Examining the impacts of plant species diversity and rooting characteristics on the accumulation of soil organic matter in temporary grasslands

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Abstract

Global soils are under increasing pressure from a range of drivers including intensive agriculture, deforestation, urbanisation and climate change. These are leading to dangerous losses of soil organic matter (SOM) which is vital for effective soil function. Soils provide a range of essential ecosystem services including food provision, flood prevention and carbon sequestration. Soil degradation through the loss of SOM could reduce the range and quality of ecosystem services that global societies receive from soil.

It has been proposed that SOM in grasslands may be enhanced by increasing the plant species diversity of swards. However, research has previously not investigated this process within the context of temporary agricultural grasslands, nor have such SOM gains been quantified down to the composite fraction level to identify how responses vary in labile and persistent SOM fractions respectively.

Two projects were conducted; firstly, a sampling exercise of an agricultural grassland which involved extracting deep soil cores for subsequent SOM fractionation and analysis; and secondly a glasshouse experiment which involved six herbaceous grassland species (selected from three functional groups determined by rooting characteristics) grown both in monoculture and 2-species diversity treatments. A subsidiary experiment was conducted concurrently allowing a detailed assessment of rooting characteristics of all six herbaceous species grown in the main glasshouse experiment.

The combined study found that SOM was not higher in the higher diversity treatment, although some individual SOM fractions were. Additionally, SOM was not consistently correlated with a specific rooting characteristic or with total root biomass. While some trends were observed linking higher SOM with more extensive root structures, these trends were inconsistent and SOM values were not proportional to root biomass. Finally, an inconsistent relationship was observed between SOM and soil depth, which may have resulted from variable water content and or bulk density at different depths between the two projects.

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Author’s declaration

I declare that this thesis is a presentation of original work and I am the sole author. This work has not previously been presented for an award at this, or any other, University. All sources are acknowledged as references

1. Introduction

The pervasive and systematic nature of global soil degradation has been widely reported in recent years (IPBES 2018, UNCCD 2017). Causes include deforestation, land use change, climate change, urbanisation and infrastructure development (EEA 1999, Kumar & Das 2014). Specific concerns have been raised about intensive agriculture, reduced soil fertility, loss of organic matter, increased instances of compaction and erosion and the impact of these deteriorating factors on the ability of soil to function resiliently (Graves et al. 2015, Lirri et al. 2012). Functioning soil systems fundamentally underpin a range of essential ecosystem services, such as nutrient cycling, food provision, flood management and climate regulation (Adhikari & Hartemink 2016). As such, attention should urgently focus on practices which support soils to develop functional resilience towards deleterious influences.

It is widely recognised that the organic matter within soil plays a crucial role in supporting soil functionality, by providing a resource for chemical and biological interactions and by improving physical properties through increased aggregation and larger pore spaces (Walsh 2012). On agricultural land, which accounts for approximately 71% of the UK's land area, increasing the soil organic matter (SOM) content of soils can also reduce the need for costly chemical fertiliser inputs (DEFRA 2009, DEFRA 2016). One pathway through which SOM increases are derived is through plant growth which results in root turnover, aboveground plant litter, root exudation and plant-associated microbial inputs (Lange et al. 2015). Conversely, SOM can be reduced through soil disruption (ploughing and burning), unrelenting vegetative extraction (overgrazing and intensive arable rotations) and environmental weathering of bare soil (bare fallow and removal of crop residue) (FAO 2005). The functional resilience of soil is subject to the dynamic state of this organic matter; therefore, SOM losses following perturbation can lead to reduced soil function (Herrick & Wander 1998).

It is important to recognise that SOM should not be treated as one homogenous compound. Von Lutzow et al (2017) describe SOM as a combination of numerous functional pools with variable stabilising mechanisms and turnover rates. A significant body of research has emerged which attempts to define and characterise these functional pools and measure respective turnover rates subject to a combination of their chemical properties and physical condition. For instance, Branco de Freitas Maia et al. (2013) presents a framework through which the variable turnover rates of SOM can be viewed through physical parameters (Table 1). They maintain that the physical size and level of protection within the organo-mineral soil matrix affects the turnover rate of SOM.

SOM pool	**Average turnover time (years)**
Live plant biomass	0.25
Plant residues	0.25
Unprotected organic matter (microbial biomass)	0.25
Labile (>63µ)	0.2-0.5
Protected organic matter (structural protection)	10-?
Colloidal Protection	10-?

Table 1: Framework of physical SOM fractionation and turnover rates (adapted from Branco de Freitas Maia et al. 2013)

SOM fraction	Density (g cm3) where applicable	Size (µm) where applicable	Turnover time (years)
fPOM			15.4
MALF-1	<1.6		41.9
MALF-2	1.6-1.85		34.4
MALF-3	1.85-2.22		37
DF	>2.22		38.9
fPOM			6
oPOM	<2.22		28.8
DF	>2.22		35.6
fPOM			1
MALF-1	<1.6		18.1
MALF-2	1.6-1.85		20.6
MALF-2	1.85-2.22		23.5
DF	>2.22		26.3
fPOM	<1.6		22
oPOM	<1.6		83
oPOM	1.6-2		49
DF	>2		63
POM	<2	>2000	4
POM	<2	200-2000	6
POM	<2	50-200	7
fPOM	<1.6	<2000	19
oPOM	<1.6	<2000	40
1.6-1.8		<2000	24
1.8-2		<2000	24
>2		<2000	41
fPOM	<1.6	<2000	27
oPOM	<1.6	<2000	49
1.6-1.8		<2000	53
1.8-2		<2000	41
>2		<2000	73
fPOM litter	<2.1	<2000	2-5
fPOM roots	<2.1	<2000	5-10
<2.1		<2000	40-100
>2.1		<2000	>100

Table 2: Framework of physical SOM fractionation and turnover rates using size and density parameters (adapted from von Lutzow et al. 2017). POM - (particulate organic matter) isolated by particle size fractionation alone or by a combination of particle size and density fractionation; fPOM - free POM; oPOM - occluded POM; LF - light fraction isolated by density fractionation alone; fLF - free light fraction; oLF - occluded light fraction; DF - dense fraction isolated by density fractionation alone; MALF - (mineral-associated light fraction).

A similar approach involves using multiple fractionation methods to isolate and categorise SOM into a series of groupings based on size, density and level of protection within the organo-mineral soil matrix. This approach is exemplified by Von Lutzow et al (2017) using a collated summary of multiple fractionation studies (Table 2). Von Lutzow et al (2017) also outline the alternative approach of chemical fractionation which can be conducted using numerous approaches; "extraction of SOM in aqueous solutions with and without electrolytes, in organic solvents, [based] on the hydrolysability of SOM with water or acids, the resistance of SOM to oxidation [and] based on destroying the different mineral phases". The challenge created from these divergent approaches to SOM fractionation is that there is no consistent way of reporting on fractionated SOM between studies when different methods are used. Accepting this, the fractionation methods adopted in the current research aimed to create a profile of SOM persistence under a range of extraction types and intensities to identify the prevalence of persistent SOM pools in contrast to more labile pools.

When considering the organic content of agricultural soils, research has found that grasslands accumulate more SOM than arable soils over a similar timeframe (Conant et al. 2001). This can be explained by a combination of reduced tillage, increased root turnover and greater protection of SOM within soil aggregates in grassland systems (Jones & Donnelly 2004). Consequently, it has been argued that converting arable land to permanent grassland or introducing temporary grass leys into arable rotations can increase SOM, which in turn may improve soil functional resilience (Garnett et al. 2017). This second suggestion is advocated in the UK's 25-year environment plan which calls for improved soil management through 'reintroduction of grass leys into arable rotations' (HM Government, 2018).

However, the net benefit of such land use change is debatable; Soussana et al. (2004) found that while the increases in soil organic content occur most rapidly in the early years following the change to a grassland system, they emphasise that such increases can be overshadowed by the subsequent losses resulting from reversion back from grassland to arable. Such concerns underline the need to maximise benefits from incorporating temporary grasslands into arable rotations by adopting management which a) increases SOM accumulation, b) considers the potential for recalcitrant SOM to persist after the reversion to arable management, and c) considers the dynamics in sub-soils below the plough line and main rooting area which may present greater stability following reversion to arable management.

Increasing SOM accumulation

In addressing the first of these points, *increasing SOM accumulation*, various approaches regularly practiced in some arable and grassland systems are documented within the literature. Approaches include minimising soil disruption through no-tillage and reduced-tillage systems, application of biochar as a soil amendment to increase long term soil carbon, and increasing the quantity of plant biota within the system through cover cropping or inter-cropping alongside or between main crops, or through the selection of vigorous species with dense rooting systems (FAO 2005). A less explored approach to boosting SOM in grassland systems is to increase the plant-species diversity of the sward.

The importance of biodiversity in environmental systems is widely accepted, although the evidence base linking biodiversity with human benefits is still unclear (Ricketts et al. 2016). The Convention on Biological Diversity's (CBD) 2020 strategy highlights how global economies and poor communities rely on biological resource diversity for medical and economic advancements and for climate change mitigation (CBD 2014). In the context of agriculture, the European Commission (EC) identifies multiple benefits of biodiversity including 'improved pollination, natural pest control, nutrient cycling, soil and water conservation and, as a consequence, a decreased demand for external inputs' (EC 2010). Substantial research has focussed on ecosystem benefits associated with plant-species diversity, with one study arguing that diversity provides 'a partial to complete substitute for many costly agricultural inputs, such as fertilizers, pesticides, imported pollinators and irrigation' (Isbell et al. 2017).

However, only limited research has focussed on the effects of grassland plant-species diversity on SOM accumulation. Two long-term grassland experiments have been conducted (The Jena Experiment

in Germany and Cedar Creek in Minnesota, USA) which investigated the effects of plant species diversity on productivity and ecosystem function. Publications have emerged from both experiments which argue that increased plant species diversity accelerates the accumulation of soil organic carbon (SOC) (Steinbeiss et al. 2008, Tilman et al. 2006). However, both studies consider SOC in the context of permanent or long-term grasslands as opposed to temporary leys within arable rotations. Grassland soil dynamics are subject to greater fluctuation in the early years following establishment, primarily due to the gradual rate at which soil structure develops, the sharp increase in root biomass (RB) following conversion and changes in established microbial communities (Soussana et al. 2004, Garnett et al. 2017). Therefore, extrapolating results from permanent grassland experiments and applying them to temporary grasslands is likely to lead to erroneous conclusions. Furthermore, the Cedar Creek site used establishment techniques which are inconsistent with typical UK farming practices (greater than typical physical disruption, burning and seedling transplantation), as such the applicability of their conclusions to a UK temporary grassland context is questionable.

It should be noted that the measurement of SOC in these two experiments refers specifically to the carbon atoms contained within organic matter, while SOM comprises a range of carbonaceous molecules into which other components such as hydrogen, oxygen, nitrogen and phosphorus may also be incorporated. Research in this field often attributes the benefits of soil organic content specifically to the SOC component rather than SOM in general (Milne 2015). The SOM content of agricultural soils typically ranges from trace amounts to around 30% (Bot and Benites 2005), while the typical ratio of SOC within SOM can vary and has been a subject of research since the early 1800's (Pribyl 2010). While it is still a matter for debate, the van Bemmelen conversion factor of 0.58 is widely used as an estimate of SOC within SOM (i.e. SOM is comprised of approximately 58% SOC). While Pribyl (2010) argues that 58% is an over-estimate and suggests that 50% is more accurate, Heaton et al. (2016) found a higher rate of 66% SOC. Clearly the proportions of SOC within SOM will fluctuate based on soil type, historic management and time of sampling and as such any standardised factor should be treated only as an estimate. However, this introduces another consideration, that while soil may be comprised of a measurable proportion of carbon atoms, these atoms are contained in a range of organic molecules with different characteristics and behaviours.

The persistence of SOM fractions

T*he persistence of SOM fractions* is also important when considering the benefits of temporary grasslands. SOM is a heterogenous collection of organic compounds which demonstrate highly variable turnover rates. These diverse organic compounds can be loosely categorised into persistent and labile pools which are respectively less/more persistent within soils when physical, chemical and biological factors are acting upon them (Six et al. 2002). Determining the persistence of SOM is particularly pertinent in the context of temporary grasslands because as Soussana et al. (2004) highlight, much of the labile, or readily reacting, organic content accumulated under grassland systems can be lost when land is converted back to arable. As such establishing profiles of SOM persistence may help determine the long-term benefits of such practices.

If it is inappropriate to treat all SOM as one homogenous compound, it is also important that SOM metabolism should not be considered in isolation but within a matrix of soil processes. The importance of the physical and chemical condition of soil has long been appreciated, however in more recent years, greater attention has been directed towards the biological condition of soil, principally the dynamics between soil chemical and physical properties and its microbial communities (Bonfante et al. 2019). Microorganisms affect various changes within the soil environment; many cycle nutrients, mineralising organic matter within the soil matrix and making it available to plant roots; nitrogen-fixing bacteria convert atmospheric nitrogen into ammonia thereby increasing soil fertility; while some microorganisms have also been described as bioengineers due to their ability to alter soil structure and pore spaces (Helliwell et al. 2014). Microbial population expansion also increases the volume of organic biomass within the soil, however one contribution which could receive greater attention is the

microbial contribution of extracellular organic substances as a significant source of persistent SOM. Two key compounds of interest are Glomalin and extracellular polymeric substances (EPS).

Most microorganisms do not exist as pure cultures but instead aggregate within biofilms, of which the microorganisms typically comprise just 10% of the overall assemblage. The biofilm matrix, which accounts for the remaining 90%, is comprised of a collection of EPS produced by the microorganisms (Flemming & Wingender 2010). Biofilms have been recognised for their highly persistent nature in response to physical and biological eradication techniques (Aggarwal et al. 2015). For over four decades EPS and biofilms have been the subject of research by various disciplines, including waste-water treatment, medicine and bioenergy (Karunakaran et al. 2011), however the role of EPS within soils has received only limited attention (Redmile-Gordon et al. 2014). Glomalin is a glycoprotein discovered by Wright & Upadhyaya (1998) and is thought to be associated with arbuscular mycorrhizal fungi (AMF). Subsequent research into Glomalin – or rather Glomalin related soil protein (GRSP) – has identified its persistent nature with lengthy turnover rates (Rillig et al. 2001, Zhang et al. 2017). In the context of the Branco de Freitas Maia et al. (2013) framework of SOM fractions (Table 1) these fractions would sit within the *colloidal protected* category as they adhere tightly to surrounding soil and plant materials forming tight bonds which are thought to contribute to soil stability (Rillig 2004).

A better understanding of how these two persistent SOM fractions accumulate and whether this is influenced by plant species composition could inform management approaches which seek to maximise the benefits of incorporating temporary grasslands into arable rotations. Previous research suggests a correlation between plant species diversity and soil microbial biomass (Zak et al. 2003), while another study linked plant species diversity with the belowground diversity of soil microbial communities (Wang et al. 2017). An increase in both the population size and diversity of microbial communities could be expected to lead to an increase in the production of microbial-derived substances such as Glomalin and EPS. There is some evidence in support of this suggestion from a study which found lower levels of AMF activity and lower quantities of Glomalin in monoculture grassland plots than were apparent in plots with greater plant diversity (Burrows 2013).

Deep soil organic content

Until recent years, research into the organic content of soils has predominately focussed on top soils, including the main rooting zone, which represents the most biologically active soil horizon (Baveye et al. 2016). However, SOC dynamics in deeper agricultural soils must be explored when seeking to maximise the full benefits of incorporating temporary grasslands into arable rotations. These deeper soil layers are less effected by disruption from ploughing, sustain a smaller proportion of RB and microbial activity, but are more likely to experience deposition of material through leaching. Although SOC typically reduces at increasing depth 'most subsoil horizons contribute to more than half of the total SOC stocks' (Rumpel & Kögel-Knabner 2011). Consequently, following the growing global commitment to remove CO_2 from the atmosphere by sequestering carbon in soils – which represent the largest single store of terrestrial carbon (Batjes 1996) – as well as an interest in accessing payments for ecosystem services; there has been a renewed effort to understanding SOC dynamics in deeper soils (Rumpel 2014).

This has prompted one school of thought which seeks to explore the potential for deep-rooting plant species to improve deep soil conditions and increase deep-deposits of SOC (Kell 2011). *Festulolium* spp. are one group of grasses with such an interest. These species are deep-rooting hybrids of *Festuca* spp and *Lolium* spp which are thought to combine the high yielding qualities and tolerance to drought and cold demonstrated by *Festuca* spp, with the *Lolium* spp's tendency to establish quickly and be readily digestible with a high sugar content (DLF, undated). Several studies which have explored the potential for Festulolium to sequester carbon in deep soil horizons have produced compelling results (MacLeod et al. 2013, Li 2017).

However, there are concerns that SOC in deep soils, which normally displays a residence time of hundreds of years (and as such can be considered highly persistent), could be disrupted and lost through the introduction of labile carbon in the form of fresh plant materials (Fontaine et al. 2007, Rumpel & Kögel-Knabner 2011). Such conclusions should prompt a cautious approach towards introducing plant species with dense and deep-rooting characteristics to increase organic content in deep soils. Certainly, new research which quantifies the variability of carbon profiles at increasing soil depth could help establish an understanding of the opportunities and risks associated with deep-rooting plants. Furthermore, as Rumpel & Kögel-Knabner (2011) suggest that the high residence SOC in deep soils is 'enriched in microbial-derived C compounds', it would be helpful to quantify the relative proportions of GRSP and EPS between soil horizons. This would improve understanding of how soil depth effects the dynamics of microbial-derived SOM.

To better understand SOC dynamics and retention, further research should be directed towards improving understanding on several key topics; A) whether plant species diversity plays an influential role in the accumulation of SOM in temporary grasslands, B) whether microbial-derived pools of reportedly-persistent SOM contribute a significant proportion of the overall SOM profile, C) whether these microbial-derived pools present any indicative associations with specific grassland species, functional groups or species/functional group compositions, D) whether the prevalence of such pools are influenced by soil depth, and E) whether SOM dynamics are differentially influenced by contrasting rooting systems (e.g. deep/shallow, tap-rooting/fibrous).

Examining these topics

To better understand the importance of both plant species diversity and species selection (based on rooting characteristics) for grassland soils, in particular the impact of these choices on SOM, two projects were designed.

The first project was a field sampling exercise; this was a single component of an established field experiment which had been designed to investigate the impact of various grass species (including multiple *Festulolium* spp. hybrids) on different aspects of soil condition. As Festulolium is a deep-rooting grass one goal was to examine soil condition below a typical sampling zone. The remit of the current research as part of the existing field experiment involved extracting a series of soil cores to a depth of 60cm to allow measurement and comparison of SOM proportions at a range of depths. This was intended to be a baseline sampling exercise which would be repeated at each sampling point annually to show change over time, however a revision in the scope of the current research resulted in only a single instance of sampling.

A glasshouse experiment was also performed (utilising methods and tools developed in the Festulolium trials) which examined how increased plant diversity affected SOM under controlled conditions. This experiment also explored changes in SOM with soil depth. A selection of six herbaceous plant species was made from those commonly found in UK agricultural grasslands. These six species were also chosen from three functional groups based on rooting characteristics (shallow-rooting, tap-rooting and dense/deep tap-rooting) to establish whether results were species/functional group dependent. All species were grown under two diversity treatments; monocultures and with Perennial Ryegrass (*Lolium perenne*).

2. Field sampling

2.1 Aims

This field sampling exercise was a single component of an established field experiment which had been designed to investigate the impact of various grass species (including multiple *Festulolium* spp. hybrids) on different soil characteristics. As Festulolium is a deep-rooting grass, one aim of the current research as part of the existing field experiment involved was to examine soil characteristics below a typical sampling zone, down to 60cm; thereby enabling measurement and comparison of SOM and its component fractions at a range of depths. This would allow analysis of the proportion of SOM stored below the typical plough line (which is therefore less susceptible to disturbance through ploughing) as well as whether SOM in deeper soil was composed of a greater proportion of purportedly persistent SOM fractions. As such the exercise provided the opportunity to test a range of SOM fractionation methods identified from the literature.

It was intended that the first instance of sampling would provide a baseline of soil characteristics and that changes over time would be measured through repeated annual sampling at each sampling point. However, a revision in the scope of the current research resulted in only a single instance of sampling.

2.2 Methodology

Experiment setup

The field site was established on heavy clay soil in Leicestershire (coordinates: 52°38'46.0"N 0°53'03.5"W) (Figure 1). Following approximately 25 years under arable management through a cereal crop rotation, the site was converted to grassland in spring 2016 since when it has been under treatment and managed by mowing. The site is oblong with dimensions 250m (Northwest to Southeast) by 150m (Northeast to Southwest). The site was sown with six grass-species treatments in 8m-wide strips along the shorter side of the field (Northeast to Southwest). From the Northerly top corner, the site descends steadily in a Southwest direction and steeply but with undulations to the Southeast. Therefore, sampling points reflect a range of elevations. Sampling occurred on 27 March 2018; on the day the weather was cold, heavily overcast but dry, following several weeks of cold, wet weather; hence the ground was wet and showed evidence of surface puddling.

Figure 1: Map of field site sampling location

Each treatment was sown as a 50:50 combination of individual test treatments and a Perennial Ryegrass (*Lolium perenne*) White Clover (*Trifolium repens*) mix, therefore Ryegrass and White Clover were present across all treatments. The six treatments included four Festulolium test varieties, Cocksfoot (*Dactylis glomerate* var. d*onata*) as a comparator and a Ryegrass/Clover control. Each treatment was sown with three replicates structured in blocks of six.

To maximise time and resources, sampling was planned to include all replicates from four of the six treatments; Cocksfoot, the control and two Festulolium varieties. The two Festulolium varieties were selected based on their contrasting hybrid profiles; var. *lofa* comprising predominantly Lolium genetics, and var. *Fojtan* comprising predominantly Festuca genetics (DLF, undated). These varieties were expected to present contrasting growth characteristics.

Hollow steel tubes (length 1m) were hand-hammered into the ground to collect soil cores to a depth of up to 60cm. However, because the site's heavy clay soil presented high penetration resistance, the corers could not all be inserted to this depth. Penetration depths (PD) and lengths of soil core (CL) inside the corers (prior to removal from the ground) were measured to calculate soil compaction.

Soil compaction was calculated:

$$CL \div PD$$

Four cores were extracted from each treatment strip at standardised points along the length of the site (70m, 120m, 170m, 220m from the Northwest edge) (Figure 2).

Figure 2: Map of treatment strips and sampling points. Ryegrass/Clover control (blue diagonal stripe), Festulolium var. Aberniche (red verticle stripe), Cocksfoot (blue vertical stripe), Festulolium var. Perseus (red chequered), Festulolium var. Fojtan (blue chequered), Festulolium var. Lofa (red diagonal stripe), sampling points successfully sampled (yellow circle), planned sampling points not sampled (black circle).

An impact-cap constructed from heavy-duty plastic was designed to fit on top of corers to disperse the impact and prevent deforming from hammer blows. Unfortunately, despite earlier testing the plastic construction was not sufficiently robust. The impact-cap fractured during sampling and was replaced with hand-held wooden blocks. Following sampling a new impact-cap was designed for future sampling exercises. The modified impact-cap used a steel construction which was rigorously tested and was found to be effective and sufficiently robust to withstand the force required to repeatedly penetrate corers to 60cm.

After cores were extracted from one complete block of four treatments (at the lowest Southwest elevation) the decision was made to halt sampling after one further strip was sampled (at the highest Northeast elevation) as a comparator. Sampling was halted when it was observed that CL may not reflect the associated soil depth (even after accounting for substantial compaction rates). This raised concerns that corers were becoming capped by dense soil, preventing the collection of more soil despite further penetration. The decision was made to review data produced from the collected samples and revisit the strategy before further sampling. These concerns were examined through analysis of data obtained during core processing and the findings are summarised in Appendix A.

Core processing

In the laboratory soil cores were extracted using a modified car jack exerting steady pressure to allow non-destructive soil removal of complete soil cores. Length and weight of each complete soil core was measured, and this was used in combination with compaction data recorded during sampling to assess whether soil capping had occurred. However, due to concerns that a delay in subsequent soil analysis could lead to labile carbon losses (Zakharova et al. 2014), it was determined that further soil processing should be conducted as rapidly as possible, hence the complete-core data analysis was conducted afterwards.

Each complete core was cut into three segments equal in length, however as complete soil cores ranged in length, these segments also ranged in length. Interpretation of the preliminary complete-core data (Appendix A) could have informed the lengths at which soil cores were segmented, and future research should consider the benefits of using this data to produce segments which reflect predetermined penetration depths. However, the concerns that delayed processing could result in labile carbon losses justified the decision to proceed.

A visual observation of segmented soil cores (hereafter referred to as soil segments) revealed that plant roots appeared to be absent below the surface 2cm. This assessment was confirmed by breaking a subset of surface soil segments by hand to make more detailed observations. Therefore, it was easy to ensure that root material wasn't included in subsequent soil analysis by removing the top 2cm. Soil segments were stored in plastic bags at 4°C until processed.

Laboratory analysis

Bulk density and water content

A 2cm length disc was cut from the mid-point of each soil segment (Figure 3). Because the measurements of soil depth used in the analysis of the field sampling exercise are estimates extrapolated from the soil compaction analysis (Appendix A), the exact depths from which bulk density discs were taken can only be approximated. As detailed in the results, soil segments were allocated within a standardised framework of depth sections (0-15cm, 15-30cm, 30-45cm, 45-60cm) to facilitate analysis of variance between samples. Because bulk density discs were consistently taken from the mid-point of each soil segment, it can be assumed that the bulk density discs are likely to represent the approximate mid-point of the depth section. Conversely, when correlating variables against depth, the measurement used for depth is the deepest point of the soil segment extrapolated from the soil compaction analysis. Therefore, because bulk density discs were consistently taken from the mid-point of soil segments it is likely that they represent a depth slightly shallower than the sample depth quoted in analysis of correlations. However, because bulk density discs were consistently taken from

the mid-point of soil segments, this overstatement would be consistent across samples and should therefore not affect the relative significance of variables correlated against depth.

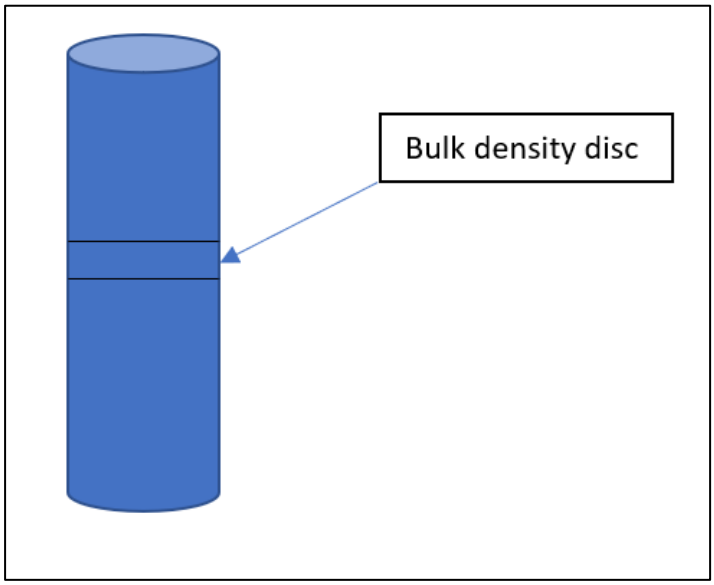

Figure 3: Depiction of the location that bulk density discs were extracted from in each soil segment

Bulk density discs were oven-dried at 70°C for 24 hours and weighed. From the weight difference between the fresh weight (SSegWet) and dry weight (SSegDry) of the dried soil disk and the volume of the soil disc (Vol) bulk density and water content were calculated.

Water content (the weight of water divided by the total weight of the soil sample) (%):

$$\left((SSegWet - SSegDry) \div SSegWet\right) \times 100$$

Bulk density (g/cm^3):

$$SSegDry \div Vol$$

pH

pH was measured by combining 3g of fresh soil with 30ml 1 M potassium chloride (Fischer Chemical, Loughborough, UK. 10684732) using deionised water. This solution was shaken for 90 minutes before reading with a pH probe (Jemway, Cambridge, UK).

Sub-sampling of pH slurry for Dissolved organic matter (DOM) and Organic acids

After allowing the pH solution to settle for one hour, 15ml of supernatant was extracted. No additional filtering took place at this stage. This decision was rectified in the subsequent glasshouse experiment, however the omission of a subsequent filtering stage here may have caused overly high measurements of DOM within field samples. 5ml of the solution was transferred to a plastic tube and stored at 4°C for subsequent organic acids analysis. The remaining 10ml was transferred to a crucible to measure dissolved organic matter (DOM).

DOM

The DOM solution was oven-dried at 70°C for 24 hours to evaporate water, leaving potassium chloride salt residue and DOM. Crucibles containing samples were weighed and then heated at 550°C for 3 hours in a furnace to ignite the organic matter. Crucibles were weighed again to determine weight of lost organic matter through loss on ignition (LoI). Crucibles containing blanks (10ml potassium chloride solution only) were also included to determine any potassium chloride weight loss from salt residue when heated at 550°C. In this write-up DOM is reported as a percentage of total soil weight.

Pyrolysable SOM and the LoI method

Variation in LoI methods was evident from a review of the literature, primarily concerning temperature range and heating duration. Various experiments have measured mass loss over increasing timescales, finding that while additional losses are recorded with greater exposure, most losses occur within the first 2-3 hours (Heiri et al. 2001, Hoogsteen et al. 2015). Regarding temperature, while not applied universally it is often argued that LoI at 550°C removes all organic matter from a sample (Heiri et al. 2001, Hoogsteen et al. 2015). While small losses of inorganic minerals can occur at a temperature of ≤550°C (Plante et al. 2009) these are small, and most of such losses typically occur at much higher temperatures (Heiri et al. 2001, Hoogsteen et al. 2015).

Considering these conclusions, a three-hour heat treatment at 550°C was chosen as the final temperature for the current experiment. However, using differential thermogravimetry, Rovira & Vallejo (2000) identified three distinct peaks of mass loss at temperatures ≤550°C. The first was attributed in part to remaining water loss between 100-200°C, the second to carbohydrates, lipids, and humic substances between 250-300°C, while the third, between 400-500°C was attributed to 'aromatic substances such as lignin, polyphenols, and the inner nucleus of humic substances'. Therefore, to measure pyrolysable soil organic matter (SOM), the oven-dried bulk density disc was homogenised using a pestle and mortar and 3g transferred to a crucible. The crucible was weighed and heated in a furnace for three consecutive three-hour cycles (at 200°C, 325°C and 550°C) and weighed again after each cycle. This stepwise process allowed the LoI to be measured at each stage. In this write-up SOM fractions are reported as a percentage of total soil weight.

This method was tested prior to use by processing a series of substances using the method described above to assess its consistency and effectiveness. Substances were chosen which reflected a range of expected persistence, each replicated five times. The results showed that the three least persistent substances; D-galacturonic acid monohydrate (Alfa Aesar, Heysham, UK. J66282.06), sucrose (Fischer Chemical, Loughborough, UK. SCD2101) and D-xylose (Sigma, Gillingham, UK. W360620), were all completely removed once crucibles were heated to 550°C. Notably, almost half of the D-galacturonic acid monohydrate was removed after the initial heating at 200°C, suggesting this substance represented the least persistent of the five. In contrast lignin (Sigma, Gillingham, UK. 471003) and humic acid (Sigma, Gillingham, UK. H16752) were far more persistent with approximately half of their masses remaining even after the 550°C heating (Figure 4).

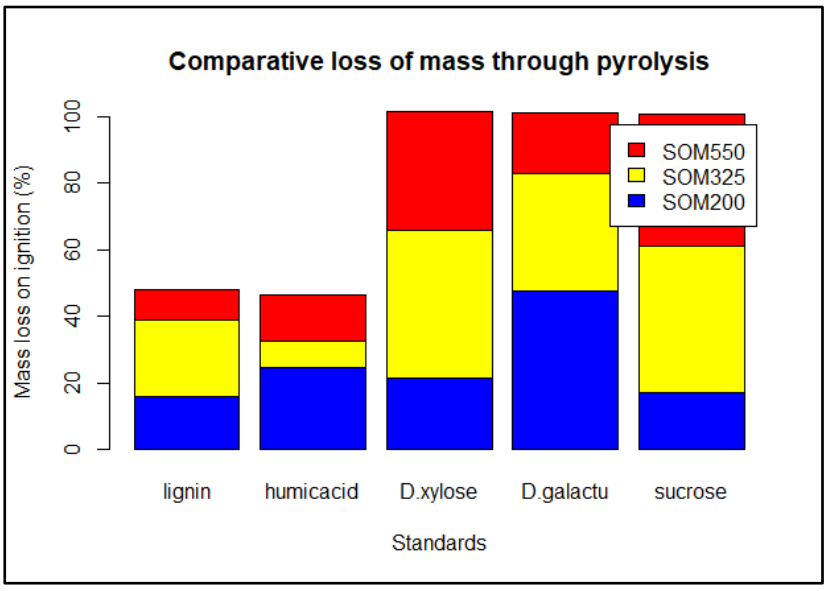

Figure 4: Comparative loss of mass through pyrolysis. D-galacturonic acid monohydrate, D-xylose and sucrose were all completely removed following a 550°C heat treatment, however only approximately 50% of the lignin and humic acid were removed by this pyrolysis treatment.

