structure under different cropping systems. Advancements in Soil Science, 12, 1-52.

Kelly EF, Chadwick OA, Hilinski TE (1998) The effect of plants on mineral weathering. Biogeochemistry. 42, 21-53.

Kirkham FW, Tallowin JRB, Dunn RM, Bhogal A, Chambers BJ, Bardgett RD (2014) Ecologically sustainable fertility management fo rthe maintenace of species-rich hay meadows: a 12-year fertilizer and lime experiment. Journal of Applied Ecology, 51, 152-161.

Klimek S, Richter gen. Kemmermann A, Hofman M, Isselstein J (2007) Plant species richness and composition in managed grasslands: The relative importance of field management and environmental factors. Biological Conservation, 134, 559-570.

Kluge G, Müller-Westermeier G (2000) Das Klima ausgewählter Orte der Bundesrepublik Deutschland: Jena. Berichte des Deutschen Wetterdienstes, 213.

Klute A, Dirksen C (1986) Hydraulic Conductivity and Diffusivity: Laboratory Methods, in Klute, A (ed) Methods of Soil Analysis Part 1: Physical and Mineralogical methods. Madison. 687-784.

Koide RT, Peoples MS (2013) Behaviour of Bradford-reactive substances is consistent with predictions for glomalin. Applied Soil Ecology, 63, 8-14.

Kong AYY, Six J, Bryant DC, Ford Denison R, van Kessel C (2005) The relationship between carbon input, aggregation, and soil organic carbon stabilisation in sustainable cropping systems. Soil Science Society of America, 69, 1078-1085.

Kouwen N (1988) Field estimation of the biomechanical properties of grass. Journal of Hydraulic Research, 26,559-568.

Kouwen N, Li RM (1980) Biomechanics of vegetative channel linings. Journal of the Hydraulics Division-Asce 106, 1085-1106.

Kutilek M, Nielson DR (1994) Soil Hydrology, Catena.

Lal R (1998) Soil erosion impact on agronomic productivity and environment quality. Critical Reviews in Plant Sciences 17,319-464.

Lal R (2001) Soil degradation by erosion. Land degradation and development, 12, 519-539.

Lal R (2003) Soil erosion and the global carbon budget. Environment International 29, 437-450.

Lavorel S, McIntyre S, Landsberg J, Forbes TDA (1997) Plant functional classifications: from general groups to specific groups based on response to disturbance. Trends in Ecology and Evolution, 12, 474-478.

Lawrance CJ, Rickson RJ, Clark JE (1996) The effect of grass roots on the shear strength of colluvial soils in Nepal.Advances in Hillslope Processes. 2, 857-868.

Le Bissonais Y (1996) Aggregate stability and assessment of soil crustability and erodibility: I. Theory and methodology. European Journal of Soil Science, 47, 425-437.

Ligdi EE, Morgan RPC (1995) Contour grass strips - a laboratory simulation of their role in soil-erosion control. Soil Technology, 8,109-117.

Lin H, (2010) Linking principles of soil formation and flow regimes. Journal of Hydrology, 393,3-19.

Loades KW, Bengough AG, Bransby MF, Hallett PD (2010) Planting density influence on fibrous root reinforcement of soils. Ecological Engineering, 36, 276-284.

Loreau M. Hector A (2001) Partitioning selection and complementarity in biodiversity experiments. Nature, 412, 72-76.

Mack RN, Simberloff D, Lonsdale WM, Evans H, Clout M, Bazzaz FA (2000) Biotic invasions: Causes, epidemiology, global consequences and control. Ecological Applications, 10, 689-710.

Macleod CJA, Humphreys MW, Whalley WR, Turner L, Binley A, Watts CW, Skot L, Joynes A, Hawkins S, King IP, O'Donovan S, Haygarth PM (2013) A novel grass hybrid to reduce flood generation in temperate regions. Scientific reports, 3, 1683.

Maestre FT, Quero JL, Gotelli NJ, Escudero a, Ochoa V, Delgado-Baquerizo M, Garcia-Gomez M, Bowker MA, Soliveres S, Escolar C, Garcia-Palacios P, Berdugo M, Valencia E, Gozalo B, Gallardo A, Aguilera L, Arredondo T, Blones J, Boeken B, Bran D, Conceicao AA, Cabrera O, Chaieb M, derak M, Eldridge DJ, Espinosa CI, Florentino A, Gaitan J, Gatica MG, Ghiloufi w, Gomez-Gonzalez S, Gutierrez JS, Hernandez RM, Huang X, Huber-Sannwald E, Jankju M, Miriti M, Monerris J, Mau RL, Morici E, Naseri K, Ospina A, Polo V, Prina A, Pucheta E, Ramirez-Collantes DA, Romao R, Tighe M, Torres-Diaz C, Val J, Veiga JP, Wang D, Zaady E (2012) Plant species richness and ecosystem multifunctionality in global drylands. Science, 335, 214-218.

Mamo M, Bubenzer GD (2001a) Detachment rate, soil erodibility, and soil strength as influenced by living plant roots part I: Laboratory study. Transactions of the ASAE, 44,1167-1174.

Mamo M, Bubenzer GD (2001b) Detachment rate, soil erodibility, and soil strength as influenced by living plant roots part II: Field study. Transactions of the ASAE 44,1175-1181.

Marquand E, Weigelt A, Temperton VM, Roscher C, Schumacher J, Buchmann N, Fischer M, Weisser WW, Schmid B (2009) Plant species richness and functional composition drive overyielding in a six-year grassland experiment. Ecology, 90, 3290-3302.

Martin C, Pohl M, Alewell C, Körner C, Rixen C (2010) Interrill erosion at disturbed alpine sites: Effects of plant functional diversity and vegetation cover. Basic and Applied Ecology, 11, 619-626.

