Rhizosheath Formation in Cover Crop Species: The Roles of Species Diversity, Water Availability, and Temporal Dynamics

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Dedicated in memory of Eric J. McBride

Declaration

I declare that the work produced for this thesis is my own and has not been submitted for another degree or qualification at any other institution.

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Statement of Authorship

This thesis has been prepared as a set of papers intended for submission to peer-reviewed journals. The chapters are presented in the format of the papers intended for submission to journals. Each paper's reference list is found in a combined reference list at the end of the thesis.

Chapter 1 provides a general introduction of the research area and the aims, objectives and key hypotheses of the thesis. It is not intended for publication.

Chapter 2 is intended for publication as:

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CMS carried out the experiment, laboratory and data analysis and prepared the original manuscript. JNQ and ICD gave advice on the experimental design, sample collection, data analysis and contributed to the revisions of the manuscript. AJK and TSG gave advice on data analysis and contributed to the revisions of the manuscript.

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CMS carried out the experiment, laboratory and data analysis and prepared the original manuscript. JNQ gave advice on equipment design, experimental design, sample collection, data analysis and contributed to the revisions of the manuscript. AJK gave advice on equipment design, experimental design, data analysis, assisted in sample collection and contributed to the revisions of the manuscript. TSG assisted with sample collection and contributed to the revisions of the manuscript. ICD contributed to the revisions of the manuscript.

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Chapter 5 comprises a general discussion and conclusions and is not intended for publication.

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Abstract

Increasing cover crop diversity has the potential to improve the resilience of agricultural systems to the extreme weather events associated with climate change. While the advantages of diverse cover crops for crop productivity are well-documented, their role in soil stabilisation remains less explored. Research on how different species contribute to soil binding through rhizosheath development is limited, and it is unclear whether multi-species cover crops provide added benefits for soil and water management.

Firstly, a mesocosm experiment assessed the rhizosheath formation capacity of four common grass and brassica cover crop species in the UK under droughted *vs* well-watered conditions, with a second mesocosm experiment assessing the diversity effect by mixing *Secale cereale* and *Brassica juncea.* Consistent across both experiments, water availability had no effect on specific rhizosheath mass and grasses had a greater soil binding capacity than brassicas. *S. cereale* maintained its soil binding capacity when grown in a mixture, suppressing the soil binding capacity of *B. juncea*.

A winter cover crop field trial then assessed the impact of cover crop diversity and associated root diversity on soil erodibility by conducting overland flow simulations on plots containing *S. cereale*, *B. juncea* and *Vicia faba* as monocultures and in all possible species combinations. Individual species identity, rather than species diversity, determined rhizosheath mass, with treatments containing *S. cereale* having 50% more rhizosheath mass per unit of root dry mass than others. Presence of above-ground cover crop biomass delayed runoff, but neither rhizosheath mass nor species diversity affected soil erodibility.

Finally, rhizosheath persistence in cover crops of varying diversity over time was assessed in the field. While overall rhizosheath mass decreased with plant age, *S. cereale* maintained the largest rhizosheath out of the three species regardless of time, diversity level or species combination and outcompeted *B. juncea* and *V. faba*. Root morphological traits in *S. cereale*, such as its fibrous rooting system and long, dense root hairs, make this cover crop exceptionally effective at binding soil. Root hair length and density explained only moderate to weak variation in specific rhizosheath mass, therefore other factors like mucilage production are likely to be important. The ability of *S. cereale* to extend and fill the space equally regardless of sowing rate coupled with its great soil binding capacity, makes this species an excellent choice to include in a cover cropping system for soil retention.

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Chapter 1. General Introduction

1.1 The external drivers for the need to build resilience in agriculture

Agricultural intensification has simplified landscapes reducing biodiversity, depleting natural resources and threatening ecosystem services (IPBES Global Assessment Report, 2019). These issues generally reduce the system's capacity to withstand or recover from abiotic stress *i.e.* resilience, threatening agricultural production and degrading the environment. More than 60% of soils in the EU are deemed unhealthy due to a combination of unsustainable management practices, increased pollution and surface sealing. This land degradation is aggravated by climate change and its associated extreme weather events (EUSO, 2023).