The standard deviation between replicates for each substance at each heating stage was <0.05 in all but two instances where it rose to <0.15. This low level of deviation confirmed that the pyrolysis method provided consistent values across replicated samples. It also successfully identified substances which were expected to be more/less persistent. The fact that part of the more persistent lignin and humic acid samples remained after the 550°C heating did raise cause to reconsider the selected temperature ranges, however concerns that increasing the final temperature could increase inorganic losses led to the decision to retain the original heating profile. One solution for future research could be to combine a pyrolysis process with a chemical decomposition process as some organic matter may be more susceptible to chemical rather than temperature decomposition, however limited resources precluded this additional stage in the current research.

Organic acids

Organic acids were to be measured using a gas chromatography – flame ionisation detector (HP 5890, Bracknell, UK). A Nukol Column (30m x 0.25mm) was used with Helium as a carrier gas, oven-ramped from 70 to 200°C over 10 minutes. 1ml of the filtered soil solution used to measure pH was transferred to a glass vial and acidified with 7.5µl of orthophosphoric acid. Volatiles within the sample were standardized against Volatile Free Acid Mix (Sigma, Gillingham, UK. CRM46975) . However, due to time restrictions chromatographs were not analysed and data produced from this method were not included in subsequent data analysis.

Glomalin Related Soil Protein

Glomalin Related Soil Protein (GRSP) was measured using a modified Wright & Upadhyaya (1998) method. GRSP was extracted in two stages using an increasingly concentrated citrate solutions (representing increasingly aggressive extraction drivers) to isolate Easily Extractable GRSP (EE-GRSP) and Recalcitrant GRSP (RE-GRSP) respectively. The citrate solution comprised citric acid (Sigma, Gillingham, UK. C0759) and tri-sodium citrate (Fischer Chemical, Loughborough, UK. 10396430) in a ratio of 18:82. Two concentrations of citrate solution were prepared, both using the 18:82 ratio. The first used citric acid and tri-sodium citrate prepared at concentrations of 20 mM, the second at 50 mM. Once citric acid and tri-sodium citrate were combined, 1 M of sodium hydroxide (Sigma, Gillingham, UK. 1064621000) was used to adjust the solutions' pH; to pH 7 for the solution prepared from 20 mM concentrations, and to pH 8 for the solution prepared from 50 mM concentrations. Approximately 1g of fresh soil (homogenised using pestle and mortar) was left at room temperature for 24 hours to air-dry and 0.2g was combined in a 2ml centrifuge tube with 1.6ml of the 20 mM citrate solution. Samples were heated at 121℃ for 30 minutes, centrifuged for five minutes at 10,000g (Heraeus Biofuge Pico, Cambridge, UK) and the supernatant removed to obtain EE-GRSP. Subsequently 1.6ml of 50mM citrate solution was added to the soil pellet which was then heated at 121℃ for one hour, centrifuged for five minutes at 10,000g and the supernatant removed to obtain RE-GRSP.

In contrast to the Wright & Upadhyaya (1998) method the 50 mM extraction was termed Recalcitrant GRSP rather than Total GRSP. It was felt that Total GRSP incorrectly implied an absolute measure of GRSP which not only should include the portion previously extracted using the 20 mM extraction solution, but also GRSP which may require an extraction concentration greater than 50 mM. Whereas, Recalcitrant GRSP more correctly implies the portion of GRSP extractable using the more concentrated of the two extraction solutions employed in the process.

GRSP within extracts was measured using a Bradford Assay with a microplate reader (BMG Labtech Clariostar, Aylesbury, UK), a method used by Burrows (2014). Bradford reagent was prepared by dissolving 100mg Coomassie Brilliant Blue G-250 dye (VWR International, Lutterworth, UK. M140-10G) in 50ml of 95% ethanol (Fischer Chemical, Loughborough, UK. 12897172), followed by the addition of 100ml of 85% phosphoric acid (Alfa Aesar, Heysham, UK. A18067), diluted to 1L using deionised water and filtered before use with 45µ Whatman filter paper. Both sets of extracts were measured by combining 150µl of extract with 150µl of Bradford reagent in a 96-well microplate and analysed with the microplate reader at 595nm. In this write-up GRSP is reported as a percentage of total soil weight.

Two sets of standards were prepared using the 20 mM and 50 mM extraction solutions as matrices with bovine serum albumin (BSA) (Fischer Chemical, Loughborough, UK. 10443834) used as a representative standard protein at concentrations of 1.25, 2.5, 5, 10 and 20 µg/ml. BSA is the standard typically used for GRSP analysis because it is inexpensive, and readily available at high purity.

The Bradford Assay was tested using standards prior to use with samples to confirm its reliable detection range. Standard curves were produced with R^2 values ranging from 0.964-0.99 suggesting the overall method was reliable. The curve for standards prepared in the EE-GRSP solution produced a straight line between 1.25-15 µg/ml, however this linear range ended above 15 µg/ml. Once adjusted to discount the 20 µg/ml concentration standard the R^2 value increased from 0.964 to 0.999. The curve for standards prepared in the RE-GRSP solution showed a similar drop off in the linear portion of the curve above a concentration of 15 µg/ml. Furthermore, it produced a negative value for the lowest concentrated standard (1.25 µg/ml) suggesting this matrix reduced the precision of the microplate reader to detect absorbance at such low concentrations. Once adjusted to discount the 1.25 µg/ml and 20 µg/ml concentration standards a clear linear range emerged between 2.5 and 15 µg/ml and the R^2 value increased from 0.99 to 0.994. Standards prepared in the EE-GRSP matrix showed a standard deviation of just <0.02 for each standard concentration, except for the 15 µg/ml concentration which showed a higher variability of 0.05. Similarly, most of the standard concentrations prepared in the RE-GRSP matrix showed a standard deviation of <0.03, except for the highest concentration (20 µg/ml) which showed a higher variability of 0.05. Therefore, it was determined that at concentrations between 1.25-15 µg/ml repeated measurements were accurate to a precision of <0.05, while RE-GRSP matrix may impair detection accuracy at low concentrations <2.5 µg/ml (Figure 5).

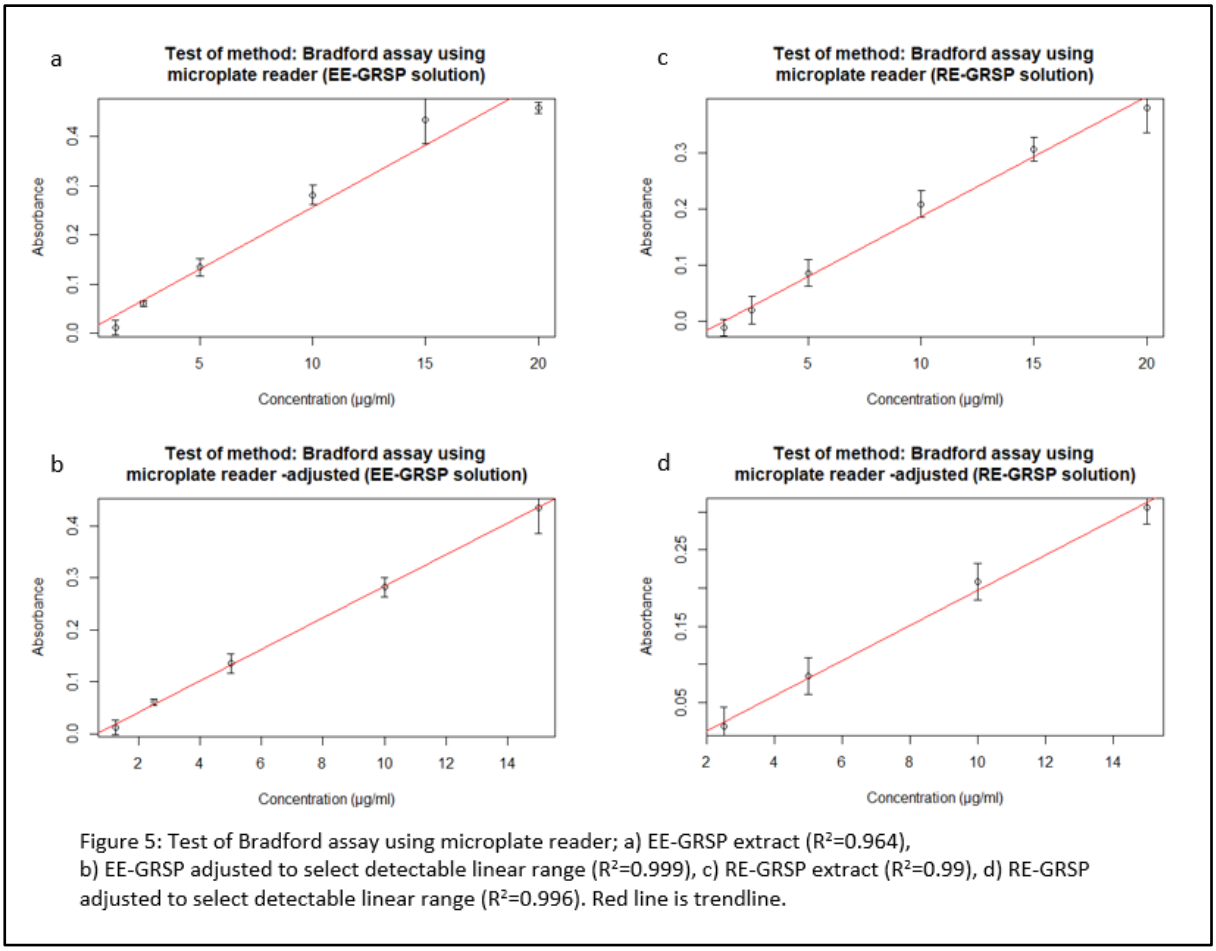

Figure 5: Test of Bradford assay using microplate reader; a) EE-GRSP extract (R^2=0.964), b) EE-GRSP adjusted to select detectable linear range (R^2=0.999), c) RE-GRSP extract (R^2=0.99), d) RE-GRSP adjusted to select detectable linear range (R^2=0.996). Red line is trendline.

Data analysis

Analysis was conducted using a combination of ANOVA and Pearson's product motion correlations.

2.3 Results & Discussion

Samples obtained from field sampling were analysed for variance between a) depth sections, b) species, and c) elevation.

Depth sections

Field sampling was halted when concerns arose that corers were becoming capped. Consequently, while whole cores were separated into soil segments (top, middle and bottom), because of variable corer penetration depths during sampling and because of the uncertainty introduced by the suspected capping phenomenon, comparing top, middle and bottom soil segments between cores may not have provided an accurate comparison of equivalent depths. Therefore, a series of calculations were conducted which provided estimates of the depths represented by each soil segment. These variable segment depths were then allocated within a standardised framework of depth sections (0-15cm, 15-30cm, 30-45cm, 45-60cm) to allow comparison. Where core segments straddled two depth sections (e.g. 10-25cm) they were allocated into the most appropriate depth section based on which depth section most of the core segment fell into (in the 10-25cm example this would be the 15-30cm depth section). However, no significant variance was detected for any measured variable between depth sections. A detailed analysis of the capping phenomenon and an explanation of how core data was assessed and converted to allow estimates of the depth profiles represented by each soil core is provided in Appendix A.

The method described above involved a degree of amelioration to allocate core segments into a standardised framework of depth sections. This may have biased the results by drawing data into prescribed depth groupings established without biological basis. Therefore, an alternative approach was also employed to analyse variables in relation to sampling depth. Using the deepest point of the estimated segment depth, a series of analyses were conducted to assess correlations between variables at differing depths. Again, this may have introduced some bias as it assumed the deepest point was representative of the total segment depth, or rather, it attributed results from the entire segment length to the deepest depth. This may have attributed soil characteristics to a depth lower than should have been the case, however because this process (allocating the lowest depth) was applied consistently to all segments the relatability of the data to other depth segments remained consistent.

Because Festulolium is a deep-rooting grass, one of the aims of the exercise was to explore SOM dynamics in relation to soil depth and to investigate changes below a typical sampling of topsoil. The data showed significant reductions in three of the measured organic matter fractions at increasing depth; SOM200 ($r=-0.55$, d.f.=58, $P<0.001$), SOM325 ($r=-0.64$, d.f.=58, $P<0.001$) and RE-GRSP ($r=-0.32$, d.f.=58, $P<0.01$). SOM550 and EE-GRSP showed no significant pattern in relation to soil depth, while DOM was the only measured organic matter fraction which significantly increased with depth ($r=0.35$, d.f.=58, $P<0.01$) suggesting that in this sampling exercise deeper soils contained more DOM (Figure 6).

Perhaps as interesting as these significant results was the fact that SOM550 was observed consistently throughout the sampled depth horizons. The fact that more labile SOM (SOM200 and SOM325) dominated the upper soil horizons, while deeper soil was dominated by more persistent SOM (SOM550) was consistent with existing research (Cambardella 2005, Rumpel & Kogel-Knabner 2011). The same research has also found that a key driver for the input of organic matter in deep soils is typically through the deposition of DOM through preferential flow pathways (Rumpel & Kogel-Knabner 2011). Our findings also support this assertion, as DOM in the current experiment was a measurement of organic matter obtained from aqueous solution followed by a pyrolysis treatment at 550°C. This is the same temperature used to establish values for the persistent SOM550, underlining a link between these two fractions which is also supported by the significant positive correlation observed between the pair.

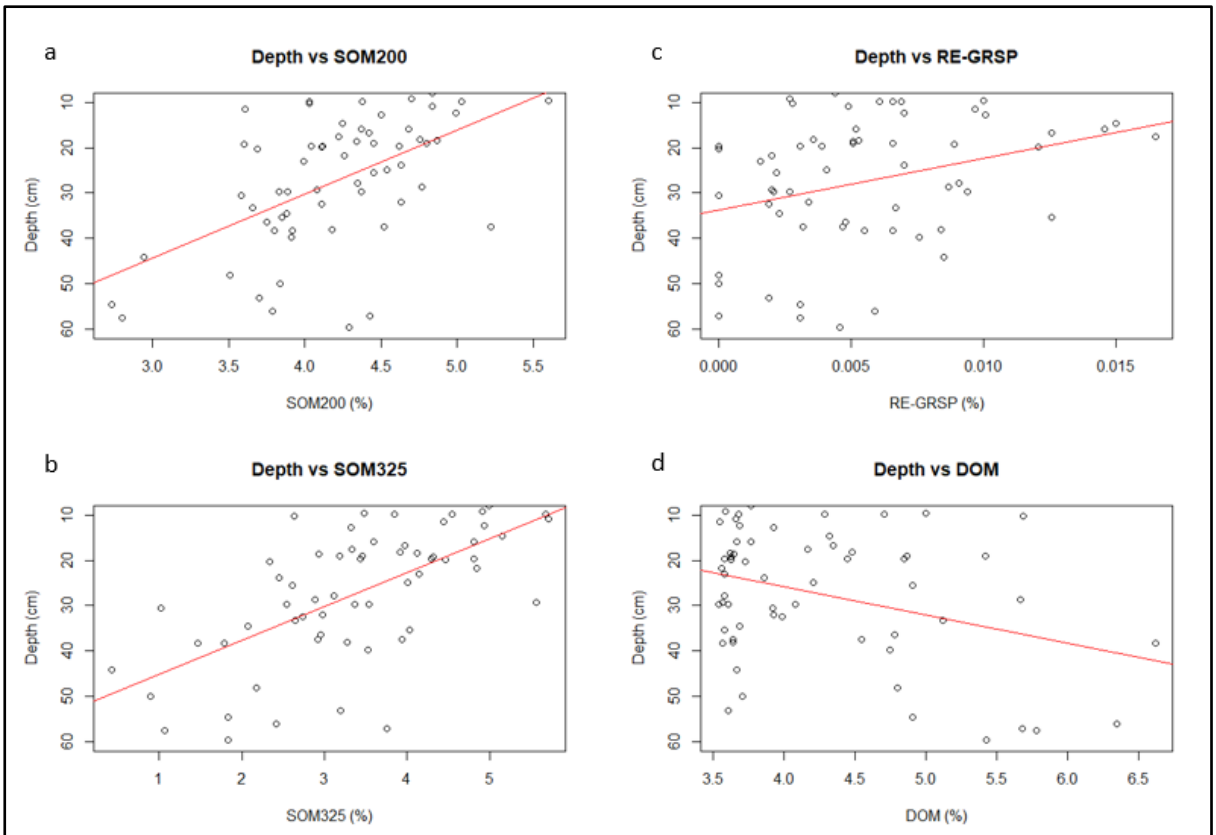

Figure 6: Correlation between depth and a) SOM200 (R^2=-0.55), b) SOM325 (R^2=-0.64), c) RE-GRSP (R^2=-0.32), d) DOM (R^2=-0.35). Red line is trendline.

Depth was also found to correlate positively with bulk density (r=0.33, d.f.=58, *P*<0.01) suggesting an increase in bulk density as depth increased (Figure 7). Conversely, a negative correlation was detected between depth and water content (r=-0.46, d.f.=58, *P*<0.001), suggesting a reduction in moisture at increasing depth (Figure 8).

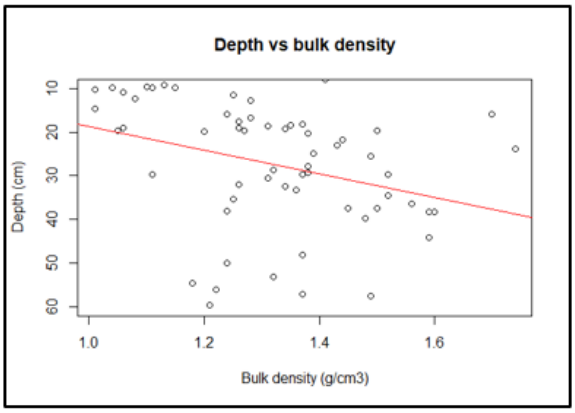

Figure 7: Correlation between depth and bulk density. Red line is trendline. R^2=0.33

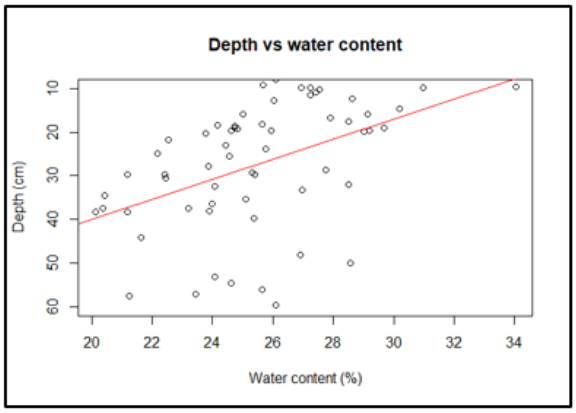

Figure 8: Correlation between depth and water content. Red line is trendline. R^2=-0.46

Having established that variation in the quantities of most measured organic matter fractions was observed between depths, the data was reviewed to establish whether a mechanism for this variation could be identified. The first explanation appeared through multiple positive correlations between measured organic matter fractions and water content, which itself was also found to decrease with soil depth. SOM200 (r=0.4, d.f.=58, *P*<0.01), SOM325 (r=0.37, d.f.=58, *P*<0.01), EE-GRSP (r=0.62, d.f.=58, *P*<0.001) and RE-GRSP (r=0.39, d.f.=58, *P*<0.01) (Figure 9) were all significantly greater within increasingly moist soil, and the same pattern was also observed for SOM550 but was only approaching significant.

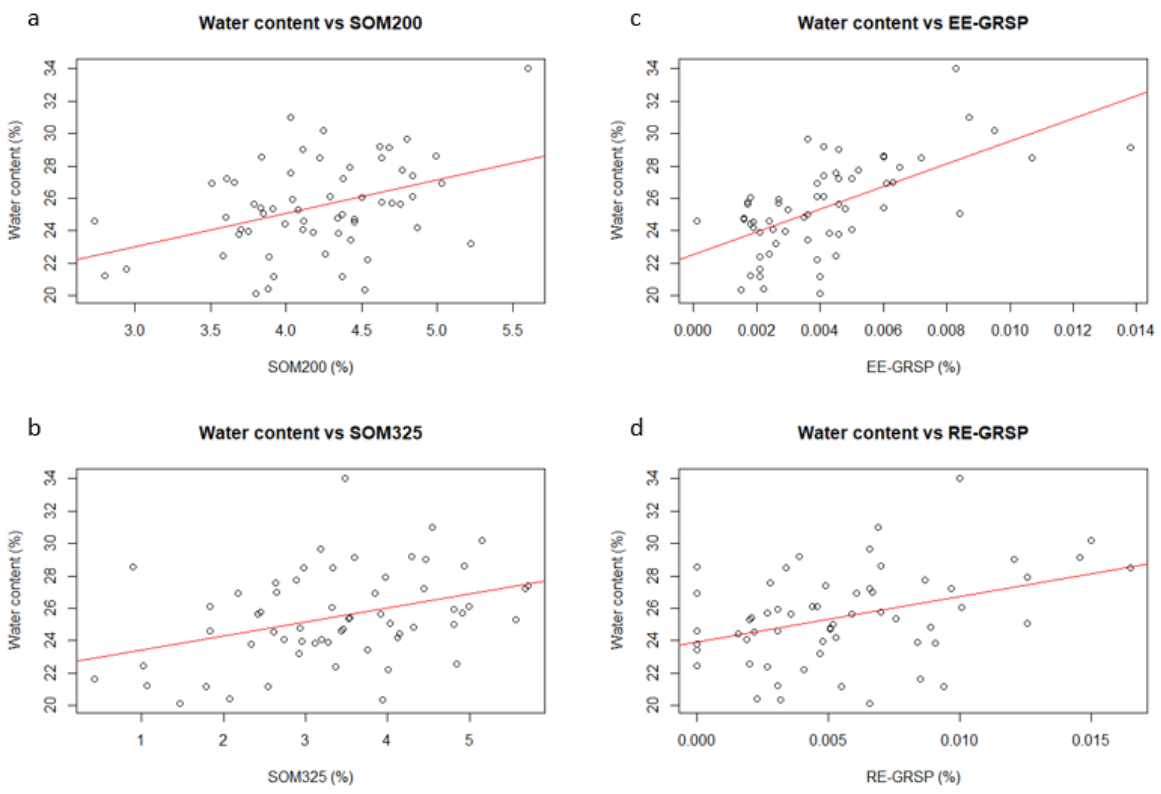

Figure 9: Correlation between water content and a) SOM200 (R^2=0.4), b) SOM325 (R^2=0.37), c) EE-GRSP (R^2=0.62), d) RE-GRSP (R^2=0.39). Red line is trendline.

An established body of research exists which suggests increased SOC can enhance the water holding capacity of soil (Rawls et al. 2003), although some debate exists around this topic (Hudson 1994). However, it is also suggested that the reverse is true, and that soil moisture can increase microbial activity and soil inputs which in turn can lead to an increase in SOM (Tulina et al. 2009) although this assertion is also disputed (Huang & Hall 2017). Microorganisms clearly play an important role in breaking down fresh organic material into more stable soil-associated organic matter (Williams & Plante 2018, Kallenbach et al. 2016). This is not a one-way process, in resource-depleted systems microorganisms can mineralise available SOM leading to a net deficit. However, in non-resource-depleted systems one may expect microbial communities to translate fresh organic inputs resulting from plant growth into SOM. Previous research suggests that microbial activity can be enhanced by soil moisture (Trivedi et al. 2018), although clearly this is only true within limits as excess soil moisture can reduce the activity of aerobic microorganisms when soil pores are saturated preventing a flow of oxygen.

The complexity of these processes which are influenced by changes in environmental conditions may explain why the link between soil moisture and SOM is the subject of debate. In the field system explored in the current research there was a significant link between water content and various measured organic matter fractions. From the available data it cannot be clearly established that increased soil moisture resulted in more organic matter, or that soil samples with more organic matter inevitably increased the water holding capacity of the soil. However, the link between the sets of variables was clear, as was the link between water content and soil depth.

Interestingly DOM was the only measured organic matter fraction which did not appear to be influenced by water content, however soil type and weather conditions prior to sampling may explain this. As stated previously, the sampling site comprised a dense clay soil which had been subjected to heavy rain for several weeks prior to sampling, resulting in surface puddling on parts of the site. This suggested that drainage on this site was poor and the soil may have been at or above field capacity. In these conditions it is possible that once the organic matter which was susceptible to water extraction was transferred into aqueous solution in the field, further percolation into deeper soils may have been impeded or severely slowed, resulting in variable rates of DOM across the sampled soil profile.

A second possible explanation for the general reduction in measured organic matter fractions in deeper soils was the observed increase in bulk density in deeper soil. SOM325 (r=-0.35, d.f.=58, $P<0.01$) and EE-GRSP (r=-0.43, d.f.=58, $P<0.001$) were both significantly negatively correlated with bulk density (Figure 10) and the same pattern was also observed for SOM200 but was only approaching significant. Because bulk density was itself significantly negatively correlated with water content it was difficult to discern which may have been the driver for any mechanism which may have influenced variable rates of measured organic matter fraction. Whether these trends resulted from enhanced moisture levels or decreased soil porosity is open for interpretation, however the physical condition of soil (allowing the circulation of water and/or air) may have had a significant impact on rates of various measured organic matter fractions. It is also possible that an increase in available moisture may have stimulated an increase in SOM through microbial processes references above, but it was also possible that compacted soil pores led to reduced biotic activity in more dense soils (Jordan 2003). It was also possible that neither an increase in bulk density or a decrease in water content was responsible for the general reduction in organic matter in deeper soils, however both present viable explanations and it seems clear that the physical condition of soil has a broad impact on SOM accumulation and turnover.

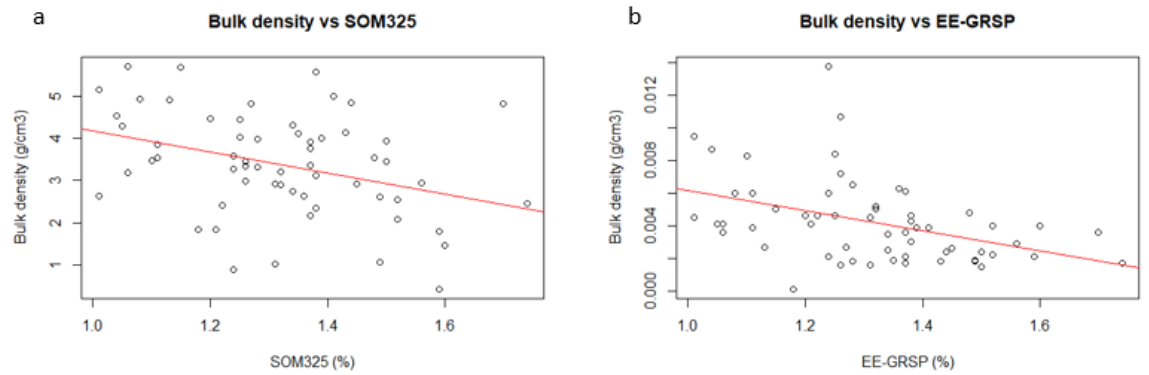

Figure 10: Correlation between bulk density and a) SOM325 (R^2=-0.35), b) EE-GRSP (R^2=-0.43). Red line is trendline.

Perhaps unsurprisingly, significant positive correlations appeared between related soil carbon fractions; EE-GRSP and RE-GRSP (r=0.55, d.f.=58, *P*<0.001); SOM200 and SOM325 (r=0.48, d.f.=58, *P*<0.001); SOM550 and DOM (r=0.29, d.f.=58, *P*<0.05). However, there were significant negative correlations observed between other related fractions, including SOM325 and SOM550 (r=-0.44, d.f.=58, *P*<0.001) and SOM325 and DOM (r=-0.33, d.f.=58, *P*<0.01) (Figure 11). This implies differences between the accumulation and/or metabolic use of both SOM200 and SOM325 in contrast to SOM550 and DOM. Given that SOM550 and DOM were both measures of LoI following pyrolysis at 550°C it could be argued that both included more persistent compounds than were present in SOM200 and SOM325. If correct, these negative correlations (lower rates of persistent fractions when less-persistent fractions are more prevalent) may be an example of soil priming, when microbial metabolism of persistent SOM is prompted by the fresh addition of labile SOM (Bird et al. 2011).

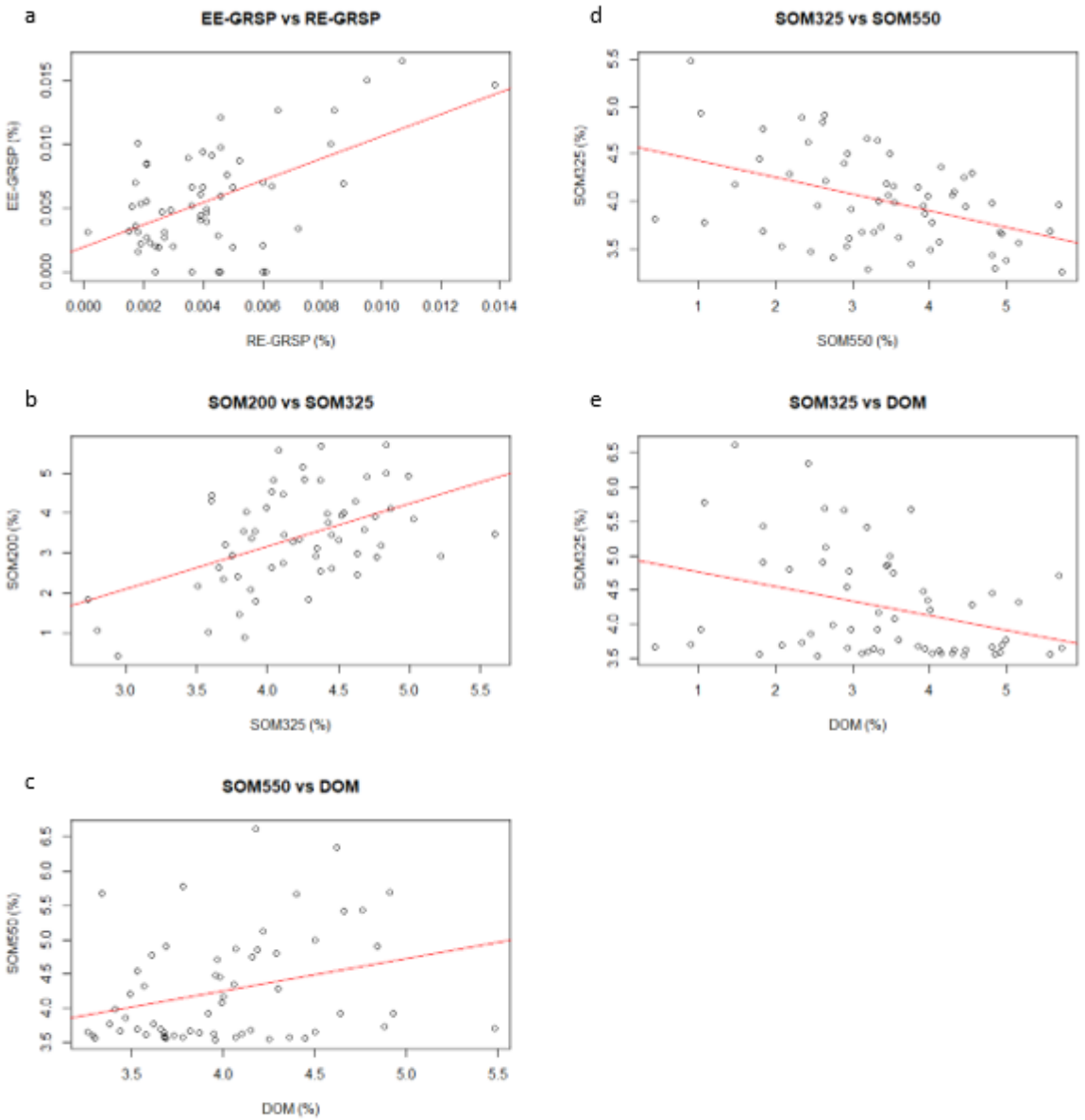

Figure 11: Correlation between organic matter fractions; a) EE-GRSP & RE-GRSP (R^2=0.55), b) SOM200 & SOM325 (R^2=0.48), c) SOM550 & DOM (R^2=0.29), d) SOM325 & SOM550 (R^2=-0.44), e) SOM325 & DOM (R^2=-0.33). Red line is trendline.

However, if this were the case then one may have expected to observe reduced rates of SOM550 in shallower soils where SOM325 was most prevalent in contrast to higher SOM550 in deeper soils were SOM325 was comparatively absent. However, this was not the case as SOM550 was recorded evenly across the measured depth profile. No further explanation was identified for the significant negative correlation which resulted in high values of SOM550 in samples which showed low values for SOM325, however this finding does underline the heterogenous nature and response of different organic matter fractions.

Species

Variance between species treatments was also analysed, however no significance differences were apparent for any measured variable between species. This may indicate that the values of measured organic matter fractions were not influenced significantly differently by any of the selected species, or that the two-year period since plant establishment was not a sufficiently long time for significant variance to present.