Masle J, Passioura JB (1987) The effect of soil strength on the growth of young wheat plants. Australian Journal of Plant Physiology, 14, 643-656.

Meadows A, Meadows PS, Wood DM, Murray JMH (1994) Microbiological effects on slope stability - an experimental-analysis. Sedimentology, 41, 423-435.

Merz A, Alewell C, Hiltbrunner E, Baenninger D (2009) Plant-compositional effects on surface runoff and sediment yield in subalpine grassland. Journal of Plant Nutrition and Soil Science, 172, 777-788.

McLaren JR, Wilson SD, Peltzer DA (2004) Plant feedbacks increase the temporal heterogeneity of soil moisture. Oikos, 107, 199-205.

McVay KA, Radcliffe DE, Hargrove WL (1989) Winter legume effects on soil properties and nitrogen fertiliser requirements. Soil Science Society of America Journal, 53, 1856-1862

Milcu A, Partsch S, Scherber C, Weisser WW, Scheu S (2008) Earthworms and legumes control litter decomposition in a plant diversity gradient. Ecology, 89, 1872-1882.

Millennium Ecosystem Assessment (2005) Ecosystems and human wellbeing.

Miller RM, Jastrow JD (1990) Heirachy of root and mycorrhizal fungal interactions with soil aggregation. Soil Biology and Biochemistry, 22, 579-584.

Miller N, Quinton JN, Barberis E, Presta M (2009) Variability in the mobilization of sediment and phosphorus across 13 European soils. Journal of Environmental Quality 38, 742-750.

Mickovski SB, Hallett PD, Bransby MF, Davies MCR, Sonnenberg R, Bengough AG (2009) Mechanical reinforcement of soil by willow roots: impacts of root properties and root failure mechanism. Soil Science Society of America Journal, 73, 1276-1285.

Mommer L, van Ruijven J, de Caluwe H, Smit-Tiekstra AE, Wagemaker CAM, Ouborg NJ, Bögemann GM, van der Weerden GM, Berendse F, de Kroon H (2010) Unveiling below-ground species abundance in a biodiversity experiment: a test of vertical niche differentiation among grassland species. Journal of Ecology, 98, 1117-1127.

Mommer L, Visser EJW, van Ruijven J, de Caluwe H, Pierik R, de Kroon H (2011) Contrasting root behaviour in two grass species: a test of functionality in dynamic heterogeneous conditions. Plant and Soil, 344, 347-360.

Montrasio L, Valentino R, Losi GL (2012) Shallow landslides triggered by rainfalls: modeling of some case histories in the Reggiano Apennine (Emilia Romagna Region, Northern Italy), Natural Hazards. 60, 1231-1254.

Morales V, Parlange JY, Steenhuis T (2010) Are preferential flow paths perpetuated by microbial activity in the soil matrix? A review. Journal of Hydrology, 393, 29-36.

Morel J, Habib L, Plantureux S, Guckert A (1991) Influence of maize root mucilage on soil aggregate stability. Plant and Soil 136, 111-119.

Morgan, RPC (2005) Soil erosion and conservation. Third Edition, Blackwell Publishing, Oxford, 11-45.

Morgan, RPC, Quinton JN, Edwards GM (1995) 3.05 Vegetation strategies for combating desertification. MEDALUS II Project 3 Managing Desertification. Contract EV5V-CT92-0165. Final Report covering the period 1 January 1991 to 30 September 1995.

Mouzai L, Bouhadef M (2011) Shear strength of compacted soil: effects on splash erosion by single water drops. Earth Surface Processes and Landforms, 36, 87-96.

Mueller KE, Tilman D, Fornara DA, Hobbie SE (2013) Root depth distribution and the diversity-productivity relationhsip in a long-term grassland experiment. Ecology, 94, 787-793.

Mytton L, Cresswell A (1992) Clover-based grassland. Journal of the Science of Food and Agriculture, 60, 400.

Naeem S, Hakansson K, Lawton JH, Crawley MJ, Thompson LJ (1996) Biodiversity and plant productivity in a model assemblage of plant species. Oikos, 76, 259-264.

Nearing MA (1997) The mechanics of soil detachment by raindrops and runoff. Eurasian Soil Science, 39, 552-556.

Norris JE (2006) Root mechanics applied to slope stability. Thesis. Nottingham Trent.

Norris JE, Greenwood JR (2000) Review of in-situ shear tests on root reinforcement of soil in A Stokes, editor. The supporting roots of trees and woody plants: form, function and physiology. Springer Netherlands, 287-293.

Novotny V (1999) Diffuse pollution from agriculture: A worldwide outlook. Water Science and Technology, 39, 1-13.

Oades JM, Waters AG (1991) Aggregate hierachy in soils. Australian Journal of Soil Research, 29, 815-828.

Operstein V, Fryman S (2000) The influence of vegetation on soil strength. Ground Improvement, 4,81-89.

Pérès G, Cluzeau D, Menasseri S, Soussana JF, Bessler H, Engels C, Habekost M, Gleixner G, Weigelt A, Weisser WW, Scheu S, Eisenhaur N (2013) Mechanisms linking plant community properties to soil aggregate stability in an experimental grassland plant diversity gradient. Plant Soil, 373, 285-299.

Pilgrim E S, Macleod CJA, Blackwell MSA, Bol R, Hogan DV, Chadwick DR, Cardenas L, Misselbrook TH, Haygarth PM, Brazier RE, Hobbs P, Hodgson C, Jarvis C, Dungait J, Murray PJ, Firbank LG (2010) Interactions among agricultrural production and other ecosystem services delivered from European temperate grassland systems. Pages 117-154 in D. L. Sparks, editor. Advances in Agronomy, Vol 109. Elsevier Academic Press Inc, San Diego.