Elevation

During sampling, core locations were loosely categorised by their respective elevations within the sampling area (high, intermediate, low). While this factor was only an estimate without definitive measurements to separate cores into statistically defined elevation zones it did provide additional context to interpret the wider dataset. Using these estimates, significant differences in bulk density, water content and EE-GRSP were identified between elevations.

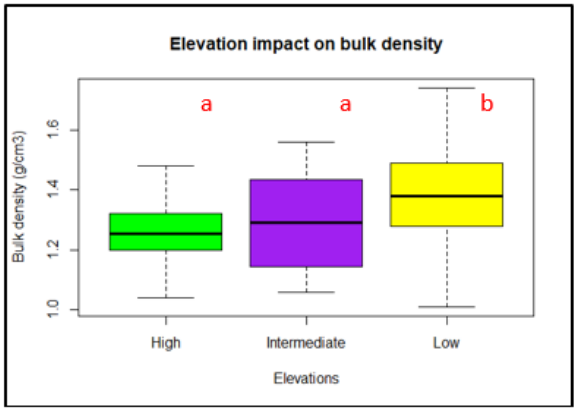

Figure 12: Elevation impact on bulk density. Bold line (median), box hinges (1st and 3rd quartile), whiskers (95% confidence interval). Red a-b denote significant variance confirmed by post-hoc test.

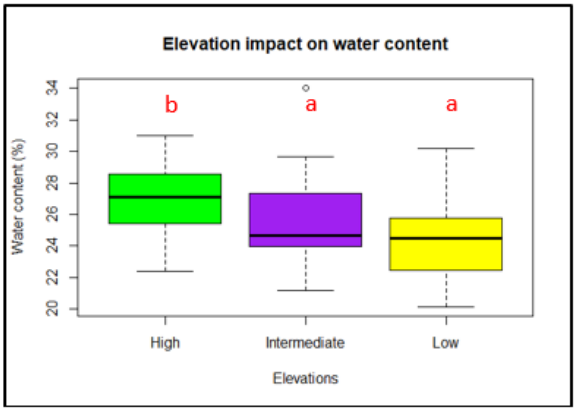

Figure 13: Elevation impact on water content. Bold line (median), box hinges (1st and 3rd quartile), whiskers (95% confidence interval). Red a-b denote significant variance confirmed by post-hoc test.

Significant variance in bulk density was observed between elevations ($F_{2,57}$=3.291, P<0.05), with higher bulk density at low elevations (Figure 12). Analysis also showed that water content varied significantly between elevations ($F_{2,57}$=6.36, P<0.01) with greater water content at high elevations (Figure 13). Given an expectation for precipitation to drain and pool at lower elevations this may appear surprising, however given the increased bulk density identified at low elevations it may be that a greater porosity of soils at high elevations resulted in more successful water retention. These two observations were further supported by the identification of a significant negative correlation between bulk density and water content (r=-0.7, d.f.=58, *P*<0.001) underlining the reduction in soil moisture in denser soils (Figure 14).

The significant variance in EE-GRSP between elevations ($F_{2,57}$=11.58, P<0.001) was shown by a post-hoc Tukey test to be driven by more EE-GRSP at high elevations compared with both low and

intermediate elevations which both showed comparable results (Figure 15). This variance may be explained by the significant correlations between EE-GRSP and bulk density (negative) and water content (positive), noted previously. As EE-GRSP has been identified as a microbial product, higher rates may be expected in somewhat moist and porous soil (Jordan et al. 2003, Trivedi et al. 2018), hence both possible explanations appeared valid.

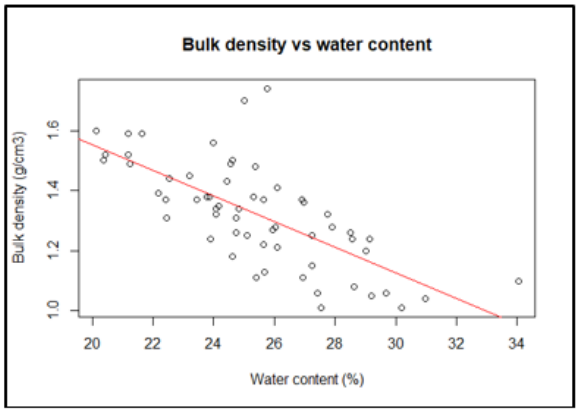

Figure 14: Correlation between bulk density and water content. Red line is trendline. R^2=-0.7

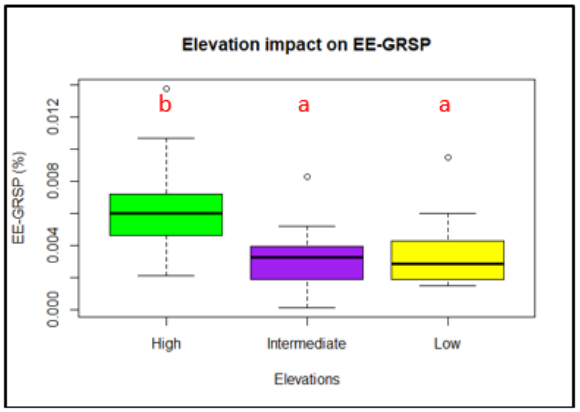

Figure 15: Elevation impact on EE-GRSP. Bold line (median), box hinges (1st and 3rd quartile), whiskers (95% confidence interval). Red a-b denote significant variance confirmed by post-hoc test.

3. Glasshouse experiment

3.1 Aims

The aims of the glasshouse experiment were to improve methods for the fractionation and measurement of SOM within soil samples and to provide evidence to support several hypothesise arising from a review of the literature:

1. Greater plant species diversity was expected to produce an increase in SOM;
In its binary design involving just two diversity treatments and with only one standardised species used for the two-species treatment (*Lolium perenne*), this experiment was designed to establish a method for testing plant diversity impacts on SOM, which could be adopted in further research to explore more complex species compositions. However, higher diversity treatments were expected to produce more SOM than monoculture treatments in line with past research (Steinbeiss et al. 2008, Tilman et al. 2006).

2. Greater SOM production was expected in species or functional groups with more substantial root structures;
Addressing this point would support findings from previous studies (MacLeod et al. 2013, Li 2017, Kell 2011) which suggest that planting species with particularly dense/extensive root systems is a practice which could be advocated to increase SOM.

3. Persistent SOM fractions which are thought to be microbial-derived were expected to occur in greater quantity in; a) higher diversity treatment, and b) species or functional groups with more substantial root structures;
Given that past research suggests that soil microbial communities are in part mediated by the presence of specific plant species (Churchland & Grayston 2014, Burnes et al. 2015) an increase in plant species diversity was expected to increase microbial community size and diversity, in line with the findings of Burrows (2013). Investigating this topic would inform whether certain plant species or species combinations demonstrated strong associations with microbial-derived SOM inputs which could then guide selection/management choices where increasing persistent SOM is an aim.

4. Both SOM and the proportions of composite SOM fractions were expected to vary at different depths;
Following a rising interest in the importance of deep deposits of SOC (Rumpel 2014), focus on this issue would help improve the understanding of the uncertain dynamics of SOM changes at depth.

3.2 Methodology

Experiment setup and plant maintenance

A glasshouse experiment was established in February 2018 at the University of York's Biology Department D-block glasshouse (coordinates: 53°56'49.9"N 1°03'28.8"W). Six herbaceous grassland species were grown in pots and soils were subsequently sampled to detect various SOM fractions. Plant species chosen were Ribwort Plantain (*Plantago lanceolata*), Yarrow (*Achillea millefolium*), Red Clover (*Trifolium pratense*), Birdsfoot Trefoil (*Lotus corniculatus*), Chicory (*Cichorium intybus*) and Dandelion (*Taraxacum officinale*). These species were selected as typical features of UK agricultural grasslands, but also as representatives of a range of functional groups based on their rooting characteristics; shallow-rooting (Clover, Birdsfoot), tap-rooting (Plantain, Yarrow), and dense/deep tap-rooting (Chicory, Dandelion). All species were grown under two treatments; monocultures (mono) containing a single herb specimen per pot, and a two-species treatment (2-spec) sown with

Perennial Ryegrass (*Lolium perenne*) containing a single herb specimen and three Ryegrass specimens per pot. Each diversity/species treatment was sown in replicates of four.

The glasshouse was equipped with light/heat lamps (Philips Master HPI – T Plus 400W/645 E40) positioned immediately above plant benches. These were used as the primary source of light and were supplemented by natural daylight. On the date of planting (22 February 2018) lamps were set to turn on automatically at 06:38 and turn off at 19:39 to mimic 1 April 2018 sunrise/sunset times (a day length of 13 hours, 1 minute). These settings were adjusted automatically each day to mimic the daily changes in sunrise/sunset times from 1 April onwards. Glasshouse heating controls were set to respond automatically to ambient temperature fluctuations only during hours that lights were turned on. Below 18°C heaters turned on automatically, at 20°C vents opened partially, at 23°C vents opened fully, at 24°C a fan turned on, at 27°C a chiller turned on.

All plants were grown in a sand/Terra-green mixture 1:1 (v/v). Terra-Green is a calcined attapulgite clay soil conditioner (Hodge et al. 2001). This homogenous growth media was chosen to provide a consistent SOM baseline. Each pot was surface sown with ten seeds (plus ten Ryegrass seeds in the 2-spec). These were subsequently thinned to three herb plants (and five Ryegrass plants), and finally to one herb plant (and three Ryegrass plants). This ratio of 1:3 herb to grass plants was chosen to reflect the dominance of grass plants in typical agricultural grasslands. Ryegrass (three plants) and non-planted controls were also included.

Except for Chicory and Dandelion, plants were grown in PVC pots (18cm diameter, reducing to 12.5cm at the base, 16cm depth) filled to 1cm below the top. Due to their extensive rooting systems Chicory and Dandelion were grown in deeper pots constructed from PVC ducting pipe (15cm diameter, 100cm depth). All pots were washed and dried prior to planting.

Pots were watered to field capacity prior to sowing. Field capacity was determined using three test pots, to each of which 100ml of water was slowly and repeatedly added until water began draining from the base where it was collected, at which point water additions ceased. Pots were left for 24 hours after which the volume of drained water was measured. The volume of drained water was subtracted from the total water added to determine how much water was still held within the soil. As ambient temperatures within the glasshouse were expected to fluctuated daily due to weather variability (despite the temperature controls in place), requirements for daily watering were also expected to fluctuate throughout the experiment. However, at the outset all pots were weighed to inform the total weight of the combined pot and soil. Using these weights and the calculated weight of water required to achieve field capacity, it was possible to conduct a weekly weighing of each pot to determine whether additional water was required to bring it to field capacity. This insured that while soil water levels may fluctuate, they would always be kept reasonably close to a measured field capacity.

Four weeks after sowing, a weekly application of Hoagland's nutrient solution was added to each pot. This application was 10ml per pot, increasing by 5ml every four weeks up to a final weekly application rate of 50ml to support increasing plant growth.

Harvesting

All plants were sown on 22/02/2019. It was planned that each treatment would be harvested once all pots within the treatment began to flower, hence a staggered harvesting schedule was anticipated. All plants in the short pots (Plantain, Birdsfoot, Clover, Yarrow) mono treatment flowered and Plantain was harvested first (28/05/19), followed by Birdsfoot (12/06/19), Clover (20/06/19), Yarrow and two non-planted control pots (3/07/19). However, despite allowing additional time for slower plant growth, none of the herbs grown in the 2-spec reached flowering

stage. Once it was judged that flowering would not occur during this growing season it was decided that these pots would be harvested prior to flowering. Plantain, Birdsfoot, Clover, Yarrow and the remaining non-planted controls were all harvested together (28/08/19). Similarly, despite healthy plant growth, neither Chicory or Dandelion in either diversity treatment (mono or 2-spec) showed signs of flowering. Additional time was given to allow flowering to occur; however, once it was judged that flowering would not occur during this growing season both diversity treatments of Chicory and Dandelion were harvested along with non-planted controls in the tall pots (24/10/2019). Subsequently, following an additional review of Chicory and Dandelion growth habits, it was identified that both species can occur as biennials. It was therefore theorised that due to the growth of extensive tap roots in this free-draining and unobstructed growth medium, the plants had adopted a biennial growth pattern without a flowering stage in the first year.

At harvesting all shoot biomass was cut at the soil surface, oven-dried at 70°C for 24 hours and weighed to determine dry weight biomass. In the 2-spec pots Ryegrass was separated from the herb and processed separately. The soil was sampled using a purpose-built double-coring device. This device was constructed using two concentric acrylic tubes, one narrow tube (internal diameter 5.4cm) and one wider tube (internal diameter 9.4cm). Each tube was cut width-ways into four 5cm sections and reassembled using masking tape. Both the inner and outer tubes were centred around the cut stem of the herb and inserted 15cm into the soil. Both tubes were then simultaneously removed from the pot and the masking tape removed from each section of the outer tube one at a time and the soil within each section separated into plastic bags. After the outer tube was dismantled the process was repeated for the inner tube. This allowed soil to be collected and separated into three depth horizons (0-5cm, 5-10cm, 10-15cm) at two different radial distances from the position of the herb stem (Figure 16). For Chicory and Dandelion an additional sampling technique was used to sample soil below 15cm. Four PVC tubes (1.8cm internal diameter, 16cm length) were inserted horizontally through pre-drilled and sealed holes at 30cm, 50cm, 70cm, and 90cm below the soil surface (Figure 17). These samples were evacuated and stored in plastic bags. All bagged soil was stored at 4°C until processing.

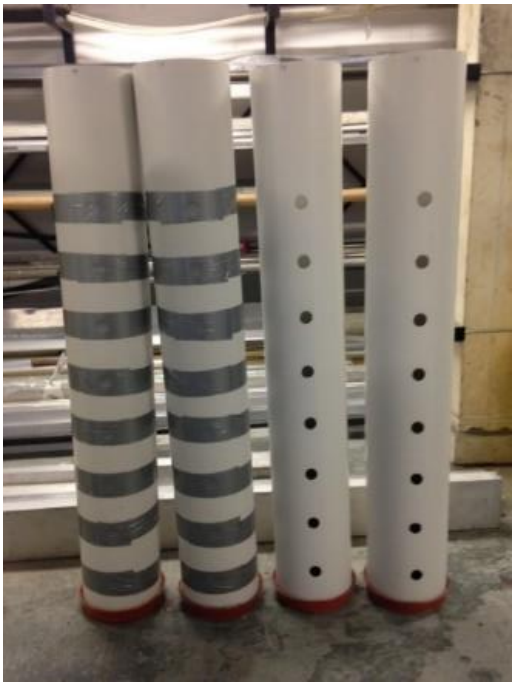

Figure 16 (Left): Double-coring device
Figure 17 (Right): Tall pots with pre-drilled and sealed holes, used for Chicory and Dandelion

Soil sample processing and laboratory analysis

Root biomass, bulk density and water content

Soil samples were weighed to determine the total fresh weight and then sifted using a 2mm sieve to remove plant roots which were collected, oven-dried at 70°C for 24 hours and weighed to determine dry weight RB. The recorded values for RB reflect the root collected from coring tubes, not the entire root stock when part of the root was outside the insertion area of the coring tubes.

A subsample (20% by weight) from each soil sample was placed inside a paper envelope and oven dried at 70°C for 24 hours to determine dry weight. The weight difference between the fresh weights (SubFW) and dry weights (SubDW) of the subsample was used along with a measurement of 20% of the volume of the coring tube segment (Vol) to calculate the bulk density and water content.

Water content (%) was calculated:

$$\big((SubFW - SubDW) \div SubFW\big) \times 100$$

Bulk density (g/cm^3) was calculated:

$$SubDW \div Vol$$

pH

pH was determined using the same method outlined in section 2.2.

DOM

DOM was determined using the same method outlined in section 2.2, however the method was improved by including a filtering stage to ensure no particulate organic matter was introduced when measuring DOM. After the solution used to measure pH was left to settle for one hour it was filtered using a 45µ filter syringe. 5ml was separated and stored in plastic tubes at 4°C for subsequent organic acids analysis. 10ml was transferred to a crucible to measure DOM following the same method outlined in section 2.2.

Organic acids

Organic acids were measured using the same method outlined in section 2.2. However, due to time restrictions chromatographs were not analysed and data produced from this method were not included in subsequent data analysis.

Pyrolysable soil organic matter and GRSP

SOM and GRSP were determined using the same methods outlined in section 2.2.

EPS

EPS was extracted using cation exchange resin (CER) and analysed to quantify protein, humic fractions and total carbohydrates following a slightly adapted method established by Redmile-Gordon et al. (2014).

EPS are primarily comprised of polysaccharides, glycoconjugates, and proteins (Redmille-Gordon et al. 2014) which coalesce into a tightly bound biofilm macrostructure. EPS extraction with CER relies upon 'a combination of shear forces and resin-Na^+ [which] cause ion exchange with multivalent cations that link the EPS' which breaks down the macrostructure (Redmille-Gordon et al. 2104). Alongside EPS within the biofilm are soluble microbial products (SMP) which 'exist as freely soluble extracellular polymers that are not bound to the cells' (Redmille-Gordon et al. 2104). SMP require a less aggressive extraction process 'using a roughly isotonic extractant with salts of equal valence to the target EPS-binding site' (Redmille-Gordon et al. 2104). As such SMP can be considered a more labile component within the overall macrostructure.

Therefore SMP/EPS extraction involved a two-stage process. Firstly, SMP were extracted using a calcium chloride solution, followed by EPS extraction using CER in a sodium-based extraction buffer. Similarly, to GRSP, this obtained two distinct organic fractions, firstly a relatively labile fraction with a low extraction tolerance, and secondly a more persistent fraction with a high extraction tolerance. SMP extraction solution was prepared by adjusting 10 mM calcium chloride (Fischer Chemical, Loughborough, UK. 10171800) to pH 7 using 10 mM calcium hydroxide (Alfa Aesar, Heysham, UK. A12650). EPS extraction solution was prepared by combining 2 mM sodium phosphate dodecahydrate (Alfa Aesar, Heysham, UK. 011590), 4 mM sodium dihydrogen phosphate monohydrate (Alfa Aesar, Heysham, UK. 010870), 9 mM sodium chloride (Fischer Chemical, Loughborough, UK. SCC2802), 1 mM potassium chloride, then adjusting to pH 7 using 1 M hydrochloric acid (Fischer Chemical, Loughborough, UK. SCO1505.

SMP was extracted by combining fresh soil (~2.5g dry weight equivalent (DWE)) with 25ml of the SMP extraction solution described above. Samples were shaken at 4°C for 30 minutes and centrifuged (Hettich Universal 32R, Salford, UK) at 3,200g for 30 minutes. The supernatant was removed for subsequent analysis. EPS was extracted by adding 25ml of EPS extraction solution and a pre-measured quantity (calculation outlined below) of CER (Sigma, Gillingham, UK. 436615) to the remaining soil pellet. This was shaken vigorously by hand to resuspend the pellet and then shaken at 4°C for four hours, centrifuged at 3,200g for 30 minutes, and the supernatant removed for subsequent analysis.

Required CER was calculated as per the original Redmille-Gordon et al. (2014) method and was based on volatile solids in the soil sample as determined previously through the method to quantify SOM.

$$(gSOM \times gDWE\ [soil\ sample\ mass]) \times 70g$$

In the original Redmille-Gordon et al. (2014) method, the remaining pellet was then washed in phosphate-buffered saline (Alfa Aesar, Heysham, UK. J67802.K2), centrifuged and retained to quantify adenosine triphosphate (ATP) as an indicator of cell lysis. However, results from Redmille-Gordon et al. (2014) showed such a small risk of cell lysis that this step was not included in the current research.

Due to the extensive time demands involved in the multiple extraction and analytical processes per sample, a subset of samples was initially chosen. The 0-5cm and 10-15cm samples from the outer coring tube were selected from three of the four replicates of all six herb species within the mono and from the four short pot species within the 2-spec, as well as two replicates of the non-planted control and three replicates of the Ryegrass control. For the tall pot species, the 30cm and 90cm samples were additionally included. These selections were intended to provide the widest range of depth comparisons across a wide range of species and diversity treatments. Radial comparisons were not included because at this stage preliminary analysis of the rest of the data suggested that variance was unlikely to be observed between the inner and out coring tubes.

Analysis of SMP and EPS extracts

Following extraction, the SMP and EPS was analysed to determine quantities of carbohydrate, protein and humic substances within the extracts. A microplate adaptation of the Lowry Assay, as devised by Redmile-Gordon et al. (2013), was used to measure protein and humic substances, while a slightly adapted phenol-sulphuric acid assay (Dubois et al. 1956) was used to measure carbohydartes.

The phenol-sulphuric acid assay was conducted by combining 1ml of SMP or EPS extract with 0.5ml of a weak phenol solution (prepared by combining 95ml of deionised water with 5ml phenol (Sigma, Gillingham, UK. P4557)) and 2.5ml of sulfuric acid (Sigma, Gillingham, UK. 30743-M) in a glass tube. Tubes were left to stand for ten minutes then moved to a water bath and heated at 30°C for 20 minutes. Then 300µl was transferred to a 96-well microplate and analysed using a microplate reader (BMG Labtech Clariostar, Aylesbury, UK) at 480 and 490 nm. The original method suggests the ideal detection point for Pentoses and Uronic acids is 480 nm, while the ideal detection point for Hexoses is 490 nm. Readings were taken at both points to assess notable variance between readings.

The method was tested prior to sample analysis to confirm its reliable detection range. Two sets of four carbohydrate standards were prepared using SMP and EPS extraction solutions as matrices. The four carbohydrate standards selected were 2-deoxy-D-ribose (Alfa Aesar, Heysham, UK. A11990), D-galacturonic acid monohydrate (Alfa Aesar, Heysham, UK. J66282.06), sucrose (Fischer Chemical, Loughborough, UK. SCD2101) and D-xylose (Sigma, Gillingham, UK. W360620) used at 10µg/ml concentration increments between 10-120 µg/ml. These standards were selected from 18 used in the original study based on their contrasting absorbance ranges. Because the nature of the carbohydrate content of the SMP/EPS extraction was unknown, selecting standards which reflect a wide absorbance range allowed for the detection of unknown carbohydrates with absorbances which fall broadly within this wide range. Standards were analysed using a microplate reader (BMG Labtech Clariostar, Aylesbury, UK) at 490 nm for 2-deoxy-D-ribose, D-galacturonic acid monohydrate and sucrose (which was the optimal wavelength adopted for hexoses in the original study) and 480 nm for D-xylose (which was the optimal wavelength adopted for pentoses in the original study).

A series of standard curves were produced with R^2 values ranging from 0.955-0.998 suggesting the overall method was reliable (Figures 18). The curves for 2-deoxy-D-ribose, D-galacturonic acid monohydrate and sucrose demonstrated a consistent linear range along the full length of the tested concentrations when prepared in both the SMP and EPS matrices. When D-xylose was prepared in EPS matrix a clear curve appeared with the linear range only present between 0-40 µg/ml. This curve was not present when D-xylose was prepared in the SMP matrix which showed a linear rage along the full length of the tested concentrations. All standard deviations were <0.04. Overall, this suggests that the method was reliable for all standards prepared in SMP across the full detection range tested, and was also reliable for all standards prepared in EPS except in the case of D-xylose where the accurate detection range was only between 10-40 µg/ml.

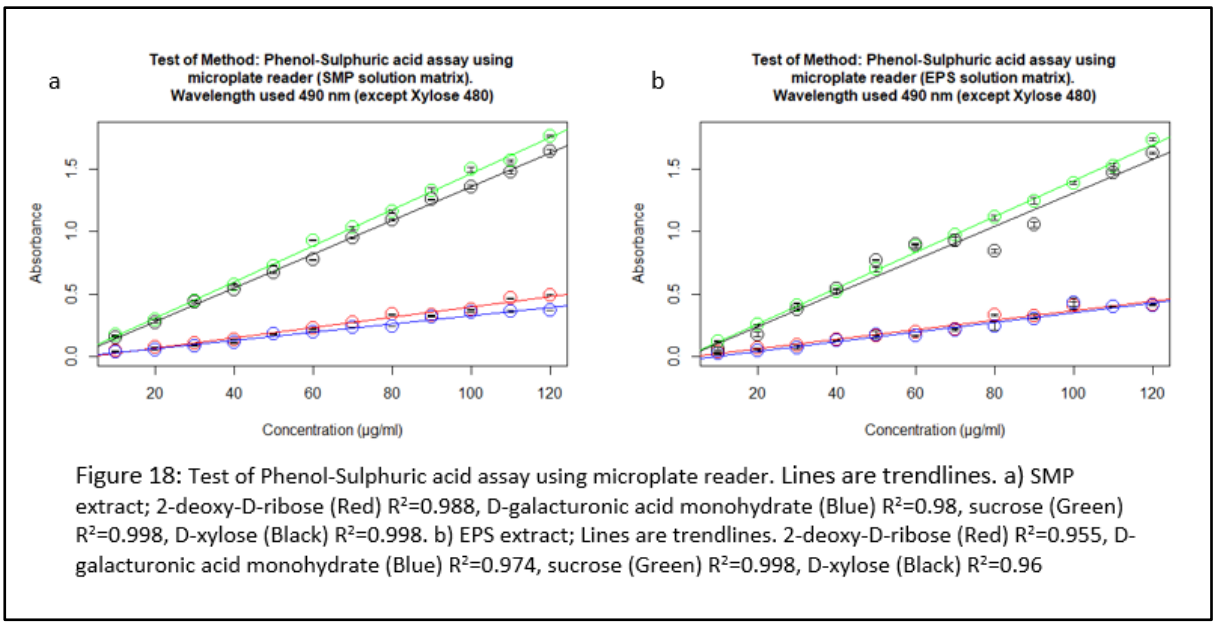

Figure 18: Test of Phenol-Sulphuric acid assay using microplate reader. Lines are trendlines. a) SMP extract; 2-deoxy-D-ribose (Red) R^2=0.988, D-galacturonic acid monohydrate (Blue) R^2=0.98, sucrose (Green) R^2=0.998, D-xylose (Black) R^2=0.998. b) EPS extract; Lines are trendlines. 2-deoxy-D-ribose (Red) R^2=0.955, D-galacturonic acid monohydrate (Blue) R^2=0.974, sucrose (Green) R^2=0.998, D-xylose (Black) R^2=0.96

The adapted Lowry Assay was conducted by first preparing two reagents from three stock solutions. Stock solution i) 3.5g copper sulphate (Fischer Chemical, Loughborough, UK. 10669163) in 100ml deionised water, ii) 7g sodium potassium tartrate (Sigma, Gillingham, UK. 217255) in 100ml deionised water, iii) 70g sodium carbonate (Fischer Chemical, Loughborough, UK. 10264540) in 1L of 0.35 N sodium hydroxide solution. Reagent A was prepared by combining the stock solutions in a ratio of 1:1:100. Reagent B was prepared in the same way except the copper sulphate was replaced with deionised water. Inclusion of the second reagent without copper sulphate allowed 'the determination of the auto-absorbance from humic compounds and chromogenic amino acids' (Redmille-Gordon et al. 2013). Subsequently 50µl of SMP/EPS extracts were transferred to two 96-well microplates and 50µl of phosphate buffered saline was added to each well. 100µl of either reagent A or B was added to each well and the microplates were left to incubate in the dark at room temperature for ten minutes. During this incubation period a 1/10 dilution of Folin-Phenol reagent (Sigma, Gillingham, UK. F9252) was prepared and 100µl subsequently added to each well before microplates were left for a second incubation of 30 minutes. Samples were analysed using a microplate reader (BMG Labtech Clariostar, Aylesbury, UK) at 750 nm. Determining the portions of absorbance attributable respectively to protein (ProtSMP and ProtEPS) and to humic compounds (HumSMP and HumEPS) was calculated using the formula in the original method.

$$AbsProtein = 1.25(AbsReagentA - AbsReagentB)$$
$$AbsHumic = AbsReagentB - (0.2\ x\ AbsProtein)$$

Before soil samples were analysed the method was tested by preparing two sets of two standards using SMP and EPS extraction solutions matrices, with BSA as a representative protein standard and humic acid (Sigma, Gillingham, UK. H16752) as a representative standard for the humic fraction. BSA was prepared at concentrations of 2.5, 5, 10, 20 µg/ml; humic acid was prepared at concentrations of 0.1, 0.2, 0.3, 0.4 µg/ml. A series of standard curves were produced; Humic acid standards produced consistent curves with R^2 values ranging between 0.993-0.999 for both extraction solutions and both reagents. However, standard curves for BSA were inconsistent; standards prepared with EPS extraction solution as a matrix produced standard curves with R^2 values of 0.999 for reagent A and 0.892 for reagent B, but standards prepared with SMP extraction solution as a matrix produced standard curves with R^2 values of 0.933 for reagent A and 0.392 for reagent B. So while the EPS extraction could be relied upon to provide sound analytical data, the SMP extraction failed to provide reliable data, leaving compounds isolated from the SMP process poorly quantified

The humic acid produced a standard deviation as follows; SMP with reagent A (0.06), SMP with reagent B (0.03), EPS with reagent A (0.01), EPS with reagent B (0.02). The BSA produced a standard deviation as follows; SMP with reagent A (0.04), SMP with reagent B (0.01), EPS with reagent A (0.01), EPS with reagent B (0.01). These curves suggested that while overall this method was a reliable way of detecting the humic fraction within soil samples and protein fractions within the EPS extraction it was flawed in detecting proteins in the SMP extraction, particularly when using reagent B. As such this data should be treated with caution (Figure 19).

Assessment of rooting characteristics

Several hypotheses posed in the aims related to rooting characteristics. Because of the disruptive nature of the soil extraction technique and the fact that plant roots were only obtained from within the coring area, an additional set of pots were used to assess rooting characteristics. These pots were prepared and maintained in the same way as those used for soil analysis, with a single specimen sown in each pot (replicated four times) for each of the six herb species. When harvested, shoot biomass was cut at the soil surface and pots were removed from around the root-soil mass which was gently rubbed to remove loose soil without damaging the roots. The root-soil mass was then submerged in water and gently rubbed to remove more soil. This process was repeated until all

soil was removed leaving an intact root ball. Each root ball was scanned using root imaging software (WinRhizo, Regent Instruments Inc., Quebec, Canada). This involved cutting the root ball into sections which were in turn laid flat onto a water-filled transparent plastic tray placed inside the flatbed scanner. Roots were cut into as few sections as possible while still ensuring minimal crossover of lateral roots. Measurements of total root length, surface area, volume and average diameter were made. The root sections were recombined and this, along with the shoot biomass, was oven-dried at 70°C for 24 hours and weighed to determine dry weight root and shoot biomass. While analysis of soils from the primary pots used root data obtained from the same pots, this additional root data provided a more complete overview of root characteristics exhibited by each herb species. This was analysed separately and was used to inform previous assumptions made about rooting characteristics, for instance the similarity of shallow rooting Clover and Birdsfoot.

Data analysis

Analysis was conducted using a combination of ANOVA, Pearson's product motion correlations and principal component analysis (PCA).

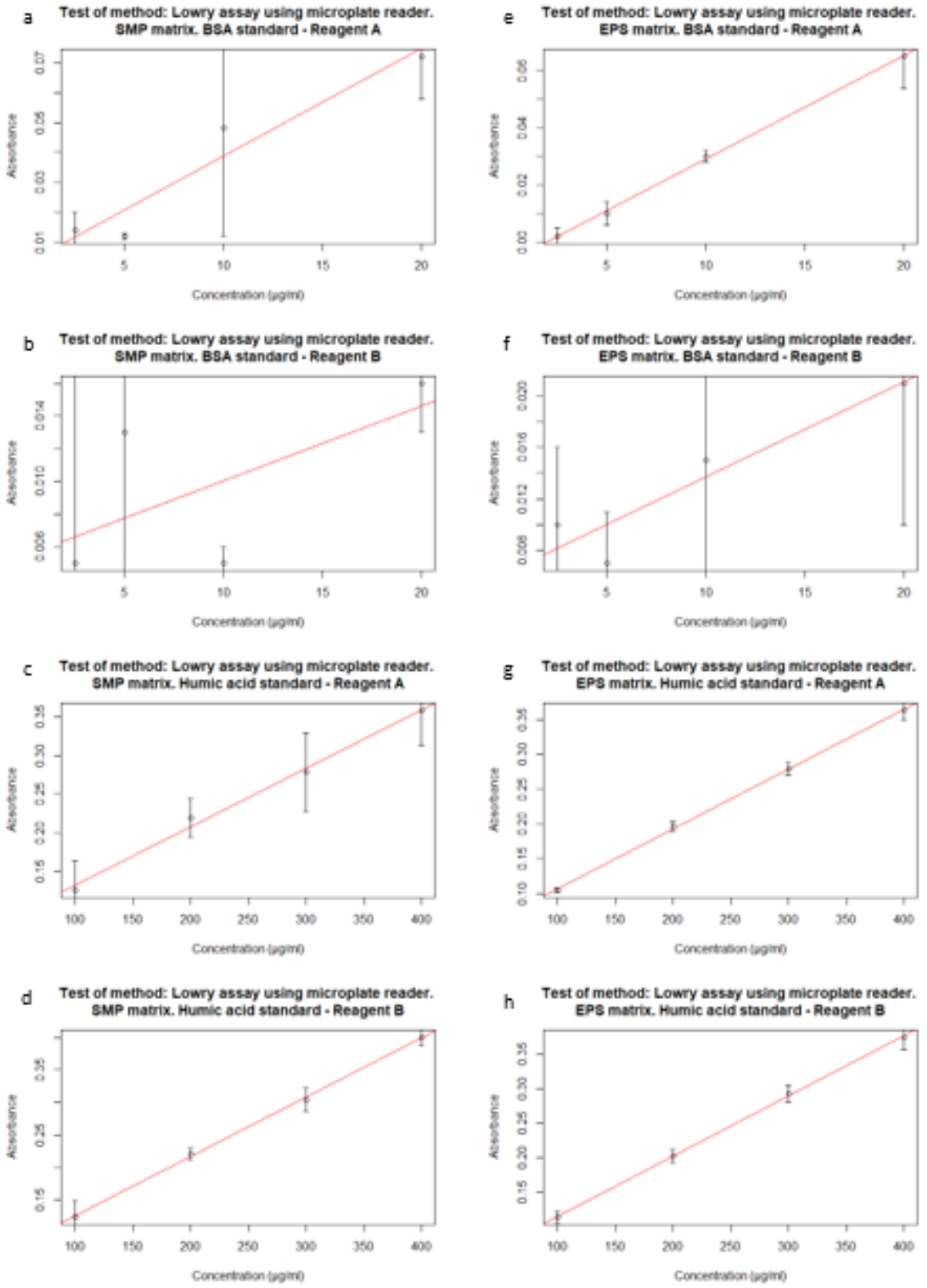

Figure 19: Test Lowry assay using microplate reader. Red line is trendline. a) SMP extract - BSA standard - Reagent A (R^2=0.933), b) SMP extract - BSA standard - Reagent B (R^2=0.392), c) SMP extract - humic acid standard - Reagent A (R^2=0.993), d) SMP extract - humic acid standard - Reagent B (R^2=0.999), e) EPS extract - BSA standard - Reagent A (R^2=0.999), f) EPS extract - BSA standard - Reagent B (R^2=0.892), g) EPS extract - humic acid standard - Reagent A (R^2=0.999), h) EPS extract - humic acid standard - Reagent B (R^2=0.999).

3.3 Results & Discussion

Root characteristics

Before addressing the main dataset associated with the glasshouse experiment, this section summarises analysis of the secondary component of this exercise, the detailed measurement of rooting characteristics. Because two of the four hypotheses posed in the aims relate in part to the variable root characters presented by the six species chosen for the glasshouse experiment, a clear understanding of how the roots of these species vary is important.

Total root length (TRL) was longest in Chicory, followed by gradual reductions in Dandelion, Yarrow and Plantain, with Clover and Birdsfoot displaying the shortest TRL (Figure 20). This variance between species was significantly different ($F_{5,12}$=4.746, P<0.01), although a post-hoc Tukey test only showed significant variance between Chicory and both Birdsfoot and Clover. However, because Chicory and Dandelion were grown in tall pots while the other four species were grown in short pots, and because pot size has been found to impact root morphology (NeSmith & Duval 1998), it was suspected that pot type may have contributed to this variance and that if all species were grown in tall pots different results may have been observed. Therefore, short pots were also analysed separately to determine whether significant variance was also apparent between species grown within the same pot conditions when the statistical bias from tall pot species was removed. This analysis also revealed significant variance ($F_{3,8}$=7.619, P<0.01) which according to a post-hoc Tukey test was driven by greater TRL in Yarrow than both Clover and Birdsfoot (data not shown).

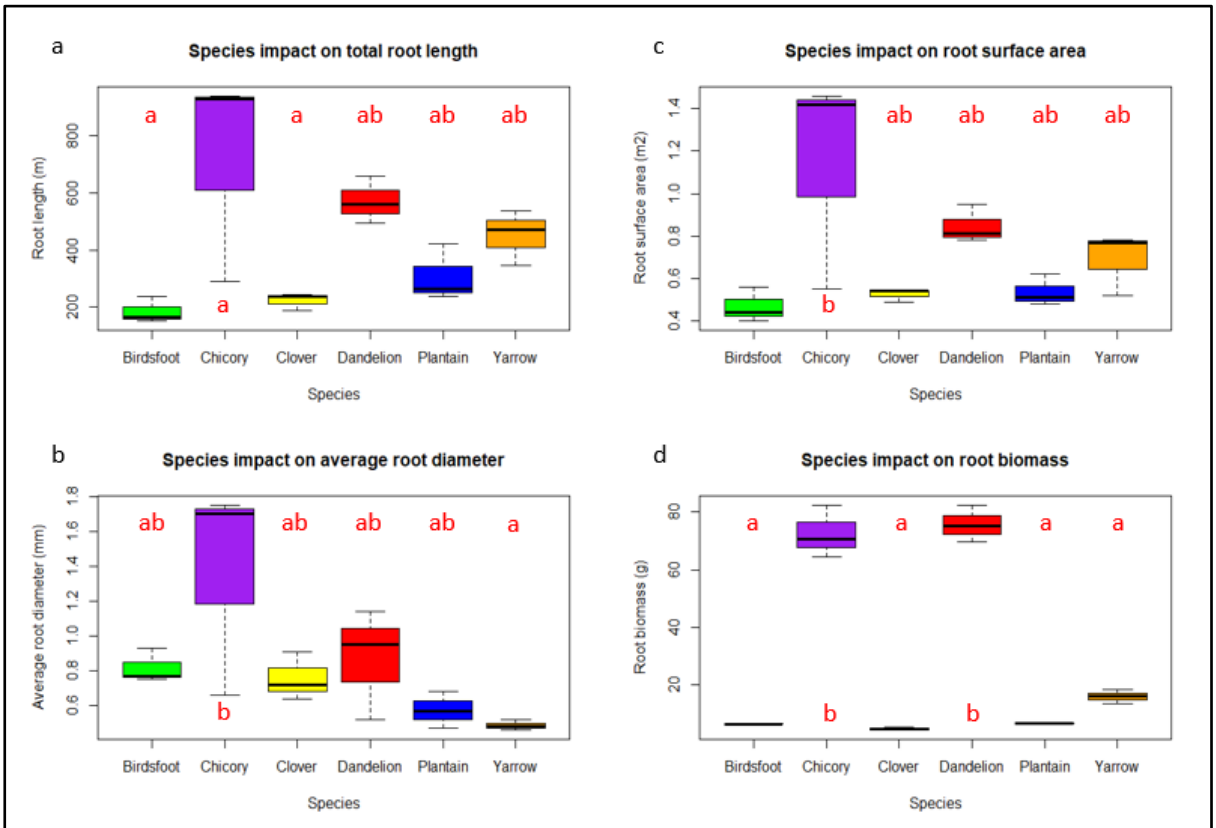

Figure 20: Species impact on rooting characteristics; a) total root length, b) average root diameter, c) root surface area, d) root biomass. Bold line (median), box hinges (1st and 3rd quartile), whiskers (95% confidence interval). Red a-b denote significant variance confirmed by post-hoc test.

Average root diameter (ARD) was widest in Chicory, followed by Dandelion, then Clover and Birdsfoot and then Plantain and Yarrow (Figure 20). ARD between species was significantly different ($F_{5,12}$=3.324, P<0.05) with a post-hoc Tukey test determining significant variance between Chicory and both Yarrow and Plantain. When short pots were analysed separately variance was again significant ($F_{3,8}$=6.98, P<0.05) with a post-hoc Tukey test revealing that Yarrow roots were significantly narrower than both Clover and Birdsfoot (data not shown). Root surface area (SA) was largest in Chicory, followed by Dandelion, Yarrow and then Plantain, Clover and Birdsfoot (Figure 20). Analysis of variance showed that SA between species was significantly different ($F_{5,12}$=3.852, P<0.05) and a post-hoc Tukey test identified the driver for this as high SA for Chicory compared with Birdsfoot, Clover and Plantain. No significant variance appeared when only short pot species were analysed. Root volume (RV) was highest in both Dandelion and Chicory, and then comparable in other species, however the difference was not statistically significant.

RB was highest in Chicory and Dandelion and comparable in other species, although slightly higher in Yarrow relative to other short rooting species (Figure 20). Variance was significant ($F_{5,12}$=160, P<0.001) with a post-hoc Tukey test confirming this was driven by higher RB in Chicory and Dandelion in contrast to the other four species. Variance was also significant within the sub-set of short pots ($F_{3,8}$=47.59, P<0.001) with a post-hoc Tukey test confirming that Yarrow was significantly higher than the remaining species (data not shown).

Herb shoot biomass (HSB) was lowest in Plantain and Birdsfoot and similar in all other species. Analysis of variance across all pots and only in short pots showed only an approaching significant variance in HSB between Chicory and Plantain and no significance between any other species. Furthermore, the lowest HSB was observed in both Plantain and Birdsfoot which represent different functional groups when using rooting habit as an indicator. The literature on root morphology suggests an interrelatedness between root and shoot growth (Bevington & Castle 1985); however, the fact that significant between-species variance was not apparent in HSB, while being present in all root variables suggested that HSB was not a clear indicator of differences in rooting characteristics in the current research. These findings were more in line with those of Faverjon et al. (2018) who found significant independence between the performance of leaf and root traits. This assertion is also supported by the fact that while a positive correlation was apparent between RB and HSB (r=0.52, d.f.=16, P<0.05) (data not shown), this disappeared when short pot species were analysed separately, and no other significant correlation was observed between HSB and any other rooting variable, whereas many rooting variables were correlated with each other (Figure 21).

For instance, RB was positively correlated with SA (r=0.75, d.f.=16, P<0.001) and with TRL, particularly in short pots when pot types were analysed separately (r=0.82, d.f.=10, P<0.001). SA was positively correlated with TRL (r=0.97, d.f.=16, P<0.001), with little change when short pots were analysed separately. A positive correlation was also observed between RV and ARD (r=0.54, d.f.=16, P<0.05) which increased when short pots were analysed separately (r=0.7, d.f.=10, P<0.01). In short pots a negative correlation appeared between RD and RL (r = -0.86, d.f. = 10, P<0.001) and another between RD and SA (r=-0.6, d.f.=10, P<0.05).

This analysis identifies two divergent rooting strategies; one driven by increasing TRL which consistently resulted in a larger SA, and another driven by increasing ARD which consistently resulted in a larger RV. These strategies are not mutually exclusive and can be observed together on the same root ball, as was the case for Chicory. Chicory and Dandelion had similar RB, however while for Dandelion this was largely driven by thick roots (>0.5cm), for Chicory the RB was evenly split between thick and thin roots (<0.5cm). While Dandelion had slightly higher RB and RV than Chicory, the SA of Dandelion was comparatively smaller than Chicory due to the large mass of long thin roots attached to the Chicory's thick central stock root. Within short pots these two rooting strategies

were best exemplified by Yarrow which had the longest TRL and the narrowest ARD, but a significantly higher RB that the other short pot species. While Plantain and Yarrow were selected to represent the same functional group (small tap-rooting species), in fact Plantain's rooting characteristics were often more similar to those of Clover and Birdsfoot than Yarrow. Clover and Birdsfoot displayed consistently similar rooting characteristics (short TRL and wide ARD), although Clover produced a higher HSB. Again, this supports the assertion that HSB was not a reliable indicator of rooting characteristics in this research.

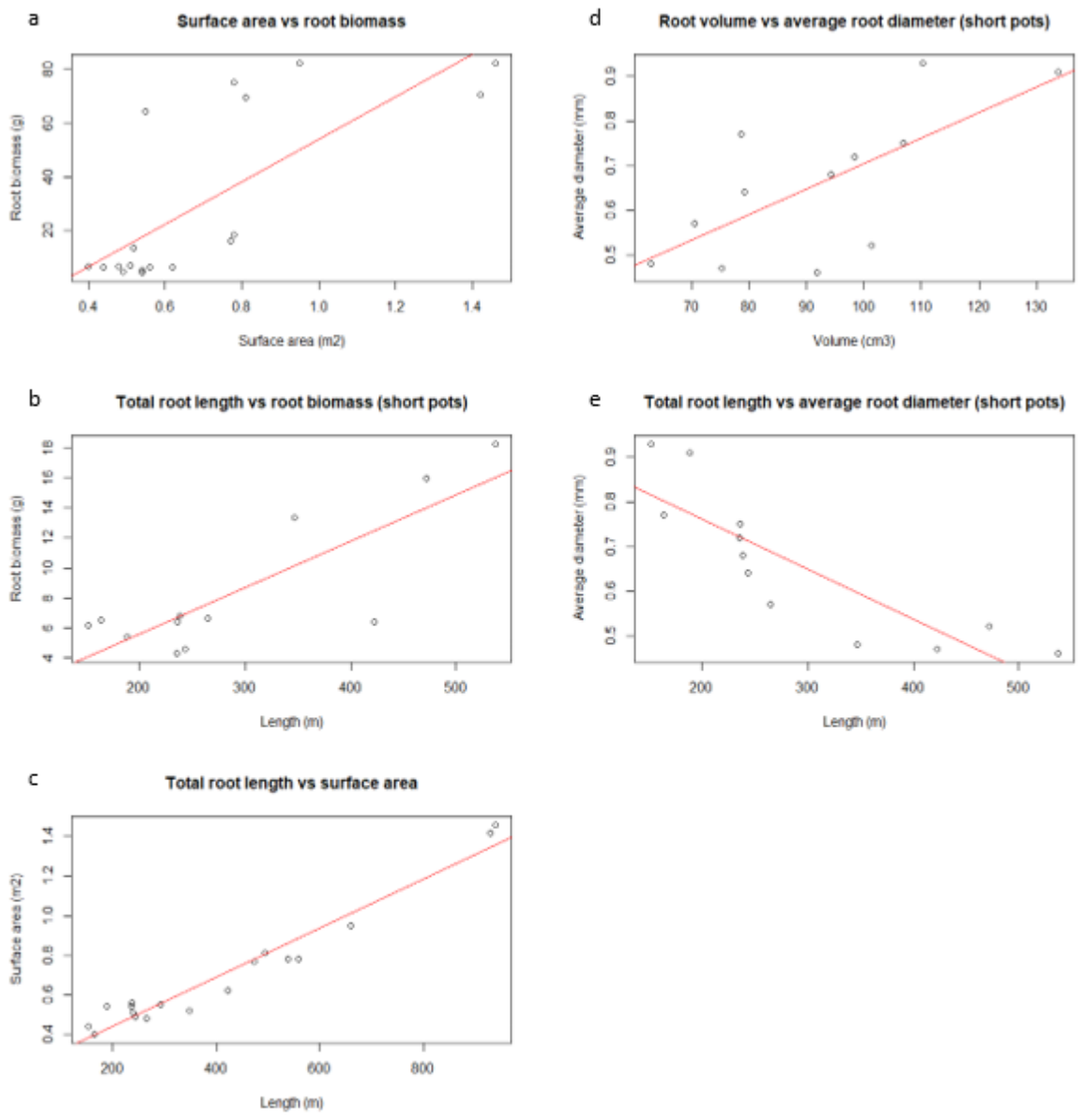

Figure 21: Correlation between rooting variables; a) surface area & root biomass (R^2=0.75), b) total root length & root biomass (short pot subset) (R^2=0.82), c) total root length & surface area (R^2=0.97), d) root volume & average root diameter (short pot subset) (R^2=0.7), e) total root length & average root diameter (short pot subset) (R^2=-0.86). Red line is trendline.

Imaging of the six herb species was conducted to establish a more detailed understanding of their respective rooting characteristics. These six species were selected as representative examples of a typical UK agricultural grassland. They were also chosen to include three functional groups based on rooting characteristics (shallow-rooting, tap-rooting and dense/deep tap-rooting) to examine

whether functional group provided an explanation for changes in net SOM. However, this categorisation of species within functional groups was based on a broadly accepted understanding of their rooting characteristics rather than being based on empirical data. Analysis of imaging data provided a sounder basis for interpreting results from the main glasshouse experiment in the context of functional groups as determined by rooting characteristics. Additionally, it provided an insight into the different rooting strategies adopted by the species selected for this experiment as well as whether and how this related to overall biomass production.

Initially it was expected that rooting behaviour within the three functional groups would be similar. This was true to a point but there was noticeable variation. Taking the dense/deep tap-rooting Chicory and Dandelion; while both produced long thick tap roots as well as comparable RV and RB, other characteristics varied. Measurement of TRL, ARD and SA suggested that Chicory was in a league of its own with substantially more vigorous growth; however, TRL and SA both exhibited high in-species variance within the three Chicory replicates. Also, Chicory exhibited high in-pot variance for ARD compared with Dandelion; this was explained by the fact that the RB of Dandelion predominantly comprised thick roots, while the RB of Chicory was evenly split between thick and thin roots demonstrating that having established a thick tap root Chicory also produced a mass of thin lateral roots. This profusion of thin lateral roots, in addition to the primary tap root, explained why the TRL and SA observed in Chicory was higher than Dandelion and the other species.

In contrast the shallow-rooting Clover and Birdsfoot were very similar in all measurements of rooting characteristic. Clover demonstrated slightly greater TRL and SA and Birdsfoot showed a slightly wider ARD (although none of this variance was significant) and their RB and RV were both comparable. The third functional group, tap-rooting, which included Plantain and Yarrow, was not as straightforward. While similar results were expected for this pair, Yarrow produced noticeably greater TRL, SA and RB than Plantain, which in turn had a wider ARD, although the RV of both was comparable. When reviewing similarities between Yarrow and Plantan in the context of the other two short pot species (Clover and Birdsfoot), Plantain was often more similar to these two than its fellow tap-rooting species. Clover and Birdsfoot produced significantly shorter TRL than Yarrow but not Plantain and they produced significantly wider ARD than Yarrow but not Plantain, while Yarrow produced significantly greater RB than Birdsfoot, Clover and Plantain.

These comparisons revealed that in this study categorising a plant into a functional group by considering only it's primary rooting feature (i.e. tap root, shallow root structure) was an insufficient method of predicting likely behavioural similarities, while consideration of a broader range of rooting characteristics can reveal more complex variation. This conclusion is supported by Faverjon et al. (2018) who found that species with similar rooting morphologies displayed quite different rooting characteristics.

Additionally, analysis of rooting characteristics identified two different rooting strategies; the first driven by a profusion of thin roots which produced high values of TRL and SA, the second driven by fewer thicker roots resulting in a higher ARD and RV. As noted already, these strategies are not mutually exclusive and can be observed together on the same root, as was the case for Chicory and to a lesser extent Dandelion. However, the four short pot species adopted just one of these two rooting strategies. Once rooting characteristics were analysed it was clear that the rooting strategy which involved a profusion of thin roots (high TRL and SA) was more successful than the method of fewer thicker roots (high ARD and RV) at producing high RB. This finding appears to contradict Faverjon et al. (2018) who found that taproot biomass was almost proportional to total RB.

The other notable finding from this analysis was the disconnect between HSB and all rooting characteristics except for RB, although a correlative link between these two variables was not consistently demonstrable throughout the analysis. While Chicory, which produced high values of all

rooting characteristics, did also record the highest HSB, the distinction was not significant, and its HSB value was only slightly higher than those of Dandelion, Clover and Yarrow which all produced comparable HSB despite representing three different functional groups. HSB showed no consistent correlation with any rooting characteristic or clear pattern with any chosen functional group. As such it seemed feasible to conclude that it was not possible to consistently use HSB to estimate root vigour. While this point was ancillary to the primary remit of the project hypotheses, it was a notable finding nonetheless.

Main glasshouse experiment

Samples obtained from the main glasshouse experiment were analysed for variance between a) species, b) diversity treatment, c) depth sections, and d) radial coring sections.

Variance between species and diversity treatment

Herb and grass shoot biomass

Analysis of variance showed that HSB between diversity treatments was significantly different ($F_{1,326}$=669.3, P<0.001) with more HSB in the mono (Figure 22). Significant variance was also apparent between species within the mono ($F_{5,164}$= 44.2, P< 0.001). A post-hoc Tukey test showed significant variance between all species except the combinations; Plantain-Birdsfoot, Clover-Chicory and Yarrow-Dandelion. This supports the previous assertion that HSB was not a reliable indicator of rooting characteristics as these three combinations of not significantly different pairs represent contrasting rooting functional groups. Significant variance in HSB was also apparent between species within the 2-spec ($F_{5,152}$= 461.4, P< 0.001). A post-hoc Tukey test showed this was primarily driven by differences between both the deep-rooting Chicory and Dandelion and each of the four other species. However, significant variance was also observed when contrasting both Clover and Birdsfoot with Yarrow (P<0.001) and with Plantain (P<0.01). This was unsurprising given that within the 2-spec HSB was highly stunted in the shallow rooting Clover and Birdsfoot, and to a lesser degree in the tap-rooting Plantain and Yarrow, while the deep-rooting Chicory and Dandelion were less effected.

When comparing variance for both Chicory and Dandelion with the other four species, it was necessary to consider that pot type may have influenced differences in measured variables due to the different growing conditions provided by tall pots. Analysis of variance showed that HSB between tall and short pots was significantly different ($F_{1,326}$=37.73, P<0.001) (data not shown), although it is unclear whether this variance was driven by pot conditions or species vigour. However, within the mono (which was not subject to additional influence from the competing Ryegrass) it is noteworthy that similar levels of HSB were recorded in Dandelion as in both Clover and Yarrow which suggests that the comparatively strong overall performance of Chicory and Dandelion was not simply an artefact of growing conditions within tall pots.

As Ryegrass was only present within the 2-spec, variance in grass shoot biomass (GSB) between diversity treatments was not analysed. However, variance in GSB between species was significantly different ($F_{6,175}$=604.9, P<0.001) and a post-hoc Tukey test showed this was driven by more GSB in both Chicory and Dandelion compared with the other four species (Figure 22). When short pots were analysed separately variance was still significant ($F_{4,115}$=5.122, P<0.001) (data not shown), however a post-hoc Tukey test only showed significant variance between Yarrow and both Birdsfoot and the Ryegrass control with least GSB recorded in Yarrow pots while GSB in all other short pots was comparable.

The significantly greater GSB in tall pots suggested that Ryegrass benefited either from the altered growing conditions in the tall pots or possibly in response to the vigorous HSB produced by Chicory and Dandelion. However, the second possibility does not explain why similar increases in GSB were not also observed in Clover and Yarrow pots which produced comparable HSB as the Dandelion pots.

Unfortunately, a Ryegrass control was not included in tall pots, so it was not possible to compare GSB in controls between pot types to see whether a significant difference was discernible. Nor was it possible to examine differences in GSB between tall pot herbs and a tall pot Ryegrass control as was possible in short pots, although here a significant difference was only observed in Yarrow pots.

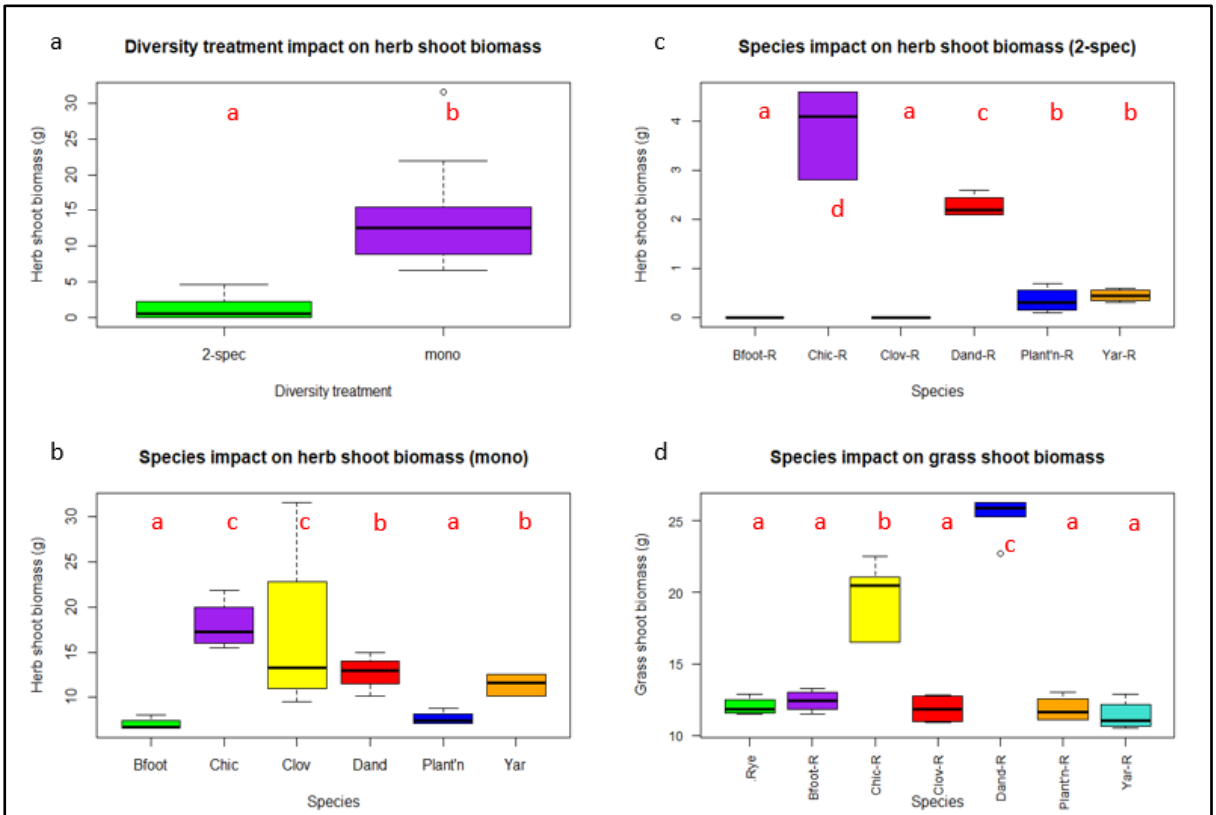

Figure 22: Diversity treatment and species impacts on herb and grass shoot biomass; a) diversity impact on herb shoot biomass, b) species impact on herb shoot biomass (mono), c) species impact on herb shoot biomass (2-spec), d) species impact on grass shoot biomass. Bold line (median), box hinges (1st and 3rd quartile), whiskers (95% confidence interval). Red a-d denote significant variance confirmed by post-hoc test.

Root biomass

In this experiment RB was calculated as g/cm^3 of soil, as the soil volume varied between the inner and outer coring tube segments (as well as the horizontal coring tubes used additionally for tall pots). Therefore, this calculation allowed direct comparison of RB between segments. When measured this way, no significant variance was apparent between diversity treatments. Analysis of variance at species level across diversity treatments did show significant difference ($F_{12,339}$=3.381, P<0.001) (data not shown). However, a post-hoc Tukey test showed this was only present between Chicory and each of Clover, Plantain and Yarrow (all mono). Considering Chicory's extensive root system some variance was unsurprising and may have been expected with Dandelion also.

There was substantial in-species and in-pot variance in RB within the 2-spec because the ryegrass formed a dense root mass in the top 5 cm and reduced considerably at lower coring depths. Despite this, average RB between species was comparable and showed no significant variance within the 2-spec. Substantial in-species and in-pot variance in RB was also observed for Chicory and Dandelion within the mono. This was driven by the distinct contrast between the thick tap root and finer lateral roots. RB in short pots within the mono showed much less in-pot and in-species variance, however

analysis of variance between species showed that RB was significantly different ($F_{3,86}$=5.303, P<0.01) (data not shown). A post-hoc Tukey test showed that this variance was driven by slightly higher RB in Yarrow compared with the other three short pot species which displayed comparable quantities of RB. This finding was in line with the RB data observed in the pots analysed to measure rooting characteristics.

This method of RB measurement was useful for understanding root associations with other variables between coring segments, however the relatively small degree of significant variation between species and diversity treatments was slightly misleading given that clear differences were apparent in the total quantity of RB per pot between species and diversity treatments. Because of this a whole-pot analysis was also conducted which shall be addressed later. Reference to RB in this section refers specifically RB (g/cm^3).

Bulk density

Bulk density was highest for Chicory and Dandelion in both diversity treatments. However, given that the bulk density for the non-planted control established in tall pots was also much higher, this suggested that the high bulk density values were an artefact of the tall pot growing conditions. This assertion was supported by a pot-hoc Tukey test which showed that only combinations which contrasted tall pots with short pots demonstrate significant variance in bulk density (Figure 23).

pH

Analysis of variance showed that pH between diversity treatments was significantly different ($F_{3,412}$=11.36, P<0.001). A post-hoc Tukey test showed that while variance between the mono and the 2-spec was significant (P<0.01), inclusion of both the Ryegrass and non-planted controls added additional context (Figure 24). pH was most alkaline in the Ryegrass control and became progressively more acidic as follows; 2-spec, mono, non-planted control. No significant variance was present between the 2-spec and Ryegrass control, or between the mono and non-planted control, whereas highly significant variance (P<0.001) appeared between the mono and Ryegrass control and between the 2-spec and non-planted control, while the most statistically significant variance was between the Ryegrass and non-planted controls. This suggested that the presence of ryegrass was the strongest driver for variance in pH. However, when examined at species level, while pH was consistent across the mono (no significant variance between species), it emerged that the significantly higher pH observed in the 2-spec was driven by Birdsfoot and Plantain (data not shown). These species displayed a pH comparable to the ryegrass control, while all other species within the 2-spec were not significantly different from the mono species. This suggests that higher pH was not

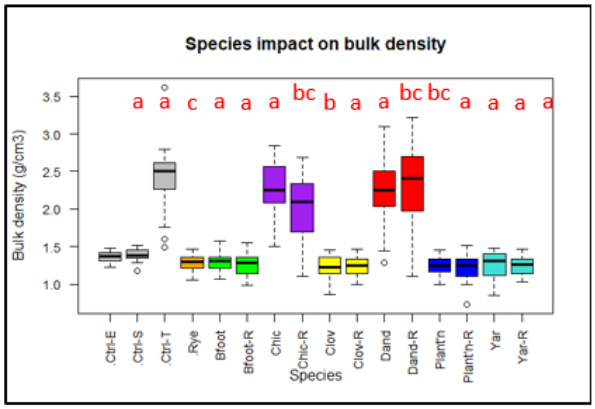

Figure 23: Species impact on bulk density. Bold line (median), box hinges (1st and 3rd quartile), whiskers (95% confidence interval). Red a-c denote significant variance confirmed by post-hoc test.

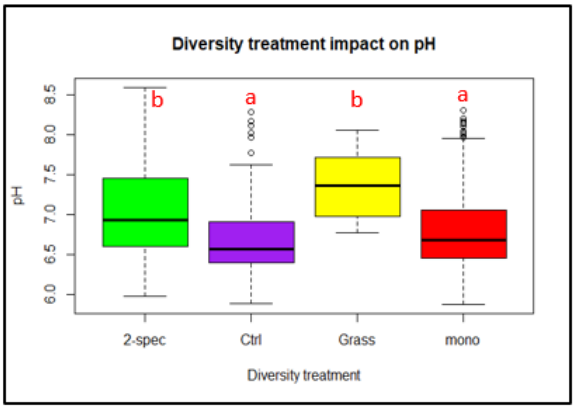

Figure 24: Diversity treatment impact on pH. Bold line (median), box hinges (1st and 3rd quartile), whiskers (95% confidence interval). Red a-b denote significant variance confirmed by post-hoc test.

simply a consequence of ryegrass being present. It is notable that Birdsfoot and Plantain (within the mono) were also the first species to be harvested and as such experienced the shortest growing time and the least watering and feeding which may explain the less acidic soil in these species. However, this does not explain why the Ryegrass control contained the least acidic soil.

SOM

No significant variance was apparent in SOM200 between diversity treatments. When examined at species level across both diversity treatments a significant variance emerged ($F_{15,398}$=3.154, P<0.001), however a post-hoc Tukey test revealed this was driven exclusively by higher SOM200 in Chicory in both treatments and it also calculated a reduced significance value (P<0.05). Except for Chicory, SOM200 was broadly similar between species across both diversity treatments (data not shown).

Analysis of variance showed that SOM325 between diversity treatments was significantly different ($F_{3,410}$=16.53, P<0.001) with lower SOM325 in the mono (Figure 25). When this pattern of variance was examined at species level across diversity treatments a post-hoc Tukey test revealed this was primarily driven by significantly lower SOM325 in the mono for Clover, Yarrow and Dandelion (P<0.001), while the same pattern was present, but not significant, for Chicory and Plantain and was reversed, but not significantly, for Birdsfoot. When analysis was conducted between species within diversity treatments significant difference was observed within the mono ($F_{5,163}$=43.76, P<0.001). A post-hoc Tukey test revealed this was driven both by higher SOM325 in Birdsfoot and Chicory, and by lower SOM325 in Clover, in contrast to Plantain, Dandelion and Yarrow which were comparable. Variance in SOM325 between species within the 2-spec was also significant ($F_{5,152}$=2.833, P<0.05), however a post-hoc Tukey test showed that this variance was only driven by lower SOM325 in Plantain (data not shown). Besides the clear contrast showing higher SOM325 in the 2-spec, no clear overall pattern for SOM325 emerged between species based on their rooting characteristics.