Pimentel D, Harvey C, Resosudarma P, Sinclair K, Kurz D, McNair M, Crist S, Shpritz L, Fitton I, Saffouri R, Blair R (1995) Environmental and economic costs of soil erosion and conservation benefits. Science, 267, 1117-1123.

Pimental D (2006) Soil erosion: A food and environmental threat. Environment, Development and Sustainability, 8, 119-137.

Pohl M, Alig D, Körner C, Rixen C (2009) Higher plant diversity enhances soil stability in disturbed alpine ecosystems. Plant Soil, 324, 91-102.

Pohl M, Stroude R, Buttler A, Rixen C (2011) Functional traits and root morphology of alpine plants. Annals of Botany, 108, 537-545.

Pojasok T, Kay BD (1990) Effect of root exudates from corn and bromegrass on soil structural stability. Canadian Journal of Soil Science, 70, 351-362.

Powis VB, Whalley WR, Bird NRA, Leeds-Harrison PB (2003) Some mechanisms responsible for the alteration of soil hydraulic properties by root activity. Pages 273-280 in H. M. Moore, H. R. Fox, and S. Elliott, editors. Land Reclamation: Extending the Boundaries. A a Balkema Publishers, Leiden

Puigdefabregas J (2005) The role of vegetation patterns in structuring runoff and sediment fluxes in drylands. Earth Surface Processes and Landforms, 30, 133-147.

Quijas S, Schmid B, Balvanera P (2010) Plant diversity enhances provison of ecosystem services: A new synthesis. Basic and Applied Ecology, 11, 582-593.

Quinton JN, Catt JA, Hess TM (2001) The selective removal of phosphorous from soil: Is event size important?, Journal of Environmental Quality, 30, 538-545.

Quinton JN, Edwards GM, Morgan RPC (1997) The influence of vegetation species and plant properties on runoff and soil erosion: results from a rainfall simulation study in south east Spain. Soil Use and Management 13, 143-148.

Quinton JN, Govers G, Van Oost K, Bardgett RD (2010) The impact of agricultural soil erosion on biogeochemical cycling. Nature Geoscience, 3, 311-314.

Reich PB, Tilman D, Naeem S, Ellsworth DS, Knops J, Criane J, Wedin D, Trost (2004) Species and functional group diversity independently influence biomass accumulation and its response to CO2 and N. Proceedings of the National Academy of Sciences, 101, 10101-10106.

Rewald B, Meinen C (2013) Plant roots and spectroscopic methods – analysing species, biomass and vitality. Frontiers in Plant Science, 4, doi: 10.3389/fpls.2013.00393

Rillig MC, Wright SF, Eviner VT (2002) The role of arbuscular mycorrhizal fungi and glomalin in soil aggregation: comparing effects of five plant species. Plant Soil, 238, 325-333.

Ritz K, Young IM (2004) Interactions between soil structure and fungi. Mycologist 18, 52-59.

Robinson D, Hodge A, Griffiths BS, Fitter, AH (1999) Plant root proliferation in nitrogen-rich patches confers competitive advantage. Proceedings of the Royal Society of London Series B-Biological Sciences, 266, 431-435.

Rockström J, Steffen W, Noone K, Persson A, Chapin III FS, Lambin EF, Lenton TM, Scheffer M, Folke C, Schellnhuber HJ, Nykvist B, de Wilt CA, Hughes T, van der Leeuw S, Rodhe H, Sorlin S, Snyder PK, Costanza R, Svedin U, Falkenmark M, Karlberg L, Corell RW, Fabry VJ, Hansen J, Walker B, Liverman D, Richardson K, Crutzen P, Foley JA (2009) A safe operating space for humanity. Nature, 461, 472-475.

Roscher C, Schumacher J, Baade J, Wilcke W, Gleixner G, Weisser WW, Schmid B, Schulze ED (2004) The role of biodiversity for elemental cycling and trophic interactions: an experimental approach in a grassland community. Basic and Applied Ecology, 5, 107-121.

Rosier CL, Hoye AT, Rillig MC (2006) Glomalin-related soil protein: Assessment of current detection and quantification tools. Soil Biology and Biochemistry, 38, 2205-2211.

Saygin SD, Cornelis WM, Erpul G, Gabriels D (2012) Comparison of different aggregate stability approaches for loamy sand soils. Applied Soil Ecology, 54, 1-6.

Schäffer B, Stauber M, Mueller TL, Müller R, Schulin R (2008) Soil and macropores under uniaxial compression. I. Mechanical stability of repacked soil and deformation of different types of macropores. Geoderma, 146, 183-191.

Scherer-Lorenzen M, Bonilla JL, Potvin C (2007) Tree species richness affects litter production and decomposition rates in a tropical biodiversity experiment. Oikos, 116, 2108-2124.

Scherer-Lorenzen M, Palmborg MC, Prinz A, Schulze ED (2003) The role of plant diversity and composition for nitrate leaching in grasslands. Ecology, 84, 1539-1552.

Schmid B, Hector A, Huston MA, Inchausti P, Nijs I, Leadley PW, Tilman D (2002) The design and analysis of biodiversity experiments. in Loreau M, Naeem S, Inchausti P (editors). Biodiversity and ecosystem functioning. Oxford University Press, Oxford, 61-75.

Scott NA (1998) Soil aggregation and organic matter mineralisation in forestes and grasslands: Plant species effects. Soil Science Society of America Journal, 62, 1081-1089.

Sharpley AN, Smith SJ, Jones OR, Berg WA, Coleman GA (1992) The transport of bioavailable phosphorus in agricultural runoff. Journal of Environmental Quality, 21,30-35.

Siemann E (1998) Experimental tests of effects of plant productivity and diversity on grassland arthropod diversity. Ecology, 79, 2057-2070.

Six J, Bossuyt H, Degryze S, Denef K (2004) A history of research on the link between (micro)aggregates, soil biota, and soil organic matter dynamics. Soil & Tillage Research 79, 7-31.

Six J, Conant RT, Paul EA, Pautian K (2002) Stablization of soil organic matter: Implications for C-saturated soils. Plant and Soil, 241, 155-176.

Six J, Elliot ET, Paustian K (2000) Soil macroaggregate turnover and microaggregate formation: A mechanism for C sequestration under no-tillage agriculture. Soil Biology and Biochemistry, 32, 2099-2103.

Six J, Elliot ET, Paustian K, Doran JW (1998) Aggregation and soil organic matter accumulation in cultivated and native grassland soils. Soil Science Society of America Journal, 62, 1367-1377.

Smilauerova M (2001) Plant root response to heterogeneity of soil resources: Effects of nutrient patches, am symbiosis, and species composition. Folia Geobotanica, 36,337-351.

Smith KA, Mullins CE (1991) Soil Analysis: Physical Methods. Marcel Dekker Inc. New York, 209-269.

Spehn EM, Hector A, Joshi J, Scherer-Lorenzen M, Schmid B, Bazeley-White E, Beierkuhnlein C, Caldeira MC, Diemer M, Dimitrakopoulos PG, Finn JA, Freitas H, Giller PS, Good J, Harris R, Hogberg P, Huss-Danell K, Jumpponen A, Koricheva J, Leadley PW, Loreau M, Minns A, Mulder CPH, O'Donovan G, Otway SJ, Palmborg C, Pereira JS, Pfisterer AB, Prinz A, Read DJ, Schulze ED, Siamantziouras ASD, Terry

AC, Troumbis AY, Woodward FI, Yachi S, Lawton JH (2005) Ecosystem effects of biodiversity manipulations in European grasslands. Ecological Monographs ,75,37-63.

Spehn EM, Joshi J, Schmid B, Alphei J, Korner C (2000) Plant diversity effects on soil heterotrophic activity in experimental grassland ecosystems. Plant and Soil, 224, 217-230.

Spehn EM, Scherer-Lorenzen M, Schmid B, Hector A, Caldeira MC, Dimitrakopolous PG, Finn JA, Jumpponen A, O'Donnovan G, Pereira JS, Schulze W-D, Troumbis AY, Korner C (2002) The role of legumes as a component of biodiversity in a cross-European study of grassland biomass nitrogen. Oikos, 98, 205-218.

Steinbess S, Bessler H, Engels C, Temerton VM, Buchmann N, Roscher C, Kreutziger Y, Baade J, Habekost M, Gleixner G (2008) Plant diversity positively affects short-term soil carbon storage in experimental grasslands. Global Change Biology, 14, 2937-2949.

Stevens CJ, Dise NB, Mountford JO, Gowing DJ (2004) Impact of nitrogen deposition on the species richness of grasslands. Science, 303, 1876-1879.

Stoate C, Boatman ND, Borralho RJ, Carvalho CR, de Snoo GR, Eden P (2001) Ecological impacts of arable intensification in Europe. Journal of Environmental Management, 63, 337-365.

Stokes A, Atger C, Bengough AG, Fourcaud T, Sidle RC (2009) Desirable plant root traits for protecting natural and engineered slopes against landslides. Plant and Soil, 324,1-30.

Tengbeh GT (1993) The effect of grass roots on shear strength variations with moisture content. Soil Technology, 6, 287-295.

Terwilliger VJ, Waldron LJ (1990) Assessing the contribution of roots to the strength of undisturbed, slip prone soils. CATENA 17,151-162.

Thomas JO, Davies LJ (1954) Common British Grasses and Legumes. Longmans Green, London.

Tilman, D (1997) Biodiversity and Ecosystem Function. Chapter 6 *in* Nature's Services and Societal Dependence on Natural Ecosystems, Island Press, Washington, 93-112.

Tilman D, Downing JA (1994) Biodiversity and stability in grasslands. Nature, 367, 363-365.

Tilman D, Wedin D, Knops J (1996) Productivity and sustainability influenced by biodiversity in grassland ecosystems. Nature, 379, 718-720.

Tisdall JM (1991) Fungal hyphae and structural stability of soil. Australian Journal of Soil Research 29, 729-743.

Tisdall JM (1994) Possible role of soil-microorganisms in aggregation in soils. Plant and Soil, 159, 115-121.

Tisdall JM, Oades JM (1979) Stabilization of soil aggregates by the root systems of ryegrass. Australian Journal of Soil Research 17,429-441.

Tisdall JM, Oades JM (1982) Organic matter and water-stable aggregates in soils. Journal of Soil Science, 33, 141-163.

Tobias S (1994) Shear strength of the soil root system: In situ shear tests. Pages 405-412 *in* R. J. Rickson, editor. Conserving soil resources: European perspectives. CAB International, Cambridge

Traoré O, Groleau-Renaud V, Plantureux S, Tubeileh A, Boeuf-Tremblay V (2000) Effect of root mucilage and modelled root exudates on soil structure. European Journal of Soil Science, 51, 575-581.

Treseder KK, Turner KM (2007) Glomalin in Ecosystems. Soil Science Society of America Journal. 71, 1257-1266.

Troumbis AY, Memtsas D (2000) Observational evidence that diversity may increase productivity in Mediterranean shrublands. Oecologia, 125, 101-108.

Tylianakis JM, Didham RK, Bascompte J, Wardle DA (2008) Global change and species interactions in terrestrial ecosystems. Ecology Letters, 11, 1351-1363.

van der Heijden MGA, Bardgett RD, van Straalen NM (2008) The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. Ecology Letters, 11, 296-310.