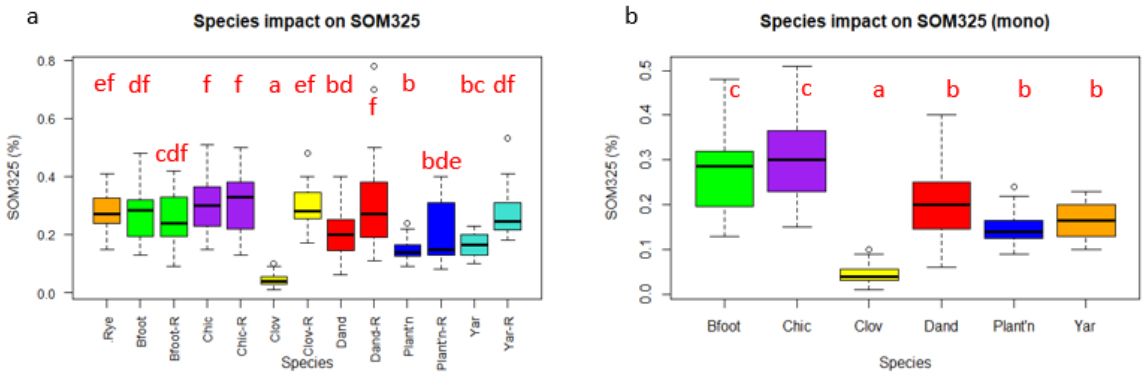

Figure 25: Species impact on SOM325; a) across both diversity treatments, b) within the monoculture treatment. Bold line (median), box hinges (1st and 3rd quartile), whiskers (95% confidence interval). Red a-f denote significant variance confirmed by post-hoc test.

In contrast to SOM325, while analysis of variance in SOM550 between diversity treatments was also significantly different ($F_{3,410}$=29.67, P<0.001), in this case lower SOM550 was recorded in the 2-spec. When this pattern of variance was examined at species level a post-hoc Tukey test revealed significantly higher SOM550 in the mono for Clover, Plantain and Birdsfoot (P<0.001) and the same pattern was present but not significant for Yarrow, while in Chicory and Dandelion SOM550 was comparable between diversity treatments (Figure 26). When analysis was conducted between species within diversity treatments significant difference was observed within the mono ($F_{5,163}$=11.08, P<0.001) with a post-hoc Tukey test showing this was driven both by high SOM550 in Dandelion and by low SOM550 in Chicory in contrast to all other species which were comparable

(data not shown). Variance in SOM550 between species within the 2-spec was also significant ($F_{5,152}$=23.14, P<0.001), with a post-hoc Tukey test showing this was driven again by high SOM550 in Dandelion and also by low SOM550 in Clover and Plantain in contrast to all other species which were comparable (data not shown). The most interesting observation from this analysis was the higher SOM550 in the mono for the four short pot species in contrast to their 2-spec counterparts, while in the tall pot species any difference between diversity treatments was negligible.

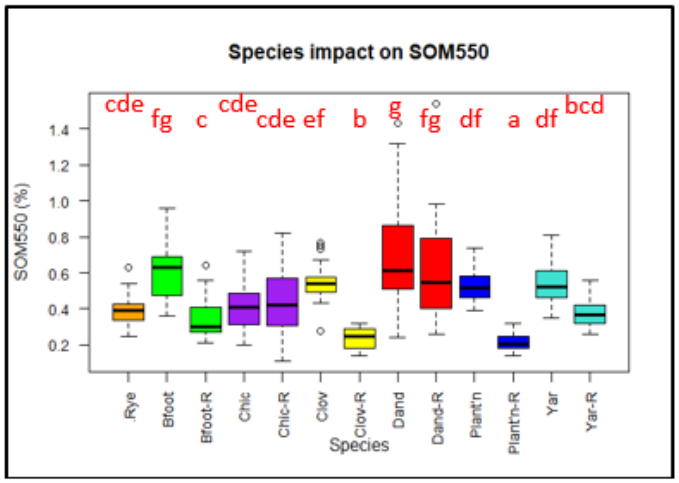

Figure 26: Species impact on SOM550. Bold line (median), box hinges (1st and 3rd quartile), whiskers (95% confidence interval). Red a-f denote significant variance confirmed by post-hoc test.

One explanation for this was that SOM550 was greater when herb species performed strongly and was lower when the four short pot species were stunted when grown with Ryegrass. The same reduction in SOM550 was not significant in the 2-spec tall pots in which the herbs were not as dramatically impacted by the presence of Ryegrass. This assertion would imply that herb species were a strong source of SOM550 while Ryegrass was not; or was to a lesser degree. Another explanation for this pattern was the presence of a priming effect from the greater amounts of more labile SOM325 in the 2-spec leading to an enhanced metabolisation of more persistent SOM550 by microbial communities in the pots containing Ryegrass. However, this theory does not explain why SOM550 was not significantly reduced in the 2-spec for Chicory and Dandelion. Therefore, the *herb-based production* explanation appears to be a better fit.

DOM

Analysis of variance showed that DOM between diversity treatments was significantly different ($F_{3,411}$=9.792, P<0.001) with more DOM in the 2-spec (data not shown). This significance was also observed when examined at the species level across both diversity treatments ($F_{15,399}$=12.09, P<0.001). A post-hoc Tukey test showed this was driven by high DOM in 2-spec Birdsfoot, Clover and Plantain. When this analysis was conducted within the 2-spec the significance remained ($F_{5,151}$=14.88, P<0.001), however a post-hoc Tukey test showed this was driven both by high DOM in Plantain and by low DOM in Yarrow, while Clover and Birdsfoot were no longer significantly different from the remaining species in the 2-spec (data not shown). Variance was also significant within the mono ($F_{5,164}$=8.011, P<0.001), a post-hoc Tukey test confirmed this was driven by low DOM in Birdsfoot, Clover and Yarrow (data not shown). Overall, analysis of DOM did not identify clear patterns between species, however DOM was higher in the 2-spec but only for some species suggesting that the standard inclusion of the Ryegrass in 2-spec did not necessarily in itself explain this pattern.

EE-GRSP

Analysis of variance showed that EE-GRSP between diversity treatments was significantly different ($F_{3,412}$=13.63, P<0.001) with more EE-GRSP in the mono. A post-hoc Tukey test showed that the most

significant variance was between the mono and the Ryegrass control (which showed the lowest EE-GRSP), although EE-GRSP in the 2-spec was also significantly lower than in the mono (P<0.001). When analysed at species level across both diversity treatments the significance remained ($F_{15,400}$=25.67, P<0.001) and a post-hoc Tukey test revealed this was driven both by low EE-GRSP in the 2-spec for Clover and Birdsfoot (as well as the Ryegrass control) and also by high EE-GRSP in the mono for Chicory and Dandelion (Figure 27). It also revealed significantly less EE-GRSP in the Ryegrass control than in the mono for Plantain and Yarrow. Interestingly this all represents a clear contrast between high EE-GRSP in tap-rooting species (particularly so in the mono) and low EE-GRSP in the shallow rooting Clover and Birdsfoot (and the Ryegrass control), this being particularly acute in the 2-spec where Birdsfoot and Clover were heavily outcompeted by the Ryegrass. This seems to suggest that when the competitive Ryegrass was present, EE-GRSP was lower (possibly due to a reduction in RB) although this was partly mitigated when tap-rooting species were present, whereas the shallow rooting species typically produced least EE-GRSP.

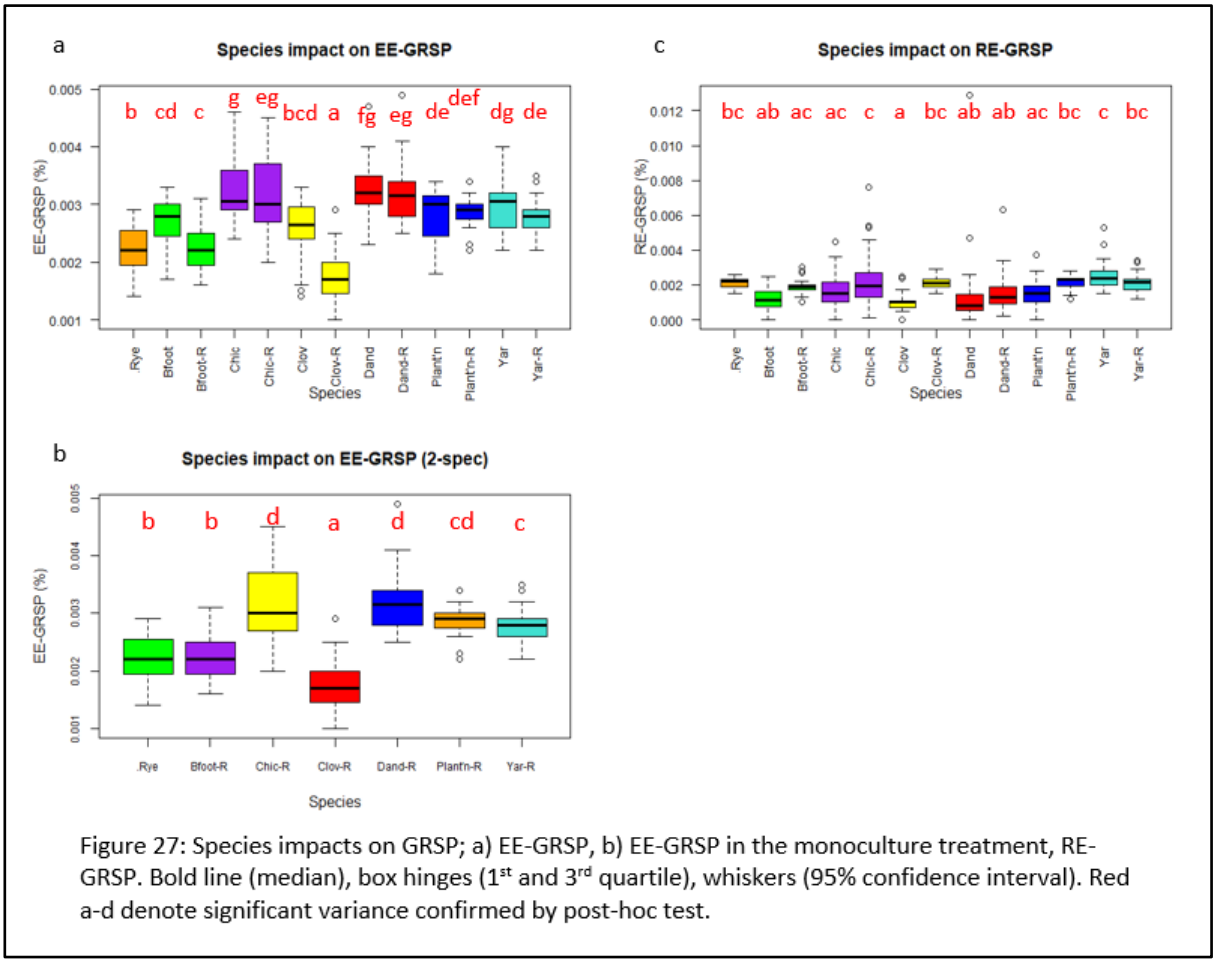

Figure 27: Species impacts on GRSP; a) EE-GRSP, b) EE-GRSP in the monoculture treatment, RE-GRSP. Bold line (median), box hinges (1st and 3rd quartile), whiskers (95% confidence interval). Red a-d denote significant variance confirmed by post-hoc test.

RE-GRSP

Analysis of variance showed that RE-GRSP between diversity treatments was significantly different ($F_{3,412}$=8.579, P<0.001) with more RE-GRSP in the 2-spec. A post-hoc Tukey test showed that the most significant variance was between the mono and the 2-spec, although RE-GRSP in the Ryegrass control was also significantly higher than in the mono (P<0.05). When analysed at species level across both diversity treatments the significance remained ($F_{15,400}$=4.679, P<0.001), however a post-hoc Tukey test revealed this was primarily driven by low RE-GRSP in Clover (mono) as well as high RE-GRSP in both Chicory (2-spec) and Yarrow (mono) and by low RE-GRSP in Birdsfoot (mono) and Dandelion (both treatments) (Figure 27). Besides the observation that RE-GRSP was consistently higher for species in the 2-spec in comparison to their mono counterparts (except for Yarrow), the only other clear finding to emerge was the particularly low RE-GRSP observed in the mono for Clover

and Birdsfoot which mirrored observations for the EE-GRSP. The fact that RE-GRSP was higher in the 2-spec and was also high in the Ryegrass control were contrary to the finding for EE-GRSP which was higher in the mono. It is possible that the presence of Ryegrass caused a transformation of EE-GRSP into more persistent RE-GRSP possibly through increased microbial metabolism following a priming effect.

Analysis of SMP/EPS extractions

Analysis of EPS and SMP extracts is reported separately because the values of these measured variables were typically very low and often below detectable levels (Appendix B).

Variance between depth sections

Root biomass

Analysis of variance showed that RB between depth sections across diversity treatments was significantly different ($F_{6,345}$=15.07, P<0.001). A post-hoc Tukey test showed this significance was driven by the variance between the top 5cm and all other depth sections. After a significant reduction in RB between the top 5cm and 5-10cm, RB gradually reduced down to 50cm at which point it tailed off to a steady rate from 50-90cm. This pattern and significance ($F_{6,151}$=62.07, P<0.001) was mirrored in the 2-spec. However, within the mono the significance disappeared, and the pattern altered; the top 5cm showed high variability while the depth sections between 5-15cm were comparable with the average value of 0-5cm. These findings underlined the variable rooting structures displayed by herb species when grown without influence from the Ryegrass as well as the dominance of the Ryegrass RB in the top 5cm (data not shown).

Bulk density

Having established that bulk density appeared artificially higher in tall pots compared with short pots; short and tall pots were examined separately when analysing variance in bulk density between depth sections. Analysis of variance in short pots showed that bulk density between depth sections across diversity treatments was significantly different ($F_{2,207}$=28.92, P<0.001). A post-hoc Tukey test showed that while significant variance was apparent between 5-10cm and 10-15cm (P<0.01), the variance between both of these two depth sections and the top 5cm was several orders of magnitude more statistically significant (P<0.001), with the lowest bulk density apparent in the top 5cm, probably due to the loosening impact of roots on the soil structure in this zone. This pattern and significance were virtually identical within the 2-spec ($F_{2,93}$=32.45, P<0.001), while within the mono the variance was reduced and was only significant between 0-5cm and 5-10cm (P<0.05). This is likely due to the reduced RB in the mono due to the absence of Ryegrass (data not shown).

Across diversity treatments, significant variance was also present in tall pots ($F_{6,134}$=27.91, P<0.001). A post-hoc Tukey test showed this was driven by a difference between the three depth sections in the top 15cm in one grouping and the four depth sections between 30-90cm in a second grouping, with depth sections within each grouping showing comparable bulk density (data not shown). Similar, patterns were observed when variance in bulk density was analysed within diversity treatments with only small variations. Interestingly, bulk density was lower at 70cm and 90cm and lowest at 30cm and 50cm, with highest bulk density in the top 15cm. One possible explanation for this result was that the thick Chicory and Dandelion roots closest to the soil surface may have laterally compressed the soil, leading to increased soil density. An alternative explanation relates to the relatively thick walls of the coring tubes (3mm). As significant soil compaction was not observed during coring, the soil below the 3mm coring tube walls must have been displaced either inside or outside of the tubes to allow its insertion. Because the tall pots were narrower with straight sides (compared with the wider sloping sides of the short pots), it was possible that outward displacement of soil in tall pots was limited, resulting in an increase in inward displacement leading to an increasingly dense soil core within the tube. This phenomenon was supported by the fact that coring tube penetration was substantially more challenging in tall pots than in short pots, suggesting possible resistance from the soil immediately below the coring tube walls. A further possible

explanation for the variance in bulk density between the top 15cm of tall pots and the lower depths was the different coring techniques used to extract the lower horizontal samples.

Water content

Because bulk density was negatively correlated with water content (explored further later) (r=-0.49, d.f.=413, *P*<0.001), short and tall pots were also examined separately when analysing variance in water content between depth sections. In short pots, variance in water content between depth sections (across diversity treatments) was significantly different ($F_{2,207}$=16.33, *P*<0.001) (Figure 28). A post-hoc Tukey test showed that this variance was driven by higher water content between 10-15cm compared with reduced water content in the top two depth sections. A similar pattern was observed within the mono where water content presented a staggered pattern, increasing in stages as depth increased (data not shown). The pattern within the 2-spec was not as clear. Water content was still highest between 10-15cm but slightly higher between 0-5cm than 5-10cm (data not shown). A comparison of the water content values between diversity treatments suggests this non-linear pattern within the 2-spec was the result of increased water content in the top 5cm rather than reduced water content between 5-10cm as the water content in this section was broadly in line with its counterpart in the mono. This may be due to lower bulk density in the top 5cm in the 2-spec caused by the Ryegrass root resulting in greater porosity in the top 5cm, and/or to greater soil aggregation around the extensive Ryegrass roots increasing the water holding potential in this zone.

Across diversity treatments, significant variance in water content was also apparent in tall pots (F6,134=40.74, P<0.001). A post-hoc Tukey test revealed a non-linear pattern; however, the primary driver was a similar grouping of depth sections as observed for bulk density. The most significant variance was between depth sections within the top 15cm and those between 40-90cm, with water content significantly higher in the latter. Variance was also observed in combinations within these two broad groupings, however none of the intra-grouping variance was as statistically significant as the inter-grouping variance. Similar patterns and significance were observed when analysed within diversity treatments.

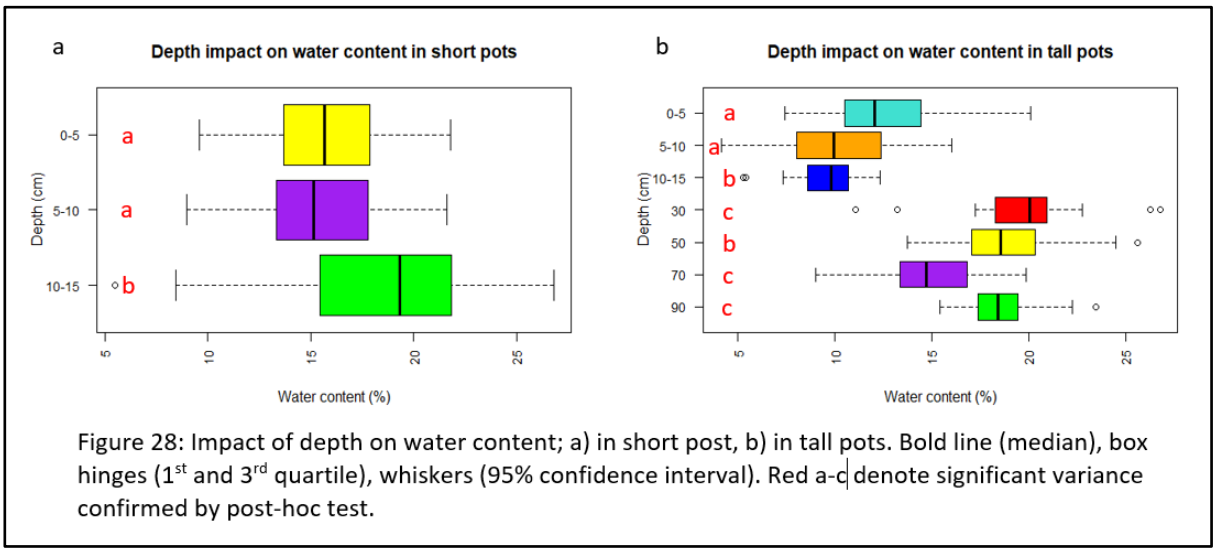

Figure 28: Impact of depth on water content; a) in short post, b) in tall pots. Bold line (median), box hinges (1st and 3rd quartile), whiskers (95% confidence interval). Red a-c denote significant variance confirmed by post-hoc test.

pH

Across diversity treatments, pH varied significantly between depth sections ($F_{6,409}$=76.83, *P*<0.001). pH reduced as depth increased down to 30cm, after which it remained relatively stable (data not shown). However, a post-hoc Tukey test revealed that significant variance was only apparent when the top two depth sections (0-5cm and 5-10cm) were contrasted with all depth sections below 10cm (and with each other). This suggests a staggered reduction in pH in the top 10cm followed by a steadier acidification below this. A similar pattern and significance were observed within the 2-spec ($F_{6,151}$=25.44, *P*<0.001) (data not shown). This was also true within the mono ($F_{6,163}$=45.88, *P*<0.001),

however a post-hoc Tukey test showed that significant variance was driven only by the high levels of pH in the top 5cm (data not shown) and the rapid reduction below this. This suggests that the presence of Ryegrass increased the depth at which pH remained more alkaline, whereas when only herb species were present, acidification occurred closer to the surface.

SOM200

Across diversity treatments, SOM200 varied significantly between depth sections ($F_{6,407}$=28.01, P<0.001). SOM200 values were loosely grouped as observed for bulk density and water content (in tall pots); the three depth sections between 0-15cm forming one group and depths between 30-90cm forming another with significantly more SOM200 (Figure 29). A post-hoc Tukey test confirmed that virtually all the significant variance was driven by inter-grouping differences. It also showed significant variance between 0-5cm and both 5-10cm (P<0.01) and 10-15cm (P<0.05), as well as between 30cm and 70cm (P<0.01). However, the inter-grouping variance was more statistically significant than these intra-grouping variances. Furthermore, when short pots were analysed separately (to discount statistical bias produced by data from the 30-90cm grouping), while variance was apparent ($F_{2,207}$=3.869, P<0.05) its significance was far lower (data not shown). Overall, these calculations suggest that variance between depth sections in the top 15cm was relatively small, particularly in contrast to that observed between 40-90cm (in tall pots). When analysed within diversity treatment, while some alterations in intra-grouping variance appeared, findings broadly mirrored the overall pattern and significance observed across diversity treatments (data not shown).

SOM325

Similar patterns to SOM200 were observed for SOM325 (Figure 29). A post-hoc Tukey test showed that the significant variance across diversity treatments ($F_{6,407}$=11.46, P<0.001) was explained by low SOM325 between 5-10cm and 10-15cm in contrast to the higher SOM325 in the 30cm, 50cm, 90cm and in this case also the 0-5cm depth sections. Unlike for SOM200, SOM325 between 0-5cm was significantly higher than the other depth sections in the top 15cm. When variance within the 2-spec was analysed, a virtually identical pattern of significant difference emerged ($F_{6,151}$=10.69, P<0.001) although the higher rate of SOM325 between 0-5cm was even more distinct. Within the mono, while the significance remained ($F_{6,162}$=4.418, P<0.001), the drivers identified by a post-hoc Tukey test were different (data not shown). Variance was only significant between 30cm and all depth sections in the top 15cm. SOM325 in the top three depth sections (0-15cm) was comparable, and although still highest between 0-5cm this was not significant. These patterns were mirrored when short pots were analysed separately. These findings support those identified previously that link higher SOM325 with the presence of Ryegrass which dominate the top 5cm. However, as with SOM200 the highest rates of SOM325 were observed below the top 15cm, at 30cm and 50cm (within both diversity treatments and overall).

SOM550

Similar patterns were observed when significant variance was identified in SOM550 ($F_{6,407}$=17.19, P<0.001). A post-hoc Tukey test showed that significant variance was mainly driven by low rates of SOM550 in the top 15cm when compared with higher rates between 30-90cm (Figure 29). There was also an approaching significant variance between 30cm and 70cm, the latter of which produced the lowest value within the 30-90cm range. Within the 2-spec a virtually identical pattern of significant difference emerged ($F_{6,151}$=23, P<0.001), albeit with a more marked variance between 0-15cm and 30-90cm. Variance within the mono was also significantly different ($F_{6,162}$=7.711, P<0.001), however in this case a post-hoc Tukey showed that this significance was driven only by the low SOM550 in the top 15cm when contrasted with the higher rates at 30cm and 50cm (data not shown). Again, the highest rates of SOM550 were observed in the 30cm and 50cm depth sections, as well as at 90cm, while at 70cm it was significantly lower. Overall this deeper group demonstrated higher rates of SOM550 than were present in the top 15cm. Within the top 15cm rates of SOM550 were comparable between depth sections, while always highest in the top 5cm, although not significantly so. Notably, SOM550 is higher in the top 15cm within the mono than in the 2-spec. This supports the

previous assertion that herb species were a stronger source of SOM550, and when their growth is stunted by dominant Ryegrass in the higher rooting zones the observed rates of SOM550 were reduced.

DOM

Higher DOM was also observed between 30-90cm compared with the top 15cm. However, this pattern was only significant within the mono ($F_{6,163}$=4.431, P<0.001) (data not shown). A post-hoc Tukey test showed this was primarily driven by contrasting 0-5cm with 50cm, while other significant and approaching significant values were also reported when contrasting other depth sections in the top 15cm with the 50cm and 90cm depth sections. When the short pots were analysed separately no significant variance was reported across or within either diversity treatment, confirming that the only significant variance was in tall pots between the top 15cm and 30-90cm.

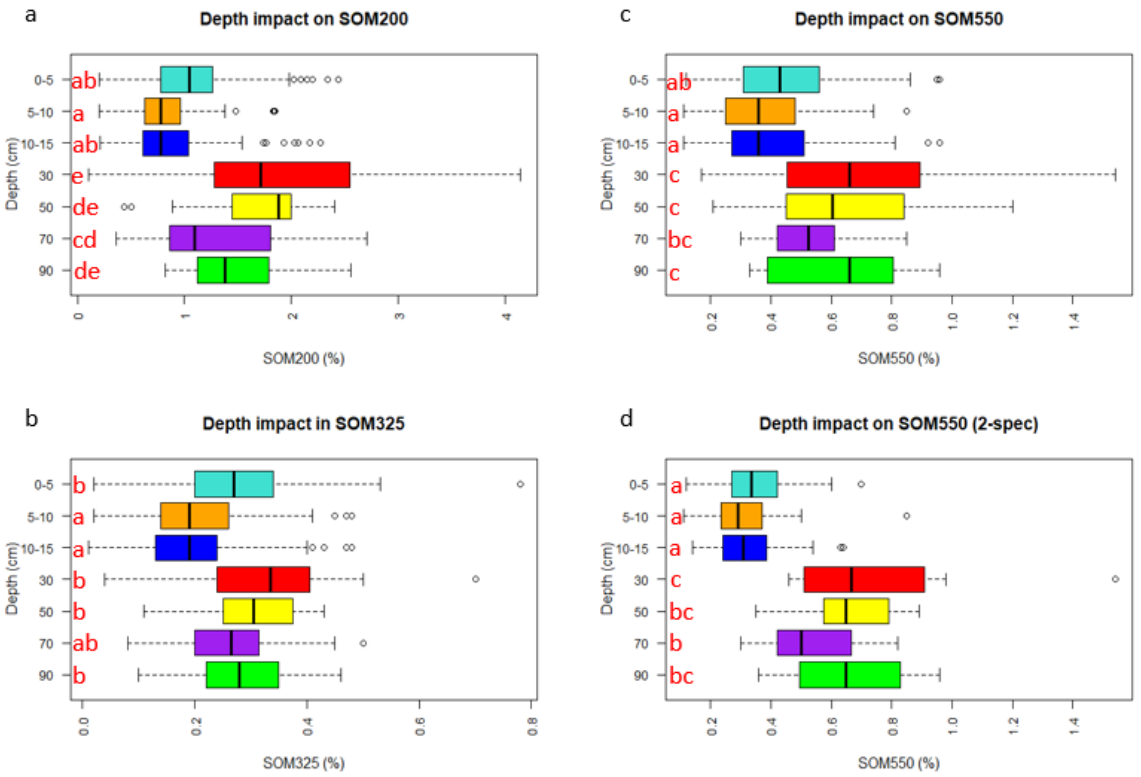

Figure 29: Impact of depth on SOM; a) SOM200, b) SOM325, c) SOM550, d) SOM550 in the 2-spec diversity treatment. Bold line (median), box hinges (1st and 3rd quartile), whiskers (95% confidence interval). Red a-e denote significant variance confirmed by post-hoc test.

GRSP

No significant variance was observed for either GRSP fraction between depth sections.

Variance between inner and outer coring sections

Analysis of variance between inner and outer coring sections only identified significant difference in RB (across both diversity treatments) ($F_{1,286}$=30.62, P<0.001) with more RB present in the inner coring tube. This pattern and significance were also observed within the mono ($F_{1,136}$=26.65, P<0.001), however within the 2-spec the significance reduced ($F_{1,124}$=4.444, P<0.05) as RB in the outer coring tube was substantially increased by the presence of ryegrass RB. No other significant variance was apparent between inner and outer coring sections suggesting that the dynamics of other measured variables were not so laterally localised as to vary between these sections. This suggests that the

impacts associated with increased/decreased root biomass were not limited to the area immediately around the root but were observed across the width of the pot (data not shown)

Correlations between measured variables

SOM

All three SOM fractions were highly correlated. However, some variations in these correlations identify possible drivers which influence the dynamics between these three fractions. SOM200 was positively correlated with SOM325 across both diversity treatments (r=0.67, d.f.=412, *P*<0.001) (Figure 30) and the R^2 value increased within the 2-spec (r=0.8, d.f.=156, *P*<0.001) (data not shown) possibly due to the more uniform RB driven by the dominant Ryegrass. When this correlation was analysed by pot type (across both diversity treatments), the significance remained in both pot types, with a high R^2 value in tall pots (r=0.79, d.f.=139, *P*<0.001) (data not shown) and a reduced one in short pots (r=0.45, d.f.=208, *P*<0.001). When short pots were further subdivided into diversity treatments the R^2 value within the mono reduced further (r=0.32, d.f.=88, *P*<0.01) while the 2-spec increased substantially (r=0.71, d.f.=94, *P*<0.001) in line with the strength of correlation observed in tall pots (data not shown). This suggests that while SOM200 and SOM325 dynamics were similar in pots dominated by ryegrass and in pots containing Chicory and Dandelion (which often produced similar results) greater variability in this correlation was observed between the short pot mono species, perhaps due to the multiple functional groups (shallow and tap-rooting) within this subset.

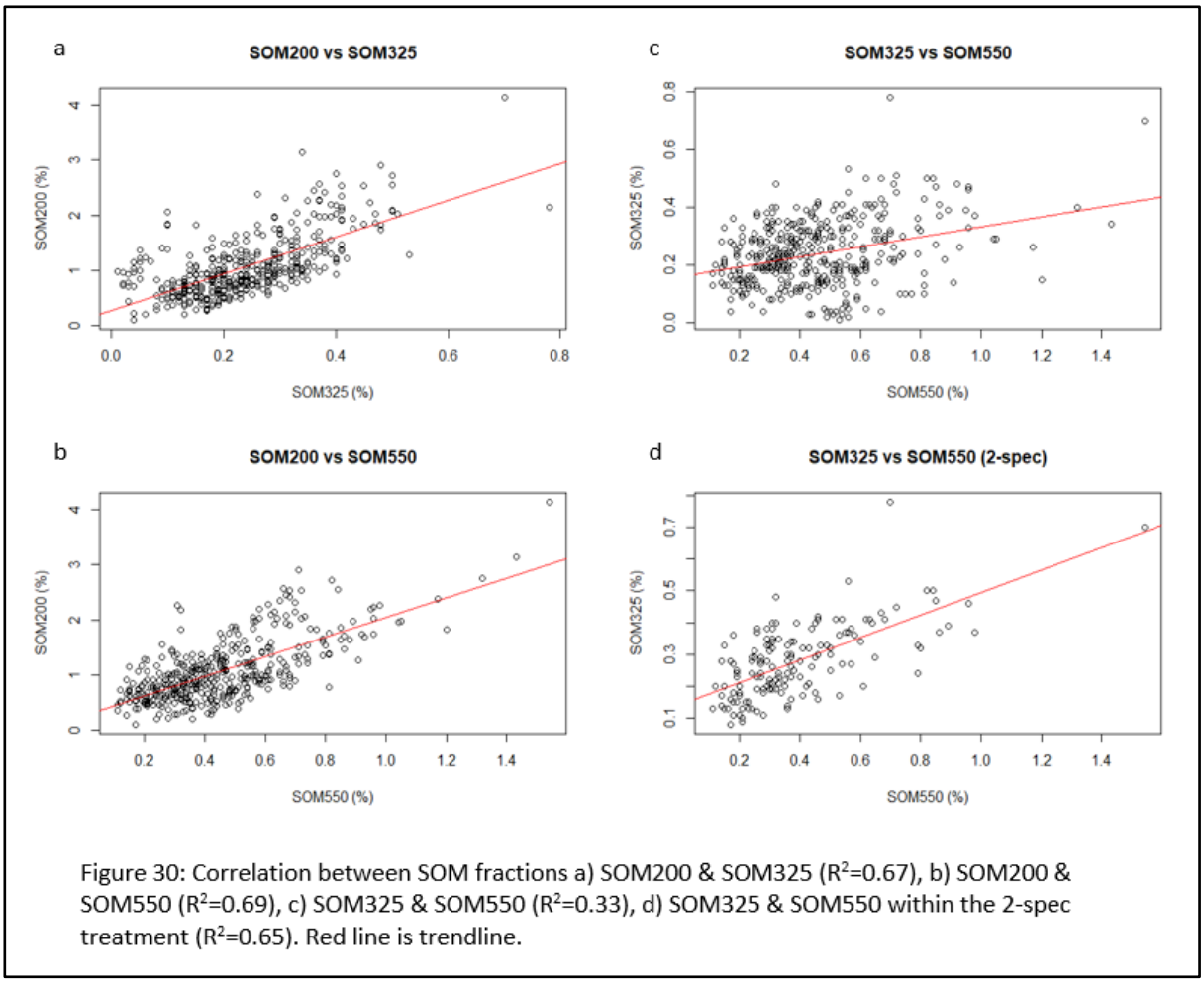

Figure 30: Correlation between SOM fractions a) SOM200 & SOM325 (R^2=0.67), b) SOM200 & SOM550 (R^2=0.69), c) SOM325 & SOM550 (R^2=0.33), d) SOM325 & SOM550 within the 2-spec treatment (R^2=0.65). Red line is trendline.