Vitousek PM, Mooney HA, Lubchenco J, Melillo JM (1997) Human domination of earth's ecosystems. Science, 277, 494-499.

Waldron L J (1977) Shear resistance of root-permeated homogenous and stratified soil. Soil Science Society of America Journal, 41,843-849.

Waldron LJ, Dakessian S (1982) Effect of grass, legume, and tree roots on soil shearing resistance. Soil Science Society of America Journal, 46, 894-899.

Weigelt A, Weisser WW, Buchmann N, Scherer-Lorenzen M (2009) Biodiversity for multifunctional grasslands: equal productivity in high-diversity low-input and low-diversity high-input systems. Biogeosciences 6,1695-1706.

Whalley WR, Dumitru E, Dexter AR (1995) Biological effects of soil compaction. Soil and Tillage Research, 35, 53-68.

Wright SF, Upadhyaya A (1998) A survey of soils for aggregate stability and glomalin, a glycoprotein produced by hyphae of arbuscular mycorrhizal fungi. Plant Soil, 198, 97-107.

Wu TH, McKinnell WP, Swanston DN (1979) Strength of tree roots and landslides on Prince of Wales Island, Alaska. Canadian Geotechnical Journal, 16, 19-33.

Yaacob O, Blair GJ (1981) Effect of legume cropping and organic-matter accumulation on the infiltration-rate and structural stability of a granite soil under a simulated tropical environment. Plant and Soil. 60, 11-20.

Yan Z, Yan C, Wang H (2010) Mechanical interaction between roots and soil mass in slope vegetation. SCIENCE CHINA Technological Sciences, 53, 3039-3044.

Yeates GM, Bardgett RD, Cook R, Hobbs PJ, Bowling PJ, Potter JF (1997) Faunal and microbial diversity in three Welsh grassland soils under conventional and organic management. Journal of Applied Ecology, 34, 453-470.

Zhang CB, Chen LH, Liu YP, Ji XD, Liu XP (2010) Triaxial compression test of soil-root composites to evaluate influence of roots on soil shear strength. Ecological Engineering, 36, 19-26.

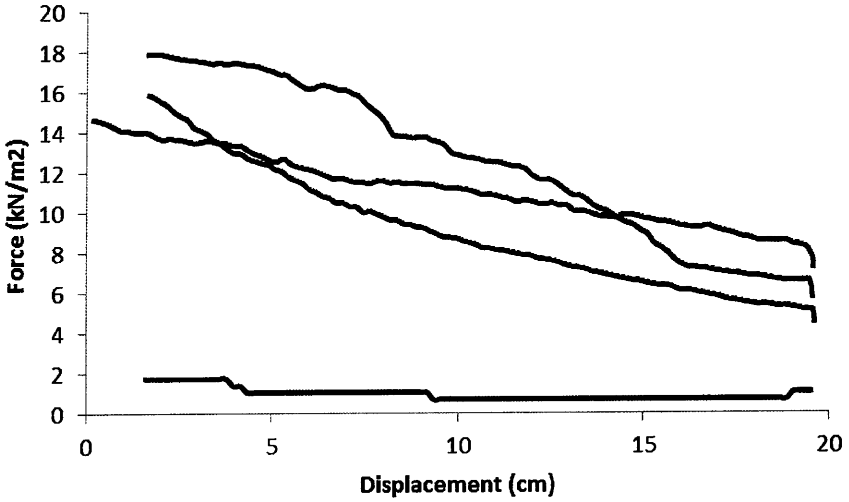
Zhou ZC, Gan ZT, Shangguan ZP, Dong ZB (2010) Effects of grazing on soil physical properties and soil erodibility of the Northern Loess Plateau (China), Catena, 82, 87-91.

Appendix A1

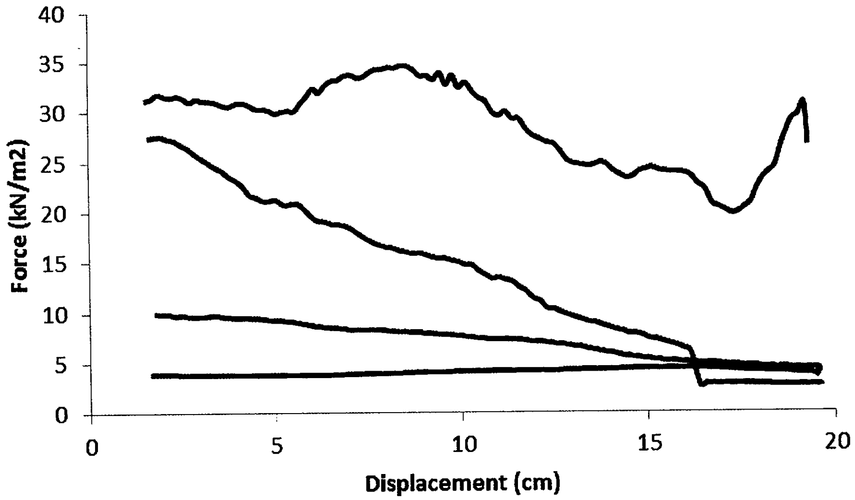
Force-Displacement graphical outputs for each mesocosm treatment in chapter 3. Lines represent each experimental replicate.

Treatment

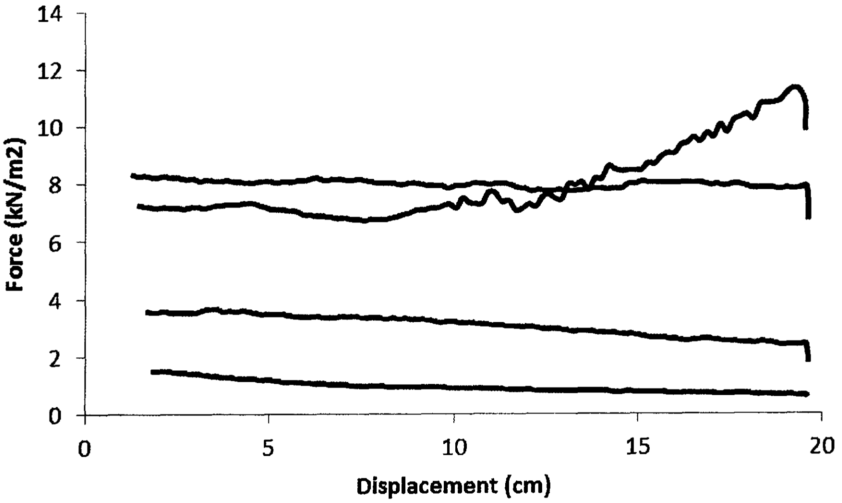
Lp



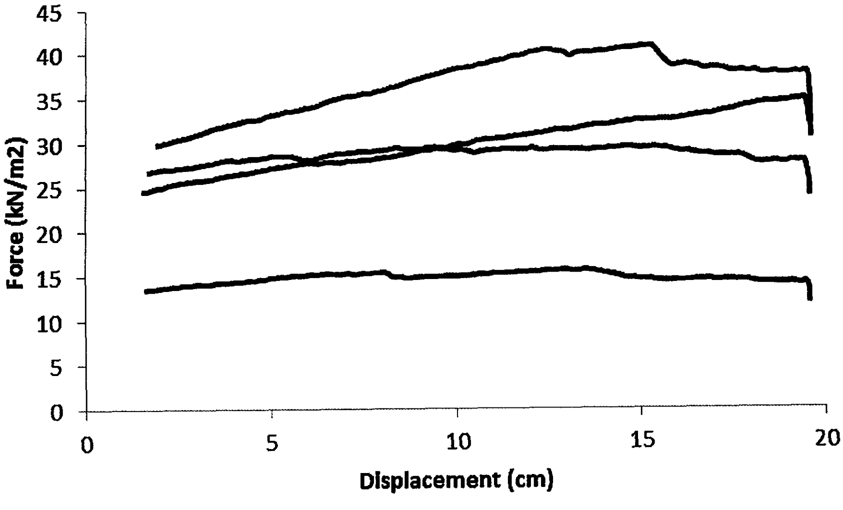
Ao



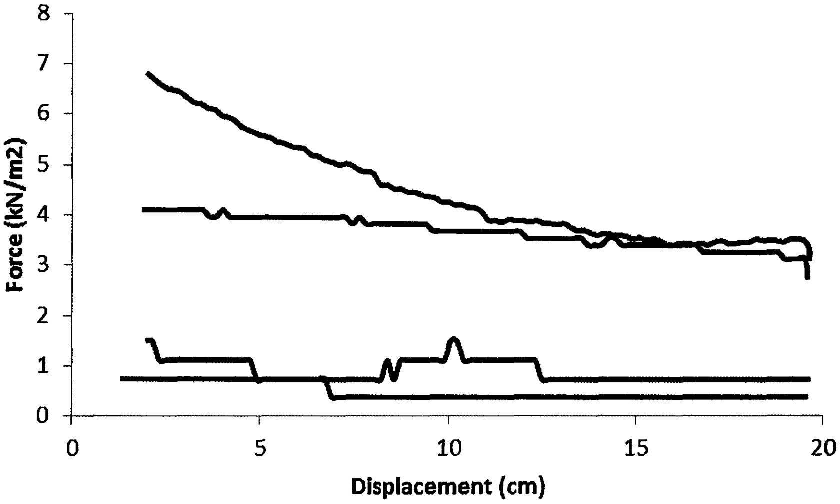
Tr


14
12
10
8
6
4
2
0
Force (kN/m2)
0
5
10
15
20
Displacement (cm)

Lc

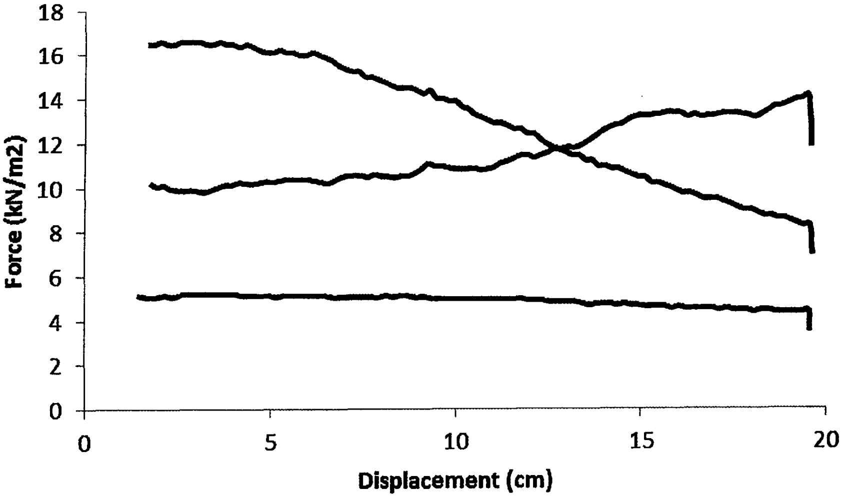

45
40
35
30
25
20
15
10
5
0
Force (kN/m2)
0
5
10
15
20
Displacement (cm)