A similar pattern emerged for SOM550 which was also positively correlated with SOM200 across both diversity treatments (r=0.69, d.f.=411, *P*<0.001) (Figure 30). This significance was also consistent within diversity treatments, while the R^2 values remained the same and increased in the 2-spec (r=0.78, d.f.=156, *P*<0.001) (data not shown). The significance also remained in both pot types across both diversity treatments, and once again the R^2 values increased in tall pots (r=0.76, d.f.=139,

P<0.001) and reduced in short pots (r = 0.47, d.f. = 208, *P*<0.001) (data not shown). However, in this case when short pots were further subdivided within diversity treatments the R^2 values increased in both the 2-spec (r=0.54, d.f.=94, *P*<0.001) and the mono (r=0.77, d.f.=88, *P*<0.001) (data not shown). Overall, this suggests that SOM200 and SOM550 were highly correlated.

Another similar pattern emerged between SOM325 and SOM550 which were positively correlated across both diversity treatments (r=0.33, d.f.=412, *P*<0.001) (Figure 30), although this was the weakest of the overall correlations between SOM200, SOM325 and SOM550. This significance was consistent within diversity treatments, while the R^2 values remained the same or increased substantially in the case of the 2-spec (r=0.65, d.f.=156, *P*<0.001). When pot types were analysed separately the significance disappeared within short pots, however when short pots were further subdivided into diversity treatments both presented similarly significant correlations; mono (r=0.62, d.f.=88, *P*<0.001), 2-spec (r=0.61, d.f.=94, *P*<0.001) (data not shown). This loss of significance which was re-established when diversity treatments were addressed separately suggests a notable variation between SOM325 and SOM550 dynamics within short pots, which may be driven by the presence of the dominant Ryegrass within the 2-spec. While a significant correlation was also apparent in tall pots (r=0.43, d.f.=139, *P*<0.001), the significance and R^2 value decreased within the mono (r=0.23, d.f.=77, *P*<0.05), but increased substantially within the 2-spec (r=0.72, d.f.=60, *P*<0.001) (data not shown). Again, this suggests that the presence of Ryegrass may have effected SOM325 and SOM550 dynamics.

Water content

Given the overall correlations between the three SOM fractions, it was unsurprising that each of these were also correlated with water content. Across both diversity treatments, water content was positively correlated with SOM200 (r=0.5, d.f.=411, *P*<0.001), SOM325 (r=0.37, d.f.=411, *P*<0.001) and SOM550 (r=0.43, d.f.=411, *P*<0.001). The significance and R^2 values were also comparable within diversity treatments. However, when further analysed by pot type, the significance and R^2 values remained consistent within short pots, while in tall pots the R^2 value increased substantially. In tall pots the correlation with water content was as follows; SOM200 (r=0.79, d.f.=138, *P*<0.001), SOM325, (r=0.49, d.f.=138, *P*<0.001), SOM550 (r=0.77, d.f.=138, *P*<0.001). This consistent pattern is exemplified by SOM200 (Figure 31). The distinct increase in water content at lower depths within tall pots offers an explanation for increased SOM at these depths. Drawing parallels with the field sampling exercise, that data showed positive correlations between water content and SOM, albeit water content was higher in shallower soil in that exercise.

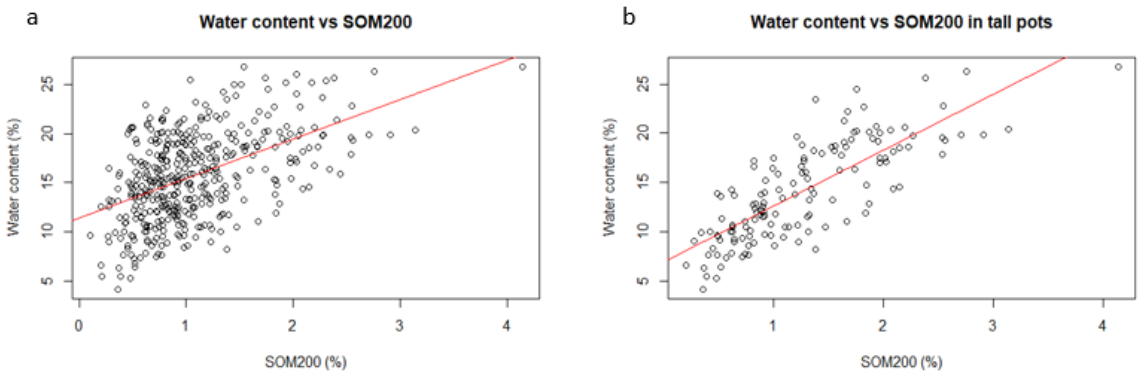

Figure 31: Correlation between water content and SOM200 a) across pot type (R^2=0.5), b) in tall pots (R^2=0.79). Red line is trendline.

Water content was weakly positively correlated with DOM across both diversity treatments (r=0.13, d.f.=412, *P*<0.01). When analysed by pot type the significant correlation disappeared within short

pots, however within tall pots the significance and R^2 value increased substantially (r=0.64, d.f.=138, *P*<0.001) and remained consistent within diversity treatments (data not shown).

SOM, bulk density and water content

While no significant correlations appeared between SOM fractions and bulk density across or within diversity treatments; when analysed by pot type significant negative correlations appeared. These were consistently strongest in tall pots; SOM200 (r=-0.24, d.f.=139, *P*<0.01), SOM325 (r=-0.43, d.f.=138, *P*<0.001), SOM550 (r=-0.56, d.f.=138, *P*<0.001) (data not shown). In tall pots, SOM fractions were consistently highest in deeper samples (between 30-90cm). These sections also consistently demonstrated lower bulk densities than in the top 15cm of tall pots. This suggested that in tall pots SOM was more prevalent in the least dense soils. An alternative explanation was apparent in the strong negative correlation observed between bulk density and water content. Across both diversity treatments and pot types this was (r=-0.49, d.f.=413, *P*<0.001), while in tall pots the R^2 value increased substantially (r=-0.74, d.f.=139, *P*<0.001) (data not shown). Therefore, within tall pots the higher SOM in less dense samples could instead be explained by increased water content.

GRSP

No clear correlations were observed between GRSP and other variables.

Principal component analysis

When PCA was applied to the glasshouse dataset, over 40% of the variance was explained by the first two principal components (22.9%, 19.3%). When these two principal components were used to establish an axes, it became apparent that there were several components which made up the primary (x) axis. Statistically SOM200 demonstrated the highest positive correlation, but this was closely followed by water content and SOM550; while pH presented a contrasting negative correlation. The secondary (y) axis contrasted the positive correlation with GSB and the negative correlation with HSB.

From the composition of the primary axis it could be interpreted that higher water content was associated with higher SOM200 and SOM550. An explanation for this was that higher water content could be stalling aerobic metabolism of the SOM compounds. Additionally, pH was lower in samples with higher water content, leading to soil acidification, perhaps through the production of organic acids through anaerobic microbial metabolism. The composition of the secondary axis can be explained more simply due to the contrasting presence/absence of Ryegrass in each half of the samples.

This PCA was plotted with samples sequentially grouped using the three independent variables which provided the most explanatory power during previous analyses; species, diversity and depth. The grouping which provided the most useful visualisation of the data was when it was grouped by species (Figure 32). This shows the Ryegrass control and the other four short pot species within the 2-spec to be closely aligned along both axes and overlapping with the 2-spec Chicory and Dandelion which were also both closely aligned. It also shows close alignment with the mono Plantain, Yarrow and Birdsfoot, and a further close alignment of the mono Chicory, Dandelion and to a degree the mono Clover. In most cases samples within these species groupings are spread in an elongated form across the length of the primary axis, but narrowly bunched along the secondary axis, demonstrating the impact that the presence/absence of Ryegrass had on this analysis.

The composition of the secondary axis , contrasting GSB with HSB, was perhaps unsurprising as this reflects the experiments contrasting diversity treatments. Consequently, in this analysis the value for GSB represents a proxy for the independent diversity variable through its presence/absence within samples. To adjust for any bias this may have caused, a second PCA was conducted which excluded the GSB data. This does not imply that the influence of GSB was unimportant, however including it alongside the fully dependent variables could mask other results.

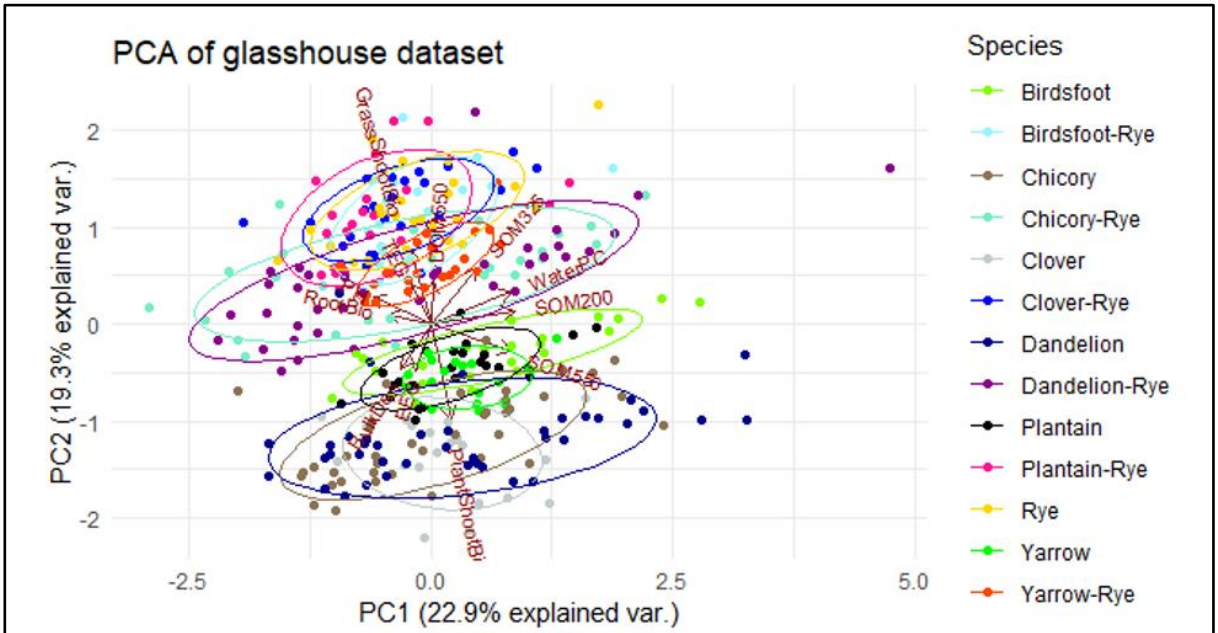

Figure 32: PCA of glasshouse dataset (grouped by species)

In the second PCA (without GSB), again over 40% of the variance was explained by the first two principal components (24.8%, 19.2%). In this PCA the same components made up the primary axis as were found in the first PCA which included GSB (water content, SOM200 and SOM550, contrasting with pH); while the secondary axis now contrasted the positive correlation with DOM and the negative correlation with bulk density, closely followed by HSB and EE-GRSP.

Several possible explanations for the composition of this new secondary axis present themselves. Firstly, DOM may be higher in less dense soils with greater soil pore volume, resulting in a larger soil surface area for the DOM to adhere to. Secondly, DOM may be higher in soils where less water was being lost through evapotranspiration due to smaller leaf area and consequently remains for longer in the soil causing compounds to dissolve. However, visualisation of the data brings this second explanation into question, as the samples with high DOM are heavily concentrated within the 2-spec diversity treatment. In this treatment HSB was often lower, however this was due to competition from the vigorous Ryegrass. Therefore, the high rates of DOM may be better explained by the presence of Ryegrass, rather than by the lower values of HSB.

Again, this PCA was plotted with samples sequentially grouped using species, diversity and depth. When the data was grouped by species there was again a close alignment between numerous similar species, however in this case the mutuality of groupings shifted (Figure 33). The Ryegrass control and the Plantain, Clover and Birdsfoot within the 2-spec were closely aligned along both axes. These groupings overlapped with Yarrow in the 2-spec which in turn overlapped with the four short pot species within the mono. Samples from both diversity treatments of Chicory and Dandelion were also loosely grouped. As already alluded to, this spread of data was partly explained when it was grouped by diversity treatment as this clearly showed a clustering of samples from the 2-spec which were positively correlated with DOM (Figure 33). However, the mutual groupings of tall spot species appear to be influenced heavily by the unusually high rates of bulk density recorded in tall pots, as referenced previously.

The results of both PCAs (including and excluding GSB data) were also examined to consider the explanatory power of the third principal component, however in both cases this produced no noteworthy findings.

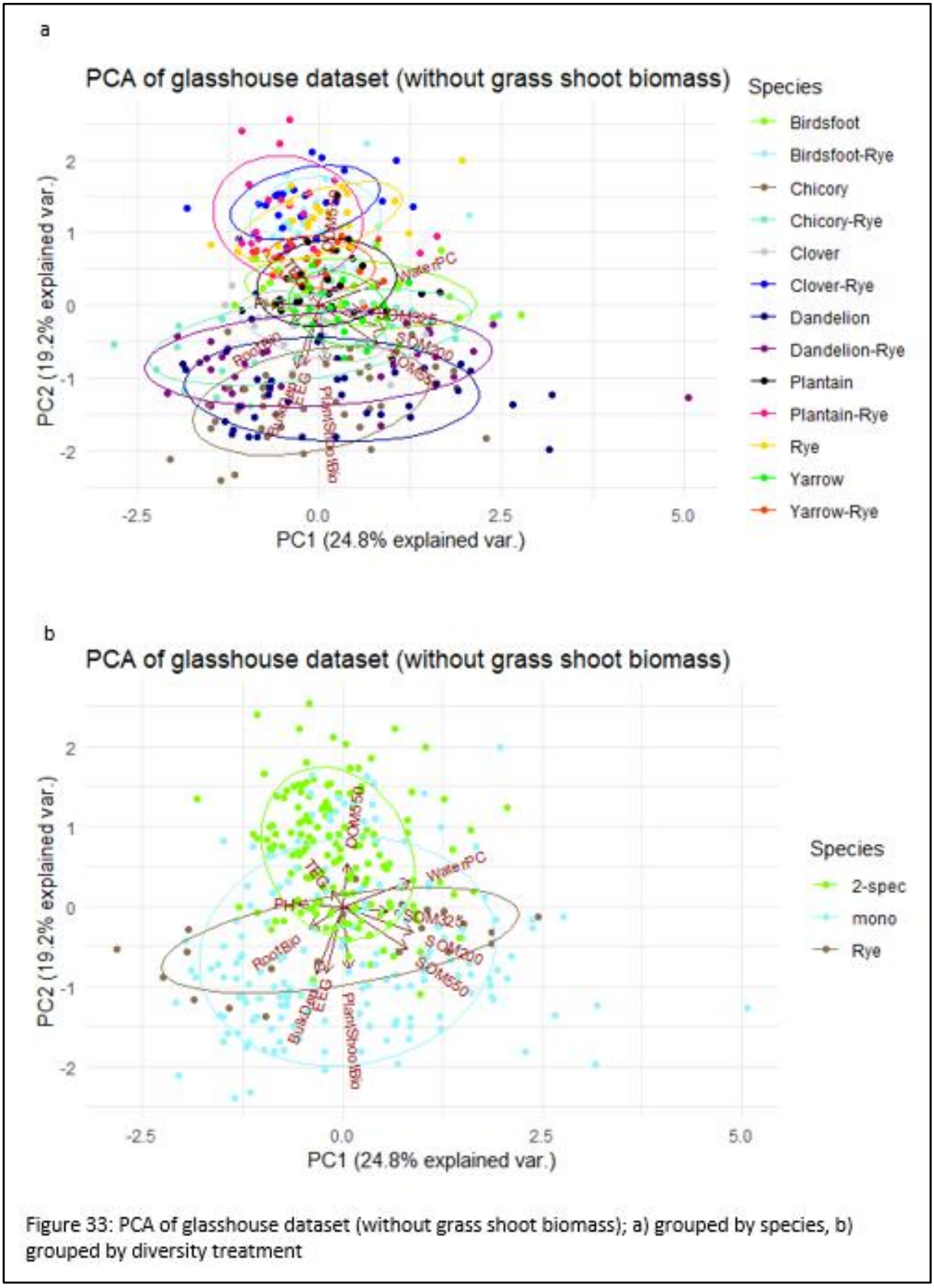

Figure 33: PCA of glasshouse dataset (without grass shoot biomass); a) grouped by species, b) grouped by diversity treatment

The result of the PCAs appear to support findings from other analyses, that water content is an influential factor in the prevalence of SOM. However, PCA analysis also identified an association between Ryegrass and higher rates of DOM, a finding which had not been clearly identified in other analyses. This suggests that Ryegrass may produce a different range of SOM products than occur in herb-dominated swards, or that there are a different set of microbial communities associated with

Ryegrass which selectively metabolise different SOM products. The latter argument is supported by the presence of the negatively correlated EE-GRSP on the secondary axis of the second PCA (without grass) in contrast to the positively correlated DOM at the far end of the axis. This suggests that microbial communities associated with EE-GRSP production were less prevalent in Ryegrass dominated soils where DOM was more prevalent.

Summary analysis of main glasshouse experiment

Species and diversity treatments

When results from the rooting characteristics analysis were compared with those for the main glasshouse experiment it was clear that values for both HSB and RB (as determined from the whole-pot analysis described later) were consistent across both exercises. One notable difference was a lower HSB value for Yarrow in the main glasshouse experiment, although despite this the overall pattern across the six species was still very similar. A second notable difference was a lower RB for Chicory and Dandelion in the main glasshouse experiment compared with the root scanning exercise, however this can be explained by the fact that root scanning accounted for the entire root stock, while the main glasshouse experiment only included root obtained from cored sections, and below 15cm only small amounts of root were obtained from the four small horizontal corers. Despite this, the overall pattern for RB was remarkably similar which demonstrates the consistency of plant growth in the two exercises. This in turn underlines the feasibility of using findings from the root scanning process to inform the understanding of root development in the main glasshouse experiment; where again no consistent correlation was observed between HSB and RB.

One clear finding from the main glasshouse experiment was the substantial inhibition of plant growth (determined not only by HSB but also by unusually sparse and sickly shoot growth) observed when herbs (particularly short pot herbs) were grown with Ryegrass. It was unclear whether this stunted herb growth resulted from an inferior competition ability for resources (water, nutrients, root space, light) or from some other influence (such as the potential for an allelopathic effect or a change in microbial communities driven by the presence of Ryegrass) (Cong & Erikson 2018, Newman & Rovira 1975). However, given that in an environmental setting these herbs are unlikely to grow without competition from Ryegrass or other competitive grasses (given their prevalence in agricultural grasslands) these impacts should be noted.

When SOM was analysed between species and diversity treatments several findings emerged. Firstly, SOM200 demonstrated minimal variance across all treatments, except for a slightly elevated value for Chicory in both diversity treatments. This relatively uniform response across species and diversity treatments suggests two possible interpretations; firstly, a standard rate of both the production and turnover of this fraction across treatments, or secondly that while SOM200 probably contained some organic matter, the primary factor in mass-loss during the LoI process at this temperature was driven by remaining water evaporation as identified by Rovira & Vallejo (2000) in their assessment of differential thermogravimetry. If this is the case, while the value for SOM200 cannot be discounted – because in all likelihood the fraction does represent some organic matter – the actual value should be treated with some caution as it may reflect a degree of additional water evaporation.

The second observation from the analysis of SOM was that SOM325 was significantly lower in the mono for Dandelion, Yarrow and particularly Clover, while SOM325 was also low in both diversity treatments for Plantain. The higher SOM325 generally observed in the 2-spec treatment suggests an increase in SOM325 may have occurred due to the increased biomass produced by Ryegrass in this treatment. The fact that the higher SOM325 in the 2-spec of Chicory was not also significant may reflect the comparative vigour of Chicory which may consequently have only enjoyed a slight increase in SOM325 production due to the accompanying Ryegrass. However, the increased biomass theory does not explain why SOM325 was higher (albeit not significantly) in the mono of Birdsfoot. Conversely, one possible explanation for the low SOM325 observed in both diversity treatments for Plantain was provided by Cong & Erikson (2018) who found that including Plantain in a Ryegrass and

Red Clover ley 'increased the relative decomposition rate of the labile C pool'. This would represent a priming effect as referenced previously.

In contrast to SOM325, an opposing dynamic was observed when SOM550 was analysis between species and diversity treatments. In this case SOM550 was higher in the mono for all short pot species while Dandelion and Chicory were comparable between treatments. SOM550 was also lower in the Ryegrass control than in all species in the mono (except for Chicory which unusually was the poorest performer). This significant trend suggested two possibilities; either herb species (in contrast to Ryegrass) produced greater quantities of the more persistent SOM550 or the absence of Ryegrass in the mono reduced the impact of a priming effect. When herb growth was inhibited by competition from Ryegrass the SOM550 input from herb growth may have been reduced. It is noteworthy that the three lowest values for SOM550 were observed in the 2-spec for Clover, Birdsfoot and Plantain, the first two of which were particularly stunted by the competitive Ryegrass (as determined by informal visual observation and comparative measurements of HSB). Conversely, if the presence of Ryegrass in the 2-spec caused a priming effect through increased microbial activity stimulated by the additional Ryegrass biomass (Bird et al. 2011), then its absence in the mono may have reduced or prevented the impact of a priming effect. While it is unclear which of these mechanisms influenced the observed results, it was clear in this case that persistent SOM550 was highest in the short pot herb monocultures. Neither of these theories address why SOM550 in tall pots was not significantly higher in the mono. One possible explanation was that for tall pot species the energy resources were directed into establishing their extensive rooting systems instead of into root exudation; or possibly that such an extensive root network may have diluted the signal sufficiently to avoid significant differences throughout.

The fact that an inverse relationship was observed between persistent SOM550 and labile SOM325 (higher SOM550 in the mono and lower in the 2-spec, and higher SOM325 in the 2-spec and lower in the mono) in several of the species reflects the findings from the field sampling exercise. This found a significant negative correlation between SOM325 and SOM550 within samples. While this was not the case in the main glasshouse experiment (in which a significant positive correlation was found between SOM325 and SOM550 within samples), the overall findings of generally higher SOM325 in the 2-spec and lower rates of SOM550 in the 2-spec (both in comparison with the mono) do underline the variable dynamics observed between these two fractions.

Interestingly, similar contrasting fraction dynamics were also observed when each GRSP fraction was analysed between species and diversity treatments. EE-GRSP was consistently higher in the mono, and while this was not significant for all species, the difference was most marked for the shallow rooting Clover and Birdsfoot. Again, this poses the question; was the lower EE-GRSP in the 2-spec the result of increased decomposition of this labile fraction in the higher biomass treatment containing the Ryegrass or was this instead a response to reduced herb vigour in the 2-spec due to Ryegrass competition (Bird et al. 2011). The fact that the lowest values for EE-GRSP were observed in the 2-spec of Clover and Birdsfoot (in which herb growth was most inhibited) as well as in the Ryegrass control (which contained no herb) suggests the second theory may better explain these findings. This assertion is further supported by the fact that within the 2-spec, EE-GRSP was highest for Chicory and Dandelion, both of which showed strong vigour despite Ryegrass competition. In contrast, RE-GRSP was consistently higher in the 2-spec for all species (except Yarrow). This suggests that RE-GRSP was highest when Ryegrass was present. Given that GRSP is thought to be AMF-associated, this may be a result of increased fungal communities prompted by the extensive Ryegrass root systems, rather than a direct consequence of increased root biomass per se.

While this contrasting fraction dynamics is similar to that observed between SOM325 and SOM550, the most notable difference is that EE-GRSP is considered the more labile of the two GRSP fractions, while RE-GRSP is considered to be the more soil-bound and more recalcitrant of the two. Therefore,

while the more labile of the pyrolysable SOM fractions (SOM325) was more prevalent in the 2-spec, it was the more persistent RE-GRSP which was found in higher quantities in this treatment.

DOM was often higher in the 2-spec, however the strength and consistency of this variance pattern, as well as the high in-species variance sometimes observed within the 2-spec made it difficult to draw strong conclusions from these results. Similar can be said of the SMP and EPS analysis as many of the samples produces values below detectable levels and when detectable results were produced there was often a high degree of in-species variance or a lack of results from other samples for effective comparison.

At the outset it was hypothesised that greater plant species diversity was expected to produce more organic matter and was also expected to produce more microbial-derived organic matter, which in this study was determined through measurements of GRSP, SMP and EPS; although results for SMP and EPS were not sufficiently robust to draw conclusions from, therefore GRSP was the sole representation of microbial-derived organic matter used to address these hypotheses. As was apparent from this analysis the answer may not be as straightforward as the hypotheses implied, as different fractions displayed variable responses to changes in plant diversity. Higher plant diversity (as represented by the 2-spec) produced higher values of SOM325 and RE-GRSP, while the same treatment produced lower values of SOM550 and E-GRSP. However, when additional values were calculated for the combined values of all three pyrolysable SOM fractions (Combined SOM) and also for both GRSP fractions (Combined GRSP), these values were in fact both consistently higher within the mono diversity treatment (data not shown).

Soil depth

When analysing results in relation to changes between depths it was clear that because species were grown in two different pot types (tall and short) results were likely to vary between these two systems. It would only have been possible to avoid this by growing all plants in the same pot type. If short pots had been used for all species, this would have unacceptably limited the natural rooting characteristics of Chicory and Dandelion. The alternative would have been to grow all plants in tall pots, however the additional cost (preparation time, processing time, pot resources, soil resources) was considered inviable and unnecessary given the rooting extent expected of the four short pot species in the given growth period. While it is conceivable that an increase in the RB of short pot species could have been observed if they too had been grown in tall pots, based on the degree of pot space utilisation observed during harvesting this seems unlikely.

The advantages of using tall pots were clear in some of the results, as were some of the disadvantages. For instance, it was immediately apparent from a preliminary review of the data that bulk density within tall pots was significantly greater than in all short pots, presumably due to the increased weight of the vertical pillar of soil held within tall pots. However, because there was minimal variation in bulk density within pot types, and because zero compaction was observed during coring, it seemed that the mineral particulate nature of the chosen soil material was resistant to plant growth process-driven fluctuations in bulk density, and that the primary factor altering bulk density in tall pots was the compressive effects when a heavy column of soil was placed within a relatively narrow, tall pot. The remaining minimal variation in bulk density was observed in the topsoil; in short pots the top 5cm was least dense, presumably due to the disruptive effect of plant roots which were typically most prevalent in the top 5cm. Conversely in tall pots the top 15cm was the most dense; it was theorised that this may be due to the significant expansion of the thick Chicory and Dandelion tap roots which may have laterally compressed the soil against the fixed sides of the comparatively narrow pot.

While in the case of bulk density some disadvantages of using tall pots emerged, benefits also emerged including the ability to observe the extensive percolation of water down the soil profile. In contrast to the field sampling exercise, in the glasshouse experiment water content increased with

soil depth, although a slightly higher water content was also observed in the top 5cm in contrast to the section immediately below, which presumably related to increased water holding capacity prompted by enhanced root-soil aggregation in the primary rooting zone. This was the case in both short and tall pots, while in tall pots the percolation trend was easier to observe due to the higher number of sampling points.

Changes at depth were also observed for SOM and DOM. Measurements for each of SOM200, SOM325 and SOM550 were highest in the top 5cm than in the rest of the top 15cm, however while this trend was apparent in both pot types, in tall pots significantly higher rates of each fraction were also observed between 30-90cm in comparison to those observed in the top 15cm. For DOM the highest rates were observed between 40-90cm, however there was no discernible difference between depths within the top 15cm as there was with SOM.

These results observed when viewing the entire profile of measured depths are largely in contrast with those seen in section 2.3. In that exercise SOM200, SOM325 and RE-GRSP all decreased as depth increased, while SOM550 and EE-GRSP showed no clear correlation with soil depth, and DOM was the only measured organic matter fraction to increase with depth. Two possible explanations for this were proposed to explain this field phenomenon; firstly, that higher bulk density at depth had compacted soil pores leading to reduced biotic dynamics in deeper soil; and secondly that increased moisture in shallower soil promoted new SOM development through increased microbial activity. Both explanations could also be applied to the results obtained from the main glasshouse experiment; this data showed a general increase in water content at depth which was in line with SOM and DOM, as well as a more complicated pattern for bulk density which was also consistent with measurements of SOM and DOM (lower bulk density in the top 5cm than elsewhere in the top 15cm, and also a lower bulk density between 40-90cm in tall pots).

While DOM followed the same pattern as SOM in tall pots, particularly in the mono, the significance of this pattern was absent in short pots. In short pots there was no correlation between DOM and either water content or bulk density. This may reflect the inhibition of natural process in short pots when considering DOM. In an environmental setting organic matter which is susceptible to dissolve in aqueous solution is likely to flow into deeper soil as water percolates through the profile, however when DOM, transported in this way, enters dryer soil below the topsoil, it is likely to be absorbed within aggregated soil pores. This would lead to more DOM in deeper soils, as was observed in section 2.3 and as was predicted by Rumpel & Kogel-Knabner (2011). However, in a depth-limited growth environment (a 16cm deep plant pot) the natural increase in DOM along a depth gradient is likely to be interrupted by the pot base or lost from the pot through drainage. This may explain why in this experiment DOM was not correlated with either water content or bulk density in short pots but only in tall pots.

Unlike in section 2.3, GRSP showed no clear pattern of variation between depths and no significant correlation with water content or bulk density. Significant variance in GRSP only appeared to be consistently influenced by species association in this experiment. Given that GRSP is thought to be AMF-associated, this influence by species association may imply a positive/negative relationship with certain rooting characteristics. This will be addressed further.

At the outset it was hypothesised that organic matter was expected to decrease in deeper soils. While the results in section 2.3 appeared to support this hypothesis (albeit not in relation to all fractions, but as an overall trend), the results of the main glasshouse experiment did not, as overall organic matter (again there was variation between fractions) was higher in deeper soil. However, an understanding of the mechanisms which were thought to be involved in the accumulation of organic matter casts this hypothesis in a fresh light. If it is correct, as has been postulated in this analysis, that variation in water content and or bulk density has a substantial impact on the accumulation of organic matter, then rather than being a subject to soil depth per se, proportions of organic matter

in soil may instead be partly dependent on these physical soil conditions. This would explain why higher rates of organic matter were generally observed in deeper soils in the main glasshouse experiment, while in the field sampling exercise higher rates were generally observed in shallower soil.

Whole-pot analysis

As indicated previously, measuring RB on a g/cm^3 basis was useful for understanding root associations with other variables between coring segments. However, the substantial in-pot variance in RB between sampling segments (due to substantially higher RB in shallower segments) made it difficult in some cases to draw conclusions regarding the impact of RB on measured soil variables. Also, the lack of significant variance observed for most measured variables (except RB) between inner and outer coring tubes suggests that the impacts of RB were not limited to localised areas within each coring tube and instead proliferated throughout the pot. Therefore, data was further analysed on a whole-pot basis, using a total value for RB within the pot (or rather within the entire coring area) and an averaged value for each measured organic matter fraction from all samples taken within each pot. While this made values for each fraction less precise it allowed an assessment of the overall impact of total-RB on pot soil in a way which avoided the substantial in-pot variance observed previously.

Briefly, this analysis showed that per gram of root, most of the organic matter fractions were actually higher in the mono and also in the species with less RB, whereas the 2-spec and the mono Chicory and Dandelion all recorded much lower values. This suggests that the amount of each quantified fractions was not directly proportional to a plant's RB.