Pl



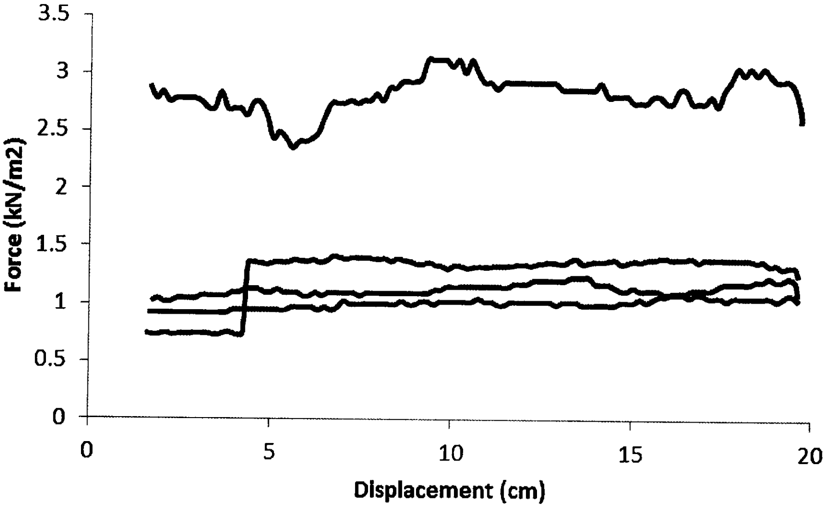
8
7
6
5
4
3
2
1
0
Force (kN/m2)
0
5
10
15
20
Displacement (cm)

Am

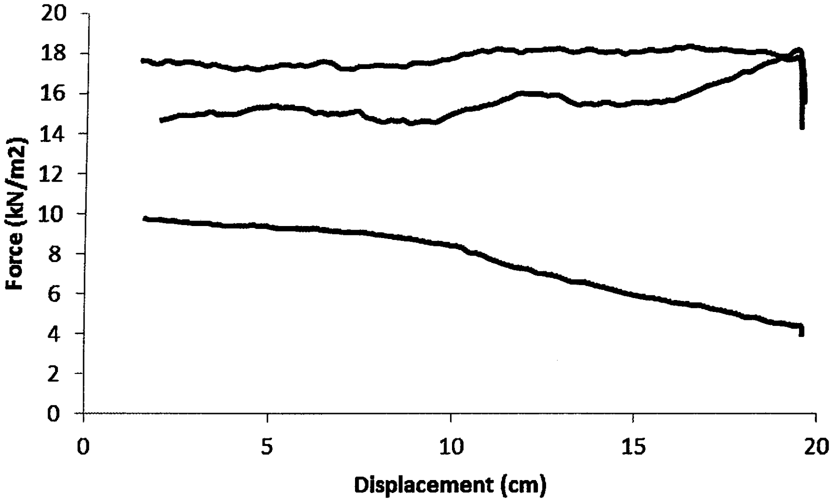


18
16
14
12
10
8
6
4
2
0
Force (kN/m2)
0
5
10
15
20
Displacement (cm)

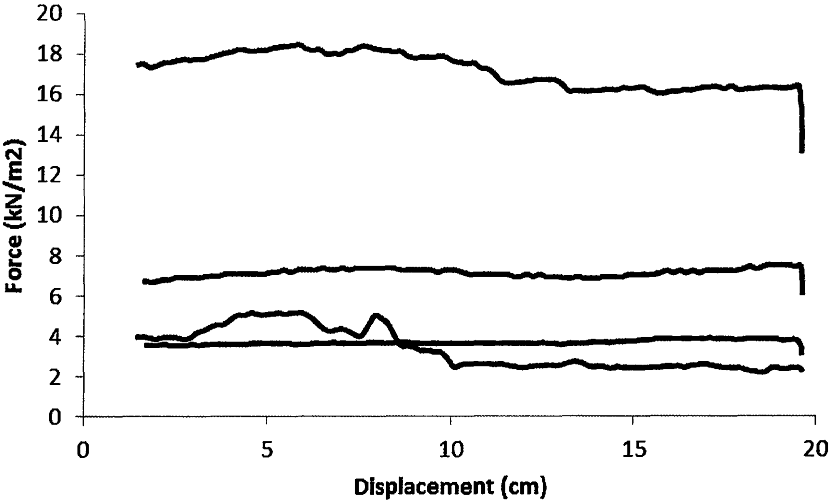
Bare Soil



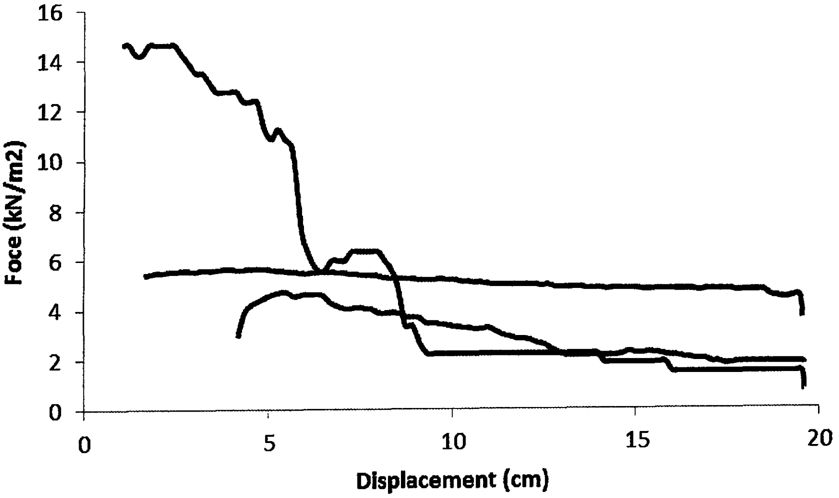
Grasses



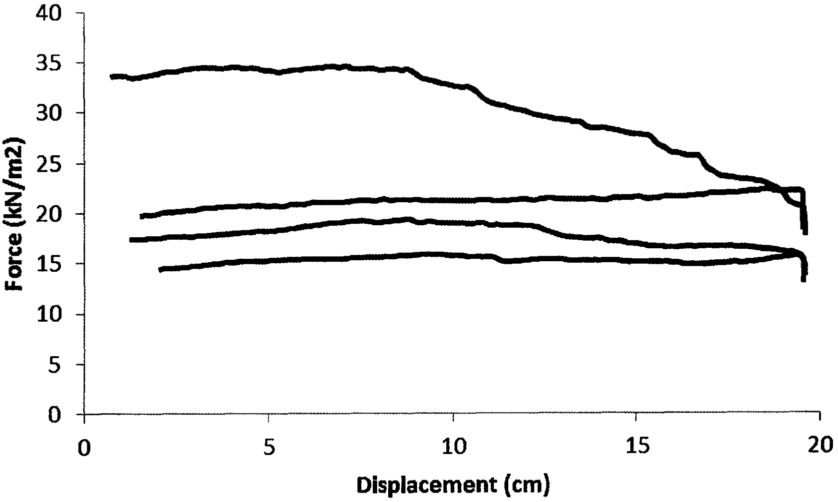
Legumes



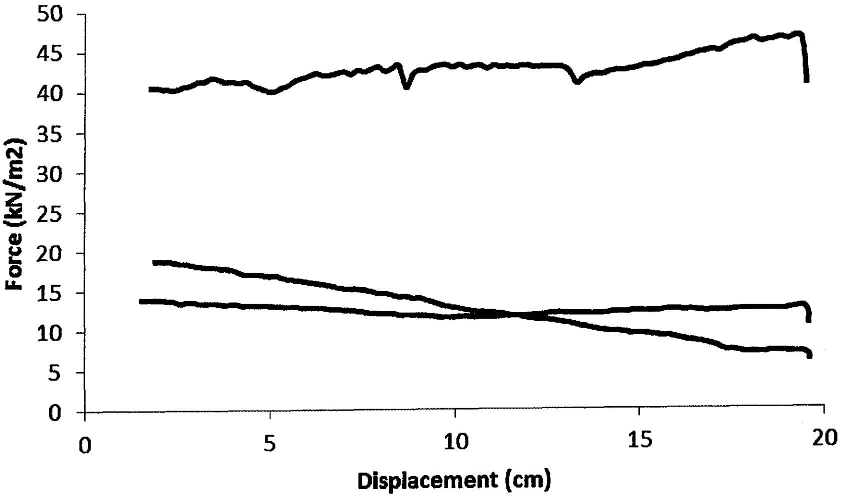
Forbs



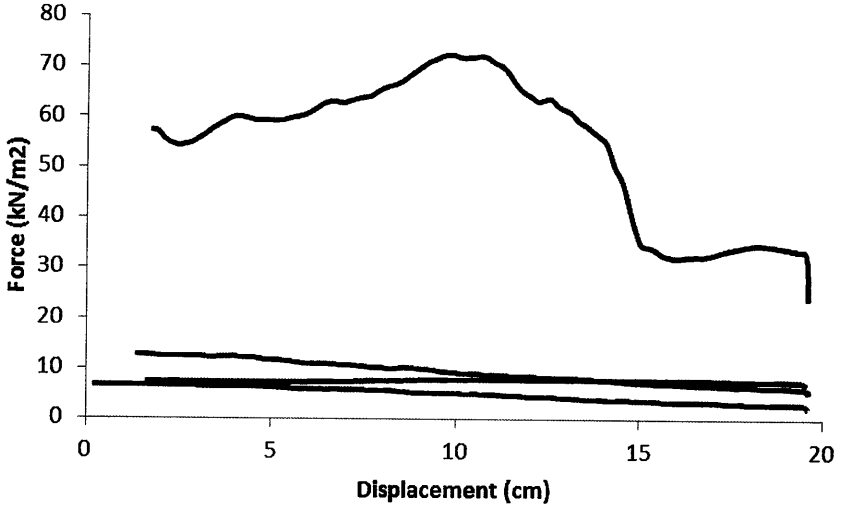
Grass and Legume



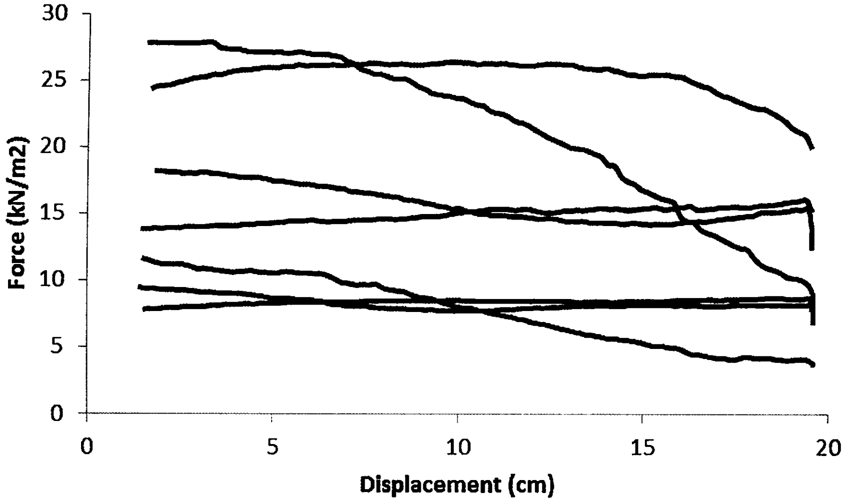
Grass and Forb



Forb and Legume



Grass, Forb and Legume



All species mixture