Root biomass

Variance in RB between diversity treatments was only approaching significant, however analysed at species level across both diversity treatments it became significant ($F_{12,37}$=20.53, P<0.001) (Figure 34). Unsurprisingly RB was higher in the 2-spec for all species (except Chicory for which the reverse

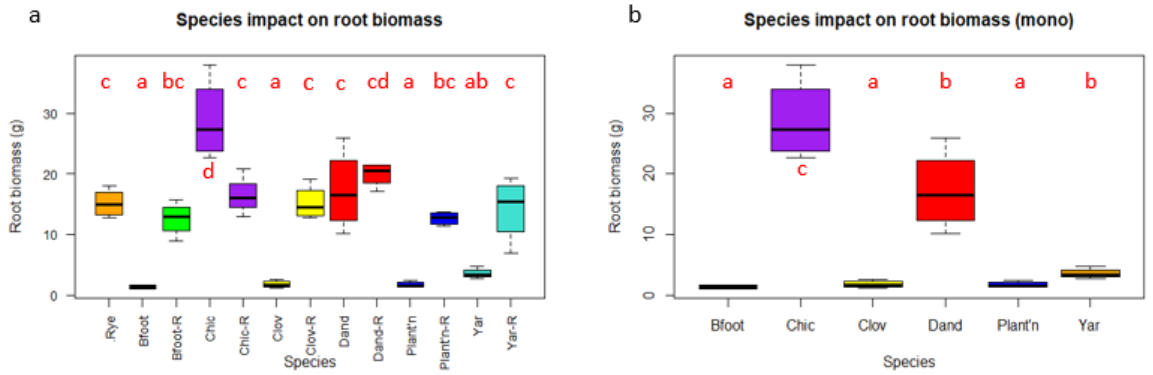

Figure 34: Species impact on root biomass; a) across diversity treatments, b) within the monoculture diversity treatment. Bold line (median), box hinges (1st and 3rd quartile), whiskers (95% confidence interval). Red a-d denote significant variance confirmed by post-hoc test.

was true), likely driven by the extensive Ryegrass root. A post-hoc Tukey test showed this inter-diversity variance was significant for Birdsfoot, Clover, Plantain, Yarrow and Chicory but not Dandelion. A post-hoc Tukey test showed that further significant variance observed within the mono ($F_{5,17}$=31.73, P<0.001) was primarily driven by higher RB in both Chicory and Dandelion in contrast to the other four species. A less significant variance was also observed within the 2-spec ($F_{5,17}$=30.59, P<0.05) which according to a post-hoc Tukey test was driven by a slight increase in Dandelion RB compared with all other species which showed comparable RB (data not shown).

SOM200

No clear pattern emerged when SOM200 was analysed. SOM200 was higher in the mono for Chicory, Dandelion, Yarrow and Clover, and higher in the 2-spec for Birdsfoot and Plantain, however none of the variance between diversity treatments was significant (Figure 35). In fact, when variance was assessed at species level, the only significant variance ($P<0.05$) between species was within the mono between Chicory (which had the most SOM200) and both Plantain and Birdsfoot. All other species combinations within and across both diversity treatments were not significantly dissimilar.

SOM325

Variance in SOM325 between diversity treatments was significant ($F_{2,47}=7.203$, $P<0.01$) with more SOM325 in the 2-spec mirroring previous findings (Figure 35). When this variance was analysed at species level across both diversity treatments the significance increased ($F_{12,37}=10.15$, $P<0.001$). A post-hoc Tukey test showed this was driven by lower SOM325 in the mono for Plantain, Yarrow and Clover. Significant variance was also observed within the mono ($F_{5,17}=17$, $P<0.001$) while a post-hoc Tukey test showed this was driven by high SOM325 in Chicory and Dandelion and by low SOM325 in Clover, compared with the remaining species. There was no significant difference in SOM325 between species within the 2-spec, suggesting that a uniformity of pot conditions driven by the presence of the dominant Ryegrass may have produced comparable values for SOM325 (data not shown).

SOM550

Variance in SOM550 between diversity treatments was also significant ($F_{2,47}=15.07$, $P<0.001$) with more SOM550 in the mono, again mirroring previous findings (Figure 35). This significance remained when analysed at species level across both diversity treatments ($F_{12,37}=12.18$, $P<0.001$). SOM550 was higher in the mono than the 2-spec for all species (except Chicory which was comparable), however a post-hoc Tukey test showed this variance was only significant for Birdsfoot, Clover and Plantain. Clover and Birdsfoot, and to a slightly lesser extent Plantain and also Yarrow were notably inhibited by competitive Ryegrass and as such the significant higher SOM550 in mono for the first three of these species again underlined a possible link between strong herb growth and SOM550. Significant variance was also observed within the mono ($F_{5,17}=6.496$, $P<0.01$), while a post-hoc Tukey test showed this was driven by an unusual divergence between the two deep-rooting species; with SOM550 highest in Dandelion and lowest in Chicory. Significant variance was also apparent between species within the 2-spec ($F_{5,17}=24.48$, $P<0.001$) and a post-hoc Tukey test showed this was driven again by high SOM550 in Dandelion and in this case also by low SOM550 in Clover and Plantain which as stated above was unsurprising if the link between herb growth and SOM550 is advocated (Figure 35).

DOM

Variance in DOM between diversity treatments was significant ($F_{2,47}=4.427$, $P<0.05$) and when analysed at species level across diversity treatments this increased ($F_{12,37}=7.69$, $P<0.001$) (Figure 36). However, a post-hoc Tukey test revealed this significance was due to extremely high DOM in the 2-spec Plantain, while all other species across both treatments were comparatively similar. Unsurprisingly, the Plantain also drove significant variance within the 2-spec ($F_{5,17}=9.661$, $P<0.001$), however variance was also significant within the mono ($F_{5,17}=5.849$, $P<0.01$) and a post-hoc Tukey test showed this was driven by high DOM in Dandelion and in Plantain (data not shown). Aside from both Plantain treatments and the mono Dandelion, DOM was not significantly different between species on a whole-pot basis. While not significant (besides Plantain) it is noteworthy that DOM between diversity treatments was highest in the 2-spec for Plantain, Clover, Birdsfoot and the Ryegrass control. Clover and Birdsfoot and to a lesser extend Plantain were the species most restricted by Ryegrass in the 2-spec, and therefore these, along with the Ryegrass control, are the pots in which Ryegrass was most dominant, implying a possible link between DOM and Ryegrass.

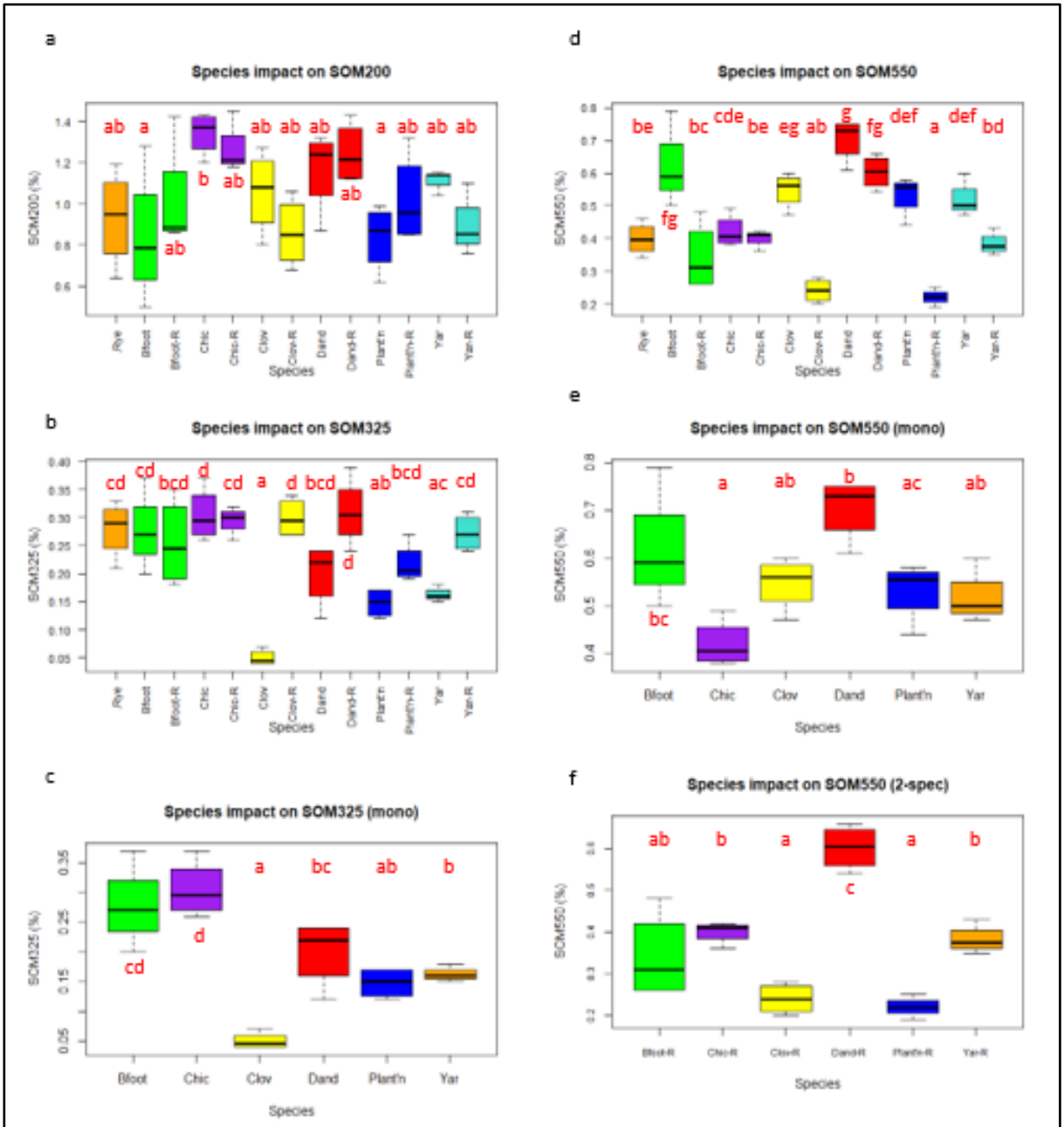

Figure 35: Species impact on SOM; a) SOM200, b) SOM325, c) SOM325 within the monoculture diversity treatment, d) SOM550, e) SOM550 within the monoculture diversity treatment, f) SOM550 within the 2-spec diversity treatment. Bold line (median), box hinges (1st and 3rd quartile), whiskers (95% confidence interval). Red a-g denote significant variance confirmed by post-hoc test.

EE-GRSP

When analysed at species level, variance in EE-GRSP between diversity treatments was significant ($F_{12,37}$=10.04, P<0.001), with higher EE-GRSP in the mono for all species (except Plantain which was comparable) (Figure 36). However, a post-hoc Tukey test revealed this significance was only driven by low EE-GRSP in the 2-spec pots of Clover and Birdsfoot. As identified previously these two species performed poorly when grown in combination with Ryegrass and the lack of EE-GRSP in the 2-spec for these two species suggests EE-GRSP may be mediated more by the presence of a herb plant than by grass.

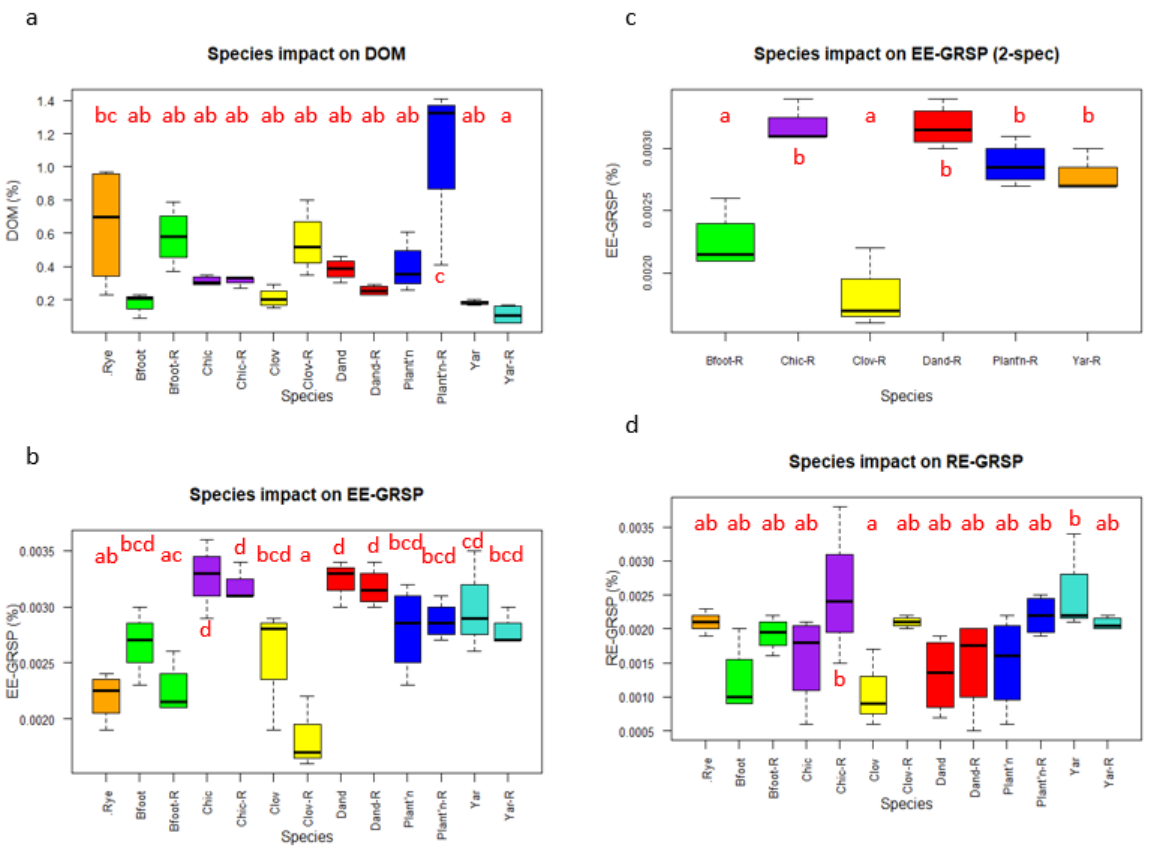

Figure 36: Species impact on DOM and GRSP; a) DOM, b) EE-GRSP, c) EE-GRSP in the 2-spec diversity treatment, d) RE-GRSP. Bold line (median), box hinges (1st and 3rd quartile), whiskers (95% confidence interval). Red a-d denote significant variance confirmed by post-hoc test.

Associations between carbon fractions and RB

Following this assessment of measured organic matter fraction variance between species and diversity treatments, this data was further analysed in the context of the total-RB data to measure the association between RB and the value of each measured organic matter fraction. To achieve this the average pot values for each carbon fraction (measured in g/cm^3 of soil) were divided by the total-RB within each pot to establish fraction per gram of root (g/g-root). A series of analyses were run to examine variance for each measured organic matter fraction.

Significant variance was observed in SOM200/RB between species across both diversity treatments ($F_{12,37}$=19.47, P<0.001). A post-hoc Tukey test revealed this variance was driven by higher SOM200/RB in the mono pots of Birdsfoot, Clover and Plantain in contrast to all other species in both diversity treatments. A similarly significant pattern was observed for SOM325/RB ($F_{12,37}$=42.56, P<0.001). A post-hoc Tukey test revealed this variance was driven by higher SOM325/RB in the mono pots of Birdsfoot and Plantain in contrast to all other species in both diversity treatments. Again, a similarly significant pattern was observed for SOM550/RB ($F_{12,37}$=31.51, P<0.001). A post-hoc Tukey test revealed this variance was driven by higher SOM550/RB in the mono pots of Birdsfoot, Clover and Plantain in contrast to all other species in both diversity treatments. For SOM200/RB and SOM325/RB, no significant variance between species was apparent within the 2-spec, and while a significant variance in SOM550/RB within the 2-spec was reported ($F_{5,17}$=31.36, P<0.05), a post-hoc Tukey test revealed no significant combinations (Figure 37).

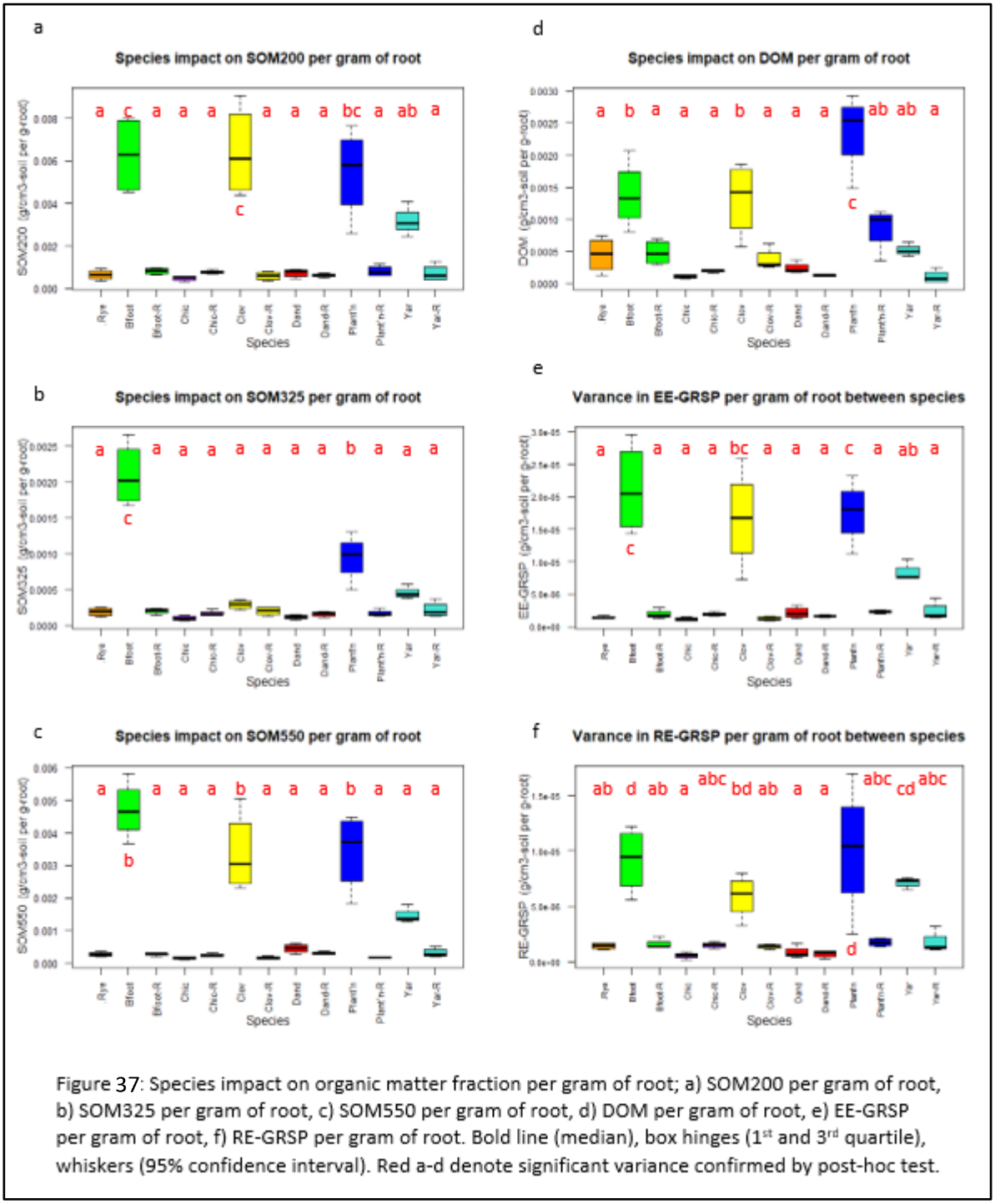

Figure 37: Species impact on organic matter fraction per gram of root; a) SOM200 per gram of root, b) SOM325 per gram of root, c) SOM550 per gram of root, d) DOM per gram of root, e) EE-GRSP per gram of root, f) RE-GRSP per gram of root. Bold line (median), box hinges (1st and 3rd quartile), whiskers (95% confidence interval). Red a-d denote significant variance confirmed by post-hoc test.

The significant patterns observed for the three SOM fractions was also shown in DOM/RB between species across both diversity treatments ($F_{12,37}$=17.14, P<0.001). A post-hoc Tukey test revealed this variance was driven by higher DOM/RB in the mono pots of Birdsfoot, Clover and Plantain in contrast to all other species in both diversity treatments. A significant variance in DOM/RB between species was also apparent within the 2-spec ($F_{5,17}$=9.525, P<0.001) and a post-hoc Tukey test confirmed this variance was driven by the extremely high DOM observed in the 2-spec Plantain referred to previously. Significant variance between species across both diversity treatments was also observed for both EE-GRSP/RB ($F_{12,37}$=19.06, P<0.001) and RE-GRSP/RB ($F_{12,37}$=11.15, P<0.001). Post-hoc Tukey tests showed these variances were both driven by higher GRSP/RB in the mono pots of Birdsfoot, Clover and Plantain (and also Yarrow in the case of RE-GRSP/RG) in contrast to all other species in both diversity treatments (Figures 37). No significant variance in GRSP/RB between species was apparent within the 2-spec.

Overall this series of analyses showed that when fractions were measured in this way (g/g-root), significantly higher rates were found in the mono pots of Birdsfoot, Clover, Plantain and to a lesser degree Yarrow. Previous analysis has already established that RB in these four species within the mono was significantly lower than Chicory and Dandelion as well as their counterparts in the 2-spec. The fact that these four species within the mono showed significantly low RB but significantly higher measures of fraction (g/g-root) suggests that RB was not a strong indicator of fraction value when measured on a whole-pot basis. If RB were a strong indicator of fraction value (g/g-root) then lower measurements of each fraction value would have been observed within the mono of Birdsfoot, Clover, Plantain and Yarrow due to their significantly lower RB, while higher rates would have been observed for Chciroy, Dandelion and to some degree for all 2-spec species due to the greater RB in these treatments. However, this was not the case which suggests that variable rates of RB did not lead to significantly variable rates of each measured organic matter fraction.

From this analysis it would appear that on a gram-root to gram-SOM basis, planting a mixture of less-extensively rooting species (Clover, Birdsfoot, Plantain, Yarrow) was a more effective way of increasing SOM.

Summary of whole pot analysis

When considering the RB data, the results provided when RB was collated in the whole-pot analysis provided a more informative perspective on the influence of RB on other variables than was provided by RB when calculated as g/cm^3 as the transformation of this data allowed for substantial in-pot variance in RB to be discounted. Unfortunately, given the variable volumes of core sections between inner and outer coring tubes, calculating g/cm^3 was the only way to allow comparison between all samples. However, given the significant in-pot variance in RB between samples this measure of RB was only helpful in interpreting RB between depths, not between species. As it was, analysis of variance between the inner and outer coring tubes produced no significant results, other than to show that RB was higher in inner coring tubes than outer coring tubes (and even this sole significant finding reduced in the 2-spec due to the presence of Ryegrass). Consequently, this suggested that any influence from RB on other measured variable was not so laterally localised as to vary between these sections. Therefore, when comparing RB between species it was deemed acceptable and informative to take a whole-pot approach to analysing the impact of RB as an alternative to concentrating on g/cm^3 within each sample within a pot.

As the data used in the whole-pot analysis was a collation of the data used for the rest of the analysis many of the initial findings were as expected from subsequent analysis; RB was typically highest in the 2-spec; SOM200 demonstrated minimal variance across both treatments except for a significantly higher rate recorded in Chicory (mono); SOM325 was typically higher in the 2-spec; while SOM550 was typically higher in the mono and was particularly low within the 2-spec of Birdsfoot, Plantain and Clover; the highest DOM was recoded in pots containing Ryegrass, however, the strength and consistency of this variance pattern was inconclusive; the lowest EE-GRSP was observed in pots containing the shallow-rooting Clover and Birdsfoot; and RE-GRSP was typically higher in the 2-spec and lowest again in the shallow-rooting Clover and Birdsfoot (mono).

However, the whole-pot analysis allowed the various measured organic matter fractions to be considered in the context of total-RB (or rather total-RB within the cored area). This produced a series of results which were inconsistent with one of the stated hypotheses, that organic matter fractions would be higher in species with more substantial rooting systems; in fact through this interpretation the reverse was true. By calculating the average weight of each fraction within the soil (g/cm^3) and dividing this by the total-RB to determine how much of each fraction was recorded per gram of root (g/g-root) it became apparent that species (or pots) with smaller rooting systems (weight of RB) produced the highest values; namely Birdsfoot, Clover, Plantain and to a lesser degree Yarrow. Mathematically this can be explained by the fact that the fraction weight (g/cm^3) was

divided by a smaller RB; however, what was inferred from this was that the amount of root (weight of RB) did not have a proportional impact on the amount of each fraction within the soil. Instead this analysis showed that the amount of each fraction within the soil was comparatively similar and in some cases a plant's RB (or a pot's combined RB) was disproportionately large for the impact it had on the soil fraction value.

This does not mean that plants with larger roots (or pots with more combined roots) did not at times produce more organic matter; in fact, fraction values were often high and sometimes highest in pots containing either Chicory or Dandelion which produced significantly more RB than the other four plants. It must also be noted that this analysis is based on fraction values as recorded at the point of harvesting, which was during a stage of active plant growth. It is feasible that if sampling had occurred at a different stage of the plants' lifecycles (for instance in a dormant phase when root and shoot biomass has senesced) then additional organic matter may be dispersed within the soil, which could go some way towards redressing the observed imbalance between RB and its proportional impact on soil fraction values. However, based on results produced from this stage in the plants' development, this data questions the supposition that using plants with extensive rooting systems necessarily has a proportionally greater impact on organic matter development within the soil.

At the outset it was hypothesised that species or functional groups with more substantial root structures were expected to produce more organic matter and were also expected to produce more microbial-derived organic matter. Once again, the answer may not be as straightforward as the hypotheses implied, as different fractions displayed variable responses in pots predominated by varying rooting styles and total root mass. Higher values of SOM325 and RE-GRSP were observed in the 2-spec which consistently contained higher RB than their mono counterparts due to the presence of Ryegrass. However, the reverse was true for SOM550 and EE-GRSP. As previously noted, when additional values were calculated for the combined values of all three pyrolysable SOM fractions (Combined SOM) and both GRSP fractions (Combined GRSP), these values were in both consistently higher within the mono which consistently contained less RB due to the absence of Ryegrass.

Even when looking beyond the influence of additional biomass produced by Ryegrass to the variable rooting characteristics observed in the mono the answer is not consistent across fractions. SOM200 was similar across rooting characteristics, although slightly higher in the dense/deep tap-rooting Chicory which produced the most RB; SOM325 was again highest in Chicory as well as the shallow-rooting Birdsfoot which produced the least RB and was lowest in Clover which was also shallow-rooting; SOM550 was again highest in Birdsfoot as well as the dense/deep tap-rooting Dandelion (which produced the second highest RB) and surprisingly was lowest in Chicory. EE-GRSP was highest in the two dense/deep tap-rooting species with high RB and lowest in Clover (a fact that was even more marked within the 2-spec when the Clover growth was significantly impeded by the competitive Ryegrass which did not seem to provide a substitute for EE-GRSP production); while RE-GRSP was highest in Yarrow. While these results show some link between high organic matter and substantial tap rooting systems as well a reduction in smaller rooting systems, this trend is inconsistent. When values for Combined SOM and Combined GRSP are used there is a marginal elevation in Combined SOM in the more extensive Chicory, dandelion and Yarrow, and another marginal elevation in Combined GRSP in Yarrow and Chicory. However, as was noted in the whole-pot analysis, these minor elevations were not proportional to RB and in fact per gram of RB the less extensive rooting Birdsfoot, Clover and Plantain were consistently associated with higher quantities of organic matter in the soil.

Understanding the link between rooting characteristics and organic matter fractions within the soil requires a more detailed investigation of the mechanisms involved in its production and depletion; with specific consideration given to the variable abundance and activity of microbial communities and further investigation of how the dynamics of labile and persistent SOM fractions are mediated by plant and microbial influence.

4. Conclusion

SOM is an integral part of soil structure, stability and nutrient value, and plays a fundamental role in the delivery of ecosystem services. Global soils are under increasing pressure from multiple drivers and as SOM depletes, so too does the ability of soil to function effectively in the provision of ecosystem services upon which human subsistence relies.

Numerous farming practices have been identified as presenting possible solutions to protect and enhance SOM; however, one theory which has not been extensively researched is that plant species diversity may enhance the rate of SOM accumulation. While this topic has been explored to some degree in permanent grassland systems, there was a knowledge gap relating to species diversity effects on SOM in temporary grasslands.

This study set out to establish a better understanding of how plant growth within temporary grasslands can influence the accumulation of SOM. Moreover, it aimed to determine how plant species and diversity and different plant functional groups (as determined by rooting characteristics) influence overall SOM and individual fractions, as well as how these factors change across different soil depths.

It was hypothesised that higher plant diversity would result in an increase of overall SOM and of persistent SOM fractions that are thought to be associated with soil microbial processes. It was also hypothesised that more extensive rooting systems would lead to increases in both overall SOM and microbial-derived persistent SOM. Finally, it was hypothesised that SOM would reduce with soil depth due to the reduction in organic inputs in deeper soil. To test these hypotheses two exercises were established.

A field sampling exercise involved soil core extraction from an ex-arable site recently converted to grassland. Samples were divided into depth layers and subsequently fractionated to determine values for several labile and persistent SOM fractions to produce a comprehensive understanding of how rates of SOM changed at varying soil depth.

This was followed by a glasshouse experiment which focussed on six herbaceous grassland plant species (representing three functional groups determined by rooting characteristics) typical of a UK agricultural grassland. These were grown under both a monoculture treatment and a 2-species diversity treatment using Perennial Ryegrass as a standardised second species. Multiple soil samples were taken from a range of depths and distances from the main rooting point, and these were fractionated to produce a comprehensive understanding of how rates of SOM fluctuated between diversity treatments and again at varying soil depth and radial distances from the plant stem. A subsidiary experiment was conducted concurrently which provided a detailed assessment of the rooting characteristics of the six herbaceous species included in the main glasshouse experiment, and the data from both experiments was used to inform an analysis of how SOM changed with differently rooting functional plant groups.

While results were not all straightforward, findings from these two projects often confounded the stated hypotheses. Within the glasshouse experiment, while some SOM fractions were more prevalent under the 2-species diversity treatment, overall SOM was typically higher when herb species grew alone. While different microbial-derived SOM fractions displayed conflicting trends across both diversity treatments, again the overall pattern showed higher proportions in monoculture treatments. The distribution of SOM under different rooting characteristics was inconclusive, although to some degree higher rates of SOM were observed alongside extensive rooting systems. However, rates of SOM were not proportional to root biomass and in fact when calculated as SOM per gram of root it was the species with less extensive rooting systems which appeared to produce proportionally more SOM.

Furthermore, comparison of the datasets produced from the two exercises identified many inconsistent patterns between the two systems (although some of this variation can be attributed to different sampling methods and focal treatments involved in the two exercises). Overall, more SOM was found in shallower soil in the field sampling exercise, while in the glasshouse experiment SOM was found in greater quantities in deeper soil. In field samples, SOM200, SOM325, SOM550 and RE-GRSP decreased with depth, while results from tall pots in the glasshouse experiment (which due to their length allowed observation of the largest depth range) showed the opposite trend with SOM200, SOM325 and SOM550 increasing at depth, and no significant trend for either GRSP fraction.

It was theorised that higher rates of SOM were associated either with lower rates of soil bulk density or higher rates of soil moisture, because in both exercises SOM was typically found to correlate with both variables, each of which demonstrated inverted patterns of behaviour (one high, one low) which showed opposite distribution patterns along a depth gradient in each of the exercises (soil water content was highest in shallow soils in the field and in deep soils in the glasshouse, while for bulk density the reverse was true). However, water content was positively correlated with EE-GRSP in the field, but in the glasshouse a significantly negative weak correlation was observed. Furthermore, in the field EE-GRSP was significantly positively correlated with pH, while in the glasshouse there was a significantly negative weak correlation between the pair, although a significant negative correlation did appear between pH and water content in both exercises. Bulk density was also significantly positively correlated with EE-GRSP in the field while these two were significantly negatively correlated in the glasshouse. Additionally, while bulk density in field samples was significantly negatively correlated with SOM325, no significant correlation was apparent in the glasshouse.

Correlations between SOM fractions were also inconsistent, SOM200 and SOM325 were positively correlated in both exercises, however SOM325 and SOM550 were negatively correlated in the field and positively correlated in the glasshouse. Furthermore, in the field DOM was negatively correlated with SOM325 and positively correlated with SOM550, however in the glasshouse it was weakly negatively correlated with SOM550 and showed no significant correlation with SOM325. Another inconsistent pattern emerged between both GRSP fractions which were positively correlated in the field but showed no significant correlation in the glasshouse.

These inconsistencies are not highlighted to question the veracity of the data, but rather to demonstrate that in the case of the experimental design and sampling strategies adopted in these exercises, results from the two do not suggest that a glasshouse can be used to reliably replicate the conditions which may be observed in the field.

Reflecting on the fractionation methods adopted in this research, the profile of SOM fractions which has been identified, bears some similarity to that of Branco de Freitas Maia et al. (2013) outlined in Table 1. While more basic in content than the highly quantified range of fractionation methods collated by Von Lutzow et al (2017) in Table 2, this approach is arguably more informative of how SOM is likely to behave than an approach which relies solely on fractionation by specific physical characteristics. For instance, identifying the particulate SOM within prescribed size and density ranges doesn't inform how the SOM will behave as it doesn't acknowledge that this specified pool could contain different functional fractions with contrasting degrees of resistance to varying extraction techniques, such as the increasingly aggressive extraction solutions used to isolate EE-GRSP and RE-GRSP.

Fractionation methods which rely solely on physical parameters also fail to account for changes in the nature of SOM when subjected to different turnover processes. For instance, when the pyrolysis method was tested, all three carbohydrate substances (D-galacturonic acid monohydrate, D-xylose,

sucrose) were still partially present following the 325°C treatment (Figure 4). Rovira & Vallejo (2000) found a LoI peak at this temperature, which was largely attributed to the removal of carbohydrates, however the test in the current research suggests that a significant portion of carbohydrate substances were still present after the 325°C treatment. One explanation for this is that the caramelising effect of the 325°C treatment on the carbohydrates may have chemically altered their structure to create more persistent substances which required the more aggressive 550°C treatment to remove them. This theory has ramifications for the current and future research as LoI is often used to quantify SOM, however if this method alters the nature and reactiveness of a substance, it may not present a reliable method for determining relative turnover responses.

It must be noted that future research should include a greater number and variety of species composition to fully understand the impacts of plant species diversity on SOM in temporary grasslands, however the methods and findings from this study have established a framework upon which further research can be based. The findings have confirmed that SOM needs to be considered not as a single homogenous unit but as a number of fractions based on their more labile or persistent nature, because different SOM fractions demonstrated contrasting patterns of behaviour which sometimes conflicted with the overall trend.

This study also established and quantified a detailed understanding of the rooting characteristics and strategies of the six herb species selected for the glasshouse experiment. This could be used to help inform species selection in subsequent research which seeks to explore the effects of different plants and plant roots on SOM and soil condition.

This study has also documented the soil-capping phenomenon encountered in the field sampling exercise which, while it is not expected to be unique to this site, does not seem to previously have been recorded in the literature.

While there were limitations to the research and inconsistent results which emerged when datasets from the two exercises were compared; learning can be extracted to inform agricultural practices which seek to maximise SOM.

First; because rates of individual SOM fraction varied between herb species, if high rates of each fraction are desired it may be advisable to plant a variety of herb species as the results from the glasshouse experiment suggested that some would contribute greater quantities of one fraction than another. This was exemplified by the higher rates of SOM325 in Chicory in contrast to Clover, and the higher rates of SOM550 in Dandelion compared to Chicory. However, this is speculative as the research only measured SOM rates when herbs were grown in monoculture or with Ryegrass, therefore it is uncertain how SOM rates may be impacted when two herb species grow together.

Second; relative planting densities of more and less competitive species should be considered, as different results from the glasshouse experiment were found when comparing monoculture herb growth with herb species growing alongside the highly competitive Ryegrass. While the presence of Ryegrass produced both higher and lower rates of different SOM fractions, in contrast to the monoculture counterparts, it is likely that the negative impacts typically observed on some SOM fractions when Ryegrass was present (e.g. SOM550 and EE-GRSP) would be less marked if the rates of Ryegrass sown was reduced.

Third, when including temporary grasslands within arable rotations, it must be noted that some of the SOM accumulated during the period under grass is likely to be lost during ploughing when it is returned to arable. Consequently, promoting persistent SOM fractions which are more likely to remain after the disruption of ploughing is desirable. Initially it appears to be difficult to draw clear conclusions from the results of the glasshouse experiment on whether Ryegrass increases or reduces persistent SOM, as both diversity treatments produced higher rates of at least one persistent SOM

fraction (SOM550 and RE-GRSP) and one labile SOM fraction (SOM325 and EE-GRSP). However, when the results of the analysis of SOM per gram of root are considered, there is a clear indication that the small pot herb species produced proportionally higher levels of all SOM fraction than the more extensive Ryegrass. This suggests higher rates of both persistent and labile fractions could be accumulated if herb species (rather than Ryegrass) are planted sufficiently densely; or that relatively high rates of SOM could still be achieved with a comparatively lower sowing density of herbs rather than grass, which could reduce the cost of grassland establishment.

Fourth; if seeking to maximise resource utilisation through diverse species selection, then choosing species which present diverse rooting characteristics may be desirable in order to minimise competition in the same rooting zone. Again, this is speculative as the research only measured SOM rates when herbs were grown in monoculture or with Ryegrass. However, results from the glasshouse experiment revealed that Chicory produces a substantial tap root in the upper-rooting zone and a substantial system of small fibrous roots below this; and that the RB of Chicory was not reduced when grown alongside the competitive Ryegrass. It is therefore likely that Chicory could be grown alongside other shallow rooting herb species, such as Clover, without the rooting structure of one herb species overshadowing that of the other. While it cannot be concluded with certainty from these results that a diversely planted herb sward produces more favourable rates of SOM than a herb monoculture, it seems reasonable to assume that this result is best achieved by selecting species which do not compete excessively for the same rooting zone.

Fifth; the positive correlation observed between water content and SOM fractions in both exercises, regardless of the variable depth rates at which this occurred, suggests that higher rates of SOM are likely to be observed when soils are well hydrated and plant roots are not limited by water stress. Conversely, the results suggest that the same trend in SOM may be expected when soil bulk density is reduced, emphasising the importance of ensuring agricultural soils are not compacted by excessive above-ground traffic.

Appendix A

Soil compaction analysis of field sampling

Field sampling was halted prematurely when substantial fluctuations were observed between cores in in-field measurements of compaction rates. While field variability was expected, this implied an extremely wide range of soil compaction. An alternative explanation was that the CL in corers did not reflect PD (even after accounting for compaction rates). This raised concerns about the effectiveness of the sampling method. Because such a wide compaction range was not expected from what was perceived as a homogenous heavy clay soil, this alternative explanation seemed more likely. It was hypothesised that at times the downward force of the corer compressed the soil below the corer resulting in a solid soil-cap which prevented deeper soil from entering the corer despite continued downward force. This phenomenon could give the impression of inflated soil compaction due to the disproportionate ratio of CL-to-PD. This phenomenon is not well documented in the literature.

To test this hypothesis, it was necessary to establish the apparent variability in compaction, and subsequently to establish whether environmental factors may adequately explain this variability. A series of ratios were calculated, and comparisons made for each core. Initially, the ratio of CL-to-PD (compaction rate) was reviewed. This ranged from 0.57:1 to 0.25:1 (a PD up to four times higher than the CL) averaging 0.4:1 with a standard deviation of 0.12 (Figure 38). This standard deviation seemed high (29% of the average CL-to-PD ratio) and only accounted for 13 of the 20 cores.

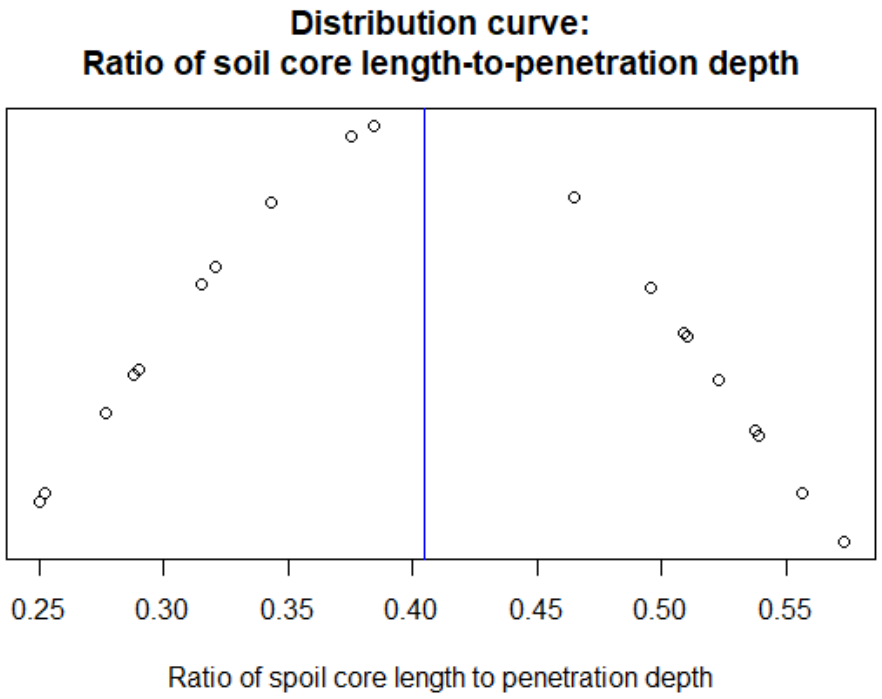

Figure 38: Distribution curve: Ratio of soil core depth-to-penetration depth. Vertical blue line denotes average ratio.

From a visual representation of these ratios, cores appeared to fall into two groupings; although exact boundaries of these groups are open to interpretation. On the right of the curve (cores displaying least compaction) are nine cores with a distribution of 0.46-0.57 (range 0.11, standard deviation 0.03), while on the left of the curve are 11 cores with a distribution of 0.25-0.38 (range 0.13, standard deviation 0.05). These core groupings are separated by a gap of 0.08 (which is >60% of either group's distribution range and twice as wide as any gaps between cores within either grouping). Therefore, this gap creates two distinctive groups. It could be argued that the core

positioned closest to the mean in the right-hand group (at 0.46) is an isolated data point which could be included in either group, as the distance to its nearest neighbour in either direction is approximately 50% of the breadth of each range (once the right-hand range has been reduced in scale following the removal of the intermediate core). However, a conservative approach was considered most appropriate, as the distance between this core and the remainder of the right-hand group was the same value as another gap between cores within the left-hand group. Even with the inclusion of this intermediate core, the right-hand group displays a tight distribution (range 0.11, standard deviation 0.03) with an average soil compaction of 0.52:1 which was in line with the typical compaction observed during corer testing. In contrast, the left-hand group displayed an average soil compaction of 0.31 :1, considerably higher than was expected.

Having established the two distinct core groupings based on compaction rates, it was necessary to establish whether the compaction variability was best explained by differences in soil density between cores. To test this, two further ratios were calculated; 1) weight-to-CL, and 2) weight-to-PD. Substantial variance in the first ratio (the weight of 1 cm^3 of soil in relation to CL) between cores would be indicative of varying soil density. Variability in the second ratio (weight of 1 cm^3 of soil in relation to PD) would highlight cores where the soil mass expected from increased PD was not reflected in the core weight, hence increased likelihood of the suspected capping phenomenon.

For further explanation of this assertion, consider two hypothetical soil cores both inserted to 60cm. The first experiences no unusual resistance and collects soil from the full PD of 60cm. Conversely, the second experiences unusual resistance at 30cm which prevents soil entering the corer. Although the corer continued to penetrate to 60cm the soil below 30cm is pushed aside by a solid cap at the base of the corer and only soil from the top 30cm is collected. Assuming a hypothetical linear soil compaction of 0.5, the first corer would hold a 30cm CL which reflects 60cm of PD, while the second corer would hold a 15cm CL which reflects only the top 30cm of PD. Assuming a uniform soil density between samples the first core should weigh twice as much as the second core. In this comparison where we are assuming both a linear and similar soil compaction and uniform soil density, both the length and weight of the cores would show that capping had occurred in the second core.

Due to the resistance of the heavy clay soil, the 20 corers deployed in during sampling were inserted to a range of depths (44-59.5cm). Given this variability and accepting that some natural fluctuation in compaction rates and soil density between sampling points is possible, the field comparison is not so straightforward as the hypothetical scenario outlined above, however the same logic can be applied. It should be noted that the linearity of the compaction rate cannot be confirmed with certainty from the available data (although this point will be revisited). Confirmation would require multiple measurements of the relationship between PD and CL for each core during sampling, a point worth noting for future research. Also, natural variance in soil density cannot be discounted, however a visual inspection of cores didn't suggest substantial differences in soil type, texture or consistency (Figure 39). Beyond these assumptions, comparing variance in both ratios (weight-to-CL and weight-to-PD) removes some of the variability introduced by the fluctuating sampling depths.

Weights of complete soil cores were calculated prior to drying as this would have affected subsequent analysis, therefore weight data are measurements of fresh weight density (including soil moisture) and not true bulk density which was calculated subsequently once complete cores were segmented. The data from the weight-to-CL ratio calculations showed the weight of 1cm^3 of soil in relation to CL ranged from 1.34-2.15g, averaging 1.81g (standard deviation 0.19). Figure 40 presents the variance of cores from this 1.81g average; cores depicted with red bars are those from the group with a higher than expected compaction rate, while blue bars are those from the group with compaction rates close to what was expected from preliminary testing. The apparently random positioning of these two groups in relation to their weight-to-CL ratio suggests no correlation between these two sets of ratios and hence little likelihood that variable soil density can explain the compaction rate groupings of the sets of cores.

Figure 39: Random selection of soil core photos taken immediately after extraction from corers

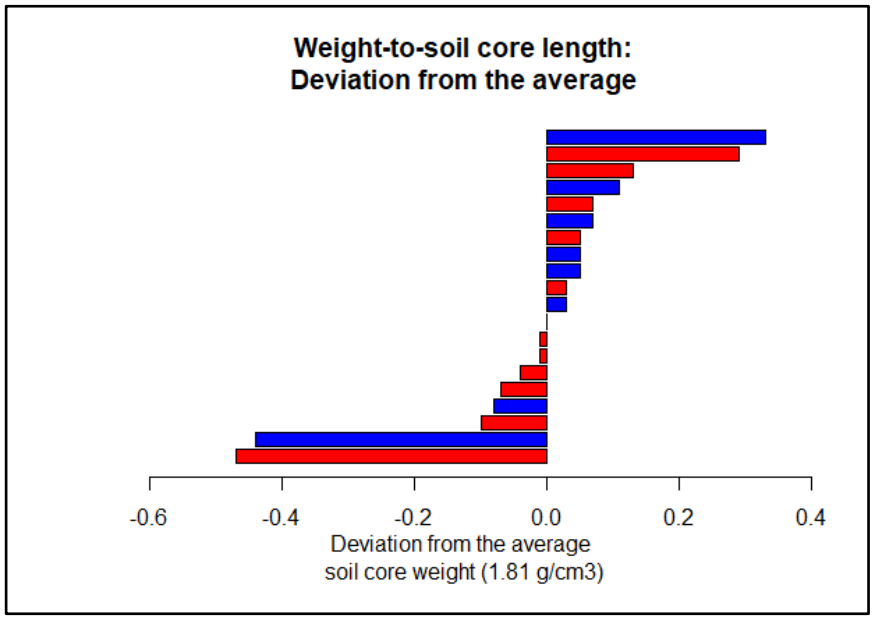

Figure 40: Deviation from average weight-to-soil core depth ratio (g/cm^3). Red bars denote soil cores which demonstrated a greater than expected compaction rate, blue bars denote soil cores which demonstrated a compaction rate close to that which was expected from preliminary testing

Furthermore, the low standard deviation shown by the weight-to-CL ratio data (10% of the average value) accounts for 80% of cores. This reflects a consistent value for the weight of 1cm^3 of soil and suggests a relatively uniform soil density. The variation present could be explained by natural soil variability or fluctuation in soil compaction caused by inconsistent corer penetration technique caused by failure of the impact-cap and its replacement with wooden blocks.

As observed above, 80% of cores presented a weight per 1cm^3 within the relatively low standard deviation range. After examining the sampling location topography of the cores which did not fit within this range it became apparent that each was sampled at points of high elevation. This may explain the two samples which demonstrated the lightest and therefore least compacted soil, as lower compaction may be expected at higher elevations for several reasons. Firstly, because slopes and high elevations (if less accessible) may experience less compaction from intensive arable farming, and secondly because small clay particles may wash downslope over time leading to lower soil density at higher elevations and higher density in gullies at the base of a slopes (Saeed et al. 2014, Peukert et al. 2012). In contrast, it was assumed that the two cores which exhibited signs of highest soil density may be a result of inconsistent corer penetration technique which can impact significantly on soil density characteristics on clay soils (Stone 1991).

In contrast, the weight-to-PD ratio showed the weight of 1cm^3 (in relation to PD) ranged from 0.39-1.09g, averaging 0.73g (standard deviation 0.22). This standard deviation was comparatively high (30% of the average weight-to-PD ratio) and accounted for only 55% of cores. This wide distribution reflects an inconsistent relationship between core weight and PD. This may be explained by vastly different compaction rates between samples, but this isn't supported by the proceeding analysis of the weight-to CL ratio which would be more variable if compaction rates were this inconsistent.

Furthermore, Figure 41 presents the variance of cores from the 0.73g average; again, red bars show cores from the group with greater than expected compaction rates, while blue bars depict the group with a compaction rate close to expectations following preliminary testing. The clear separation of these two groups by their weight-to-PD ratio supports the argument that the cores depicted in red experienced the proposed capping phenomenon as this ratio highlights cores where the soil mass expected from the PD was not reflected by core weight, suggesting an obstruction during coring.

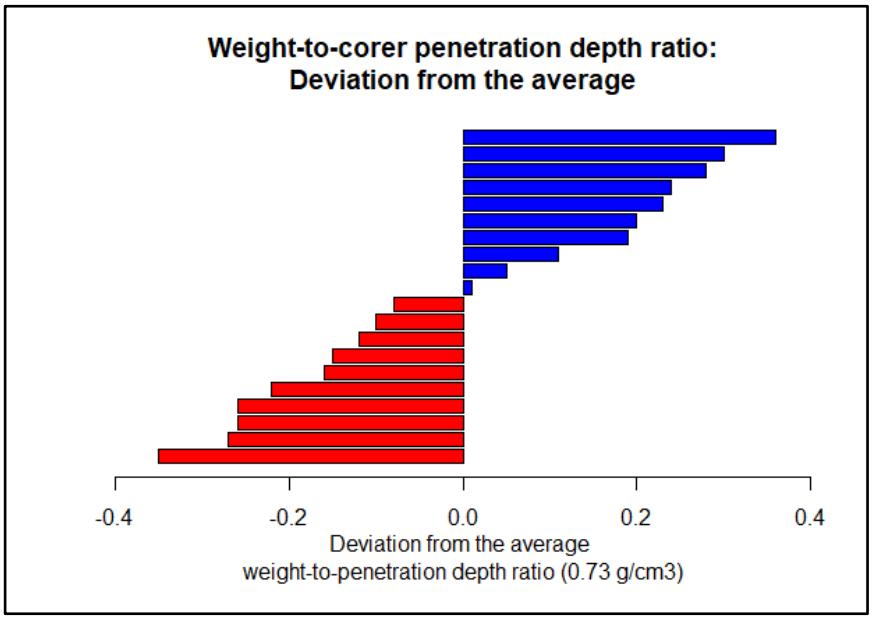

Figure 41: Deviation from average weight-to-corer penetration depth ratio (g/cm^3) . Red bars denote soil cores which demonstrated a greater than expected compaction rate, blue bars denote soil cores which demonstrated a compaction rate close to that which was expected from preliminary testing

By reviewing these ratios, the likelihood of variable soil density can be plausibly discounted as an explanation for the presence of two distinct groups of compaction rates. Consequently, this supports the hypothesis that the coring method was hampered by the formation of a soil-cap below the base of the corer preventing deeper soil from entering. Therefore, it should be assumed that those soil cores suspected of experiencing this phenomenon don't reflect the recorded PD.

Interpreting core depth data

Having established that the capping phenomenon probably did occur and having identified which cores were the likely subjects of capping, this preliminary data was further reviewed to estimate the depth capping occurred. This was necessary to interpret the full data set prior to analysis.

As outlined above, the suspected group of uncapped cores shows a compaction rate of 0.46-0.57, while the suspected capped group showed a compaction rate of 0.25-0.38. The average compaction rate of the uncapped group was 0.52 which was similar to rates observed during testing and can be considered within this method's expected range (albeit tests were conducted on a slightly lighter clay-loam soil). The average compaction rate of the capped group was considerably higher at 0.31. The soil density for the capped and uncapped groups was similar (uncapped 1.37-2.15 g/cm^3, capped 1.34-2.1 g/cm^3). Once the core's respective compaction rates are considered in the calculation to determine the density per cm^3 of actual depth sampled these values reduced; with the uncapped group ranging between 0.79-1.09 g/cm^3 and the capped group considerably lower, 0.39-0.75 g/cm^3. However, when the average compaction rate for the uncapped group was substituted as a standard factor for the compaction rates of the capped group this range increases to 0.7-1.01 g/cm^3. This brings these ranges into line; the uncapped group averaging 0.95 g/cm^3 (standard deviation 0.1) and the capped group averaging 0.94 g/cm^3 (standard deviation 0.1). These averages are comparable to the lower end of the 1-1.6 g/cm^3 density range expected for clay soil (Chaudhari et al. 2013).

Taking a similar approach, by applying the average compaction rate from the uncapped group to the CL of the capped cores it was possible to estimate the likely PD that these soil cores represent, and therefore an approximate capping depth. Using 0.52 as the expected compaction percentage to infer the PD from the CL of the capped group, the estimated PD ranged from 24-38cm, averaging 31cm (standard deviation 4cm). This standard deviation accounts for 73% of cores in the capped group.

This suggests that once corers penetrated the soil to a depth of approximately 30cm the chance of corer capping increased due to the naturally compacted nature of the site's heavy clay soil. Instances of capping were primarily observed at lower elevations (73%) which may therefore represent the most compacted soils. This compares to 33% for the uncapped cores, 67% of which were instead found at higher elevations. The occurrence of capping at approximately 30cm could be explained by the presence of a soil pan caused by repeated ploughing at this depth. Repeated ploughing at the same depth can lead to a hardened soil pan (Finch et al. 2014) which may have acted as a bung, capping the base of the core and preventing further soil from entering and subsequently compressing or displacing the soil below the corer as it continued to penetrate. This assertion seems credible given that this site is an ex-arable field and repeated ploughing at this depth is typical of arable soils.

It should be noted that this hypothesis is based on certain assumptions regarding the homogeneity of soil density across the site and the linearity of compaction rates. As stated previously the variable dynamics of soil compaction at increasing depths could be explored further by taking multiple measurements of the ratio between PD and CL during sampling. However, considering the current data a review of the ultimate compaction rates of all uncapped soil cores (suspected capped cores being discounted as the occurrence of capping distorts the measurements of PD) could reveal whether a consistent trend in compaction rate was present. As outlined in Figure 42, there is an increase in compaction rate at greater depths, however the correlation is not significant (r=-0.23,

d.f.=7, P=0.56) and the data points are widely dispersed with a very low R^2 value (0.072), suggesting that increasing soil depth is not a satisfactory explanation for higher rates of compaction in this instance. Therefore, the assumption of a linear compaction rate seems acceptable until further data becomes available.

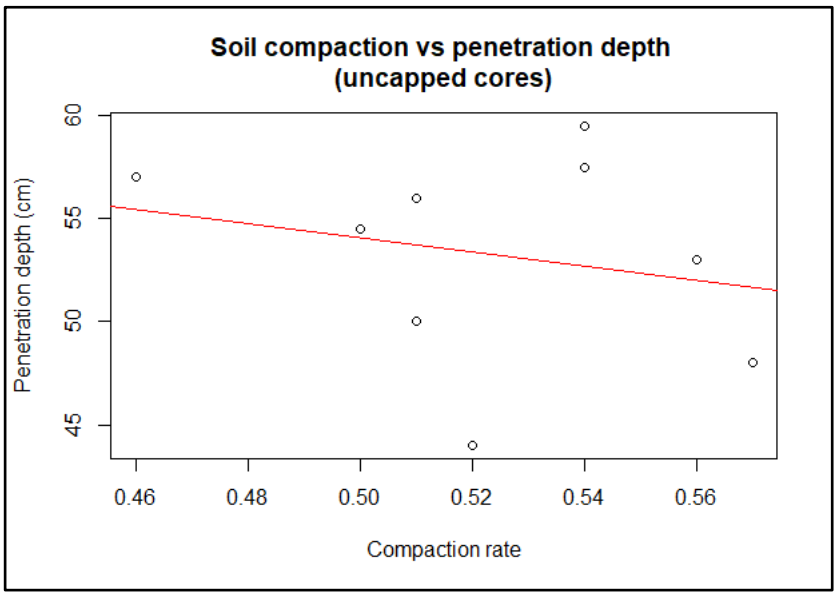

Figure 42: Soil compaction rates correlated with corer penetration depth (uncapped cores). Red line is trendline. R^2=0.072.

Appendix B

Analysis of SMP/EPS extractions

Analysis of EPS and SMP extracts is reported separately because the values of these measured variables were typically very low and often below detectable levels. Protein and humic fractions within the SMP extraction (ProtSMP, HumSMP) were present at a detectable level in only a few samples and often only appeared in single replicates within a species treatment. As a result, there was no significant variance between species or diversity treatments. Whereas, variance for the humic fraction in the EPS extraction (HumEPS) between diversity treatments was significant ($F_{2,74}$=5.903, P<0.01) (data not shown). However, when this variance was analysed at species level across both diversity treatment this significance reduced to only approaching significant, and a post-hoc Tukey test showed no significant variance between any species combinations.

Similarly, significant variance was also observed for protein in the EPS extraction (ProtEPS) between diversity treatments ($F_{2,74}$=3.726, P<0.05) (data not shown). However, when analysed at species level across both diversity treatments, again this significance reduced to only approaching significant, and a post-hoc Tukey test showed that significant variance was only driven by higher ProtEPS in the 2-spec Birdsfoot (P<0.05). Carbohydrates were found at only negligible levels in all species treatments within both SMP and EPS extractions (except Chicory within the mono). Analysis of variance was also conducted at various depths, however the low return of detectable results in many cases resulted in no clear variance between depths, and when detectable results were available, no significant variance was observed.

Because measurements of ProtEPS and HumEPS provided the most complete sets of detectable results from all variables measured from the SMP and EPS extractions, these two variables were further analysed to detect correlations with other measured organic matter fractions. With SOM200 both a positive and negative correlation appeared respectively between HumEPS (r=0.4, d.f.=78, P<0.001) and ProtEPS (r=-0.34, d.f.=78, P<0.01) (data not shown). However, in both cases, when analysed within diversity treatments the significance disappeared within the 2-spec and reduced within the mono; HumEPS (r=0.41, d.f.=45, P<0.01), ProtEPS (r=-0.33, d.f.=45, P<0.05) (data not shown). Initially no significant correlation was apparent between SOM325 and either HumEPS or ProtEPS, however when analysed within diversity treatments a significant positive correlation was observed for HumEPS within the 2-spec (r=0.44, d.f.=21, P<0.05), while significant negative correlations appeared for ProtEPS within both diversity treatments; mono (r=-0.32, d.f.=45, P<0.05), 2-spec (r=-0.42, d.f.=21, P<0.05) (data not shown). A positive correlation appeared between SOM550 and HumEPS (r=0.47, d.f.=78, P<0.001) (data not shown) but this reduced when analysed within diversity treatments; mono (r = 0.3, d.f. = 45, P<0.05), 2-spec (r=0.43, d.f.=21, P<0.05) (data not shown). Conversely, a negative correlation appeared between SOM550 and ProtEPS (r=-0.4, d.f.=78, P<0.001) (data not shown), but this disappeared when analysed within diversity treatments. Finally, a negative correlation was observed between ProtEPS and EE-GRSP (r=-0.41, d.f.=78, P<0.001) (data not shown). When this correlation was analysed within diversity treatments its significance reduced within the mono (r=-0.4, d.f.=45, P<0.01) and disappeared in the 2-spec (data not shown).

Overall, SOM was positively correlated with HumEPS. The EPS extract was expected to contain the more persistent compounds (in contrast to SMP); however, HumEPS was not more strongly correlated with the more persistent SOM550 and RE-GRSP than it was with the more labile SOM200/325 and EE-GRSP. Conversely SOM and EE-GRSP were consistently negatively correlated with ProtEPS. This finding was surprising, particularly when considering that EE-GRSP, which was negatively correlated with ProtEPS, because both were measurements of soil protein which are thought to derive from microbial activity. Overall, HumEPS and ProtEPS did not behaviour in a way which matched expectations based on the theory and on other findings within this research. This may have reflected the low return of detectable results.

Appendix C

Root scans from plant root imaging

Due to the size of the mature plant roots, these had to be cut into sections allowing the roots to be laid flat with minimal overlap on top of the scanning plate. Therefor each root was cut into between 11 and 35 separate sections which were separately scanned before the data was combined to create total values for each root ball. Three replicates were scanned for each of the six herb species; as such a large number of images were captured during this process. A small representative subset is included here including two images respectively showing thin <0.5cm and thick >0.5cm roots from a Chicory plant (images 1 and 2) and one image showing a combination of thick and thin roots from a Birdsfoot plant (image 3).

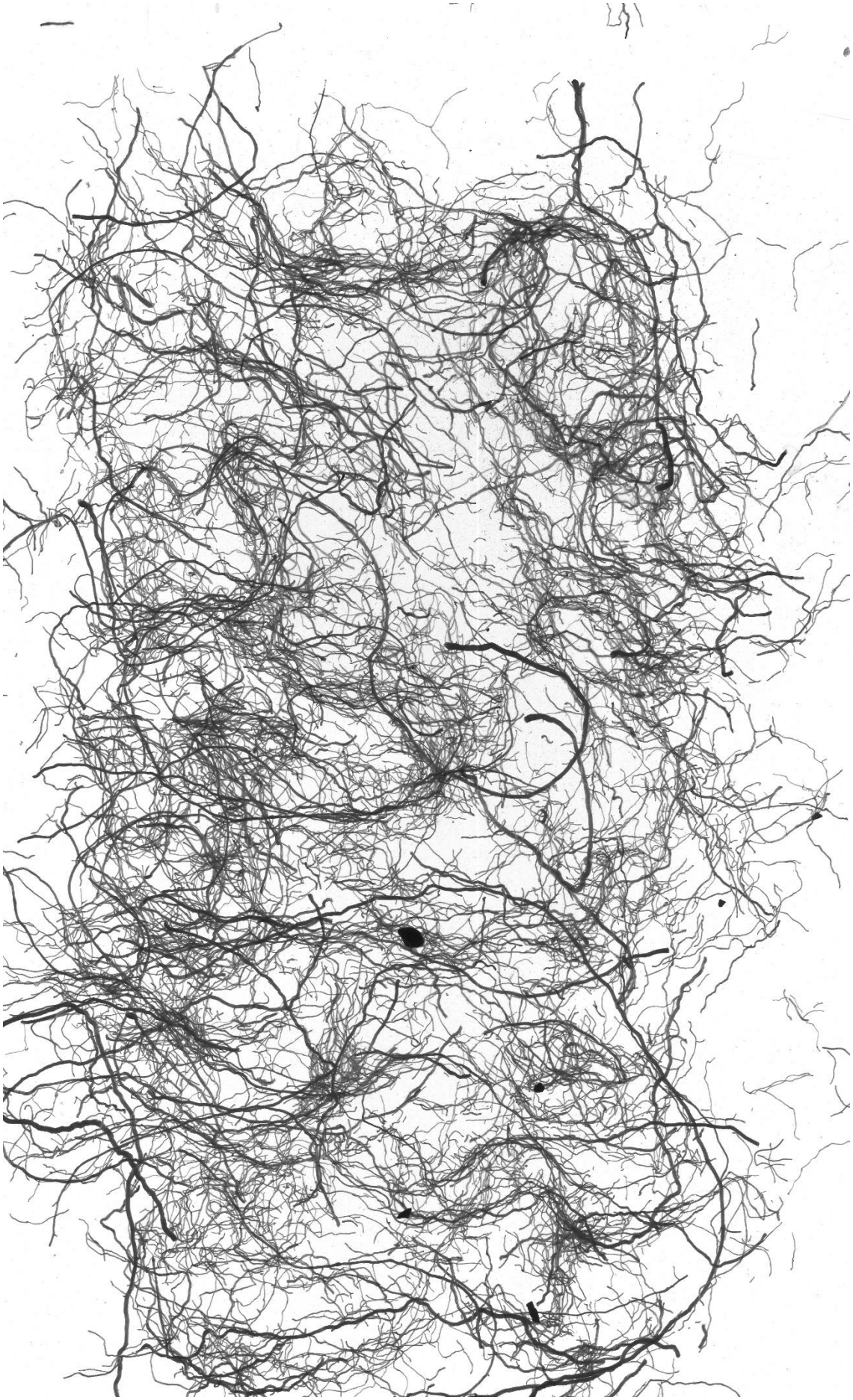

Image 1: Thin Chicory roots

Image 2: Thick chicory roots

Image 3: Birdsfoot roots

Abbreviations

2-spec	2 species diversity treatment
Abs	Absorbance
AMF	Arbuscular mycorrhizal fungi
ARD	Average root diameter
BSA	Bovine serum albumin
CBD	Convention on Biological Diversity
CER	Cation exchange resin
CL	Length of soil core
DOM	Dissolved organic matter
DWE	Dry weight equivalent
EC	European Comission
EE-GRSP	Easily extractable Glomalin related soil protein
EPS	Extracellular polymeric substances
GRSP	Glomalin related soil protein
GSB	Grass shoot biomass
HSB	Herb shoot biomass
HumEPS	Humic component of EPS extraction
HumSMP	Humic component of SMP extraction
LoI	Loss on ignition
Mono	monoculture diversity treatment
ProtEPS	Protein component of EPS extraction
ProtSMP	Protein component of SMP extraction
PD	Penetration depth
RB	Root biomass
RE-GRSP	Recalcitrant extractable Glomalin related soil protein
RV	Root volume
SA	Surface area
SMP	Soluble microbial products
SOC	Soil organic carbon
SOM	Soil organic matter
SOM200	Soil organic matter removed through LoI at 200°C
SOM325	Soil organic matter removed through LoI at 325°C (minus SOM200)
SOM550	Soil organic matter removed through LoI at 550°C (minus SOM200 and SOM325)
SSegDry	Soil segment dry weight
SSegWet	Soil segment wet weight
SubDW	Subsample dry weight
SubFW	Subsample fresh weight
TRL	Total root length
Vol	Volume

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