In the UK, summers are predicted to become hotter and drier, and winters and springs are predicted to become wetter (Kendon et al., 2023). Drier summers may improve sowing and harvesting conditions but also increase drought risk. For example, in 2018 drought contributed to a yield reduction of 8.6% compared to the previous year (Defra, 2019). On the other hand, the predicted wetter weather may increase the waterlogging risk and cause floods, such as the 2023 floods that contributed to a wheat production loss of 11% (Defra, 2024). This variability in water supply, both drought and excess, coupled with the increased frequency of extreme weather events leading to severe erosion events associated with climate change, is exposing soils to irreversible damage.

Soil erosion, accelerated by agricultural intensification and climate change, is degrading the environment and reducing its resilience to abiotic stress, threatening agricultural production and water quality globally (FAO and ITPS, 2015). Soil erosion rates in conventionally managed arable land are 1-2 orders of magnitude greater than soil formation rates (Montgomery, 2007) and, in some areas, topsoil could be eroded away in less than 140 years (Evans et al., 2019). Approximately 3.2 million hectares of European agricultural land are at risk of erosion caused by the interaction of drought, floods, wind, and water erosion. Water erosion alone, caused by inter-rill and rill processes (Panagos et al., 2015), affects at least 30% of land in Europe (Borrelli et al., 2022). This form of erosion is particularly harmful for soil fertility as, through the soil erosion processes of mobilisation, transport and deposition, both soluble nutrients and those attached to soil particles are lost from arable land to water bodies (Quinton et al., 2010). Over 4 million tonnes of soil are lost to erosion annually in the UK alone (Graves et al., 2015; Rickson et al., 2020) leading to an annual cost of £50 million in Scotland (Rickson et al., 2020) and £177 million in England and Wales (Graves et al., 2015). Identifying the tools to improve soil and water management is therefore of upmost importance to build the resilience of agricultural systems to abiotic stress. Various strategies, including no-till farming, contour ploughing and terracing, are used to manage soil erosion, and cover cropping is of particular interest, not only for their potential to enhance soil stability but also for benefiting additional ecosystem functions.

1.2 Cover crops as a solution to erosion mitigation and better water management

Cover cropping is a common agricultural practice for crop production and field resource management that is currently gaining traction for building resilience to climate change, and, up to March 2025, has been a key component in UK (Defra, 2024) and EU agricultural policy (CAP Strategic Plan Regulation, 2021). Cover crops are any non-cash crop introduced into the agricultural system between cash crop harvests to protect the soil over the winter months, as well as intercropped with the cash crop to protect the ground between rows or underneath crop plants that would otherwise be left bare and bring benefits to the cash crop (companion cropping).

Farmers have a wide range of cover crop species to choose from (Hallama et al., 2019) - mainly from the grass, brassica and legume families. These groups have different above- and below-ground traits that can support different ecosystem functions, such as large canopy cover of rye, the capacity of mustard tap roots to penetrate and improve the structure of compacted soil and the nitrogen fixing capacity of clover (Griffiths et al., 2022). Cover crop effects on ecosystem services vary depending on soil type, elevation, climatic conditions (Poeplau and Don, 2015) and species selection (Osipitan et al., 2019), therefore species selection should consider the desired ecosystem function and its compatibility with environmental factors.

Cover crops have been found to benefit several ecosystem services e.g. pest management (Snapp et al., 2005), weed suppression (Teasdale and Daughtry, 1993), and soil fertility (Saleem et al., 2020). Although comparatively less investigated, cover crops also influence soil properties such as structure (De Baets et al., 2011) and hydrology (Colla et al., 2000) and can therefore improve soil and water management.

1.2.1 How does aboveground cover mitigate erosion and improve water management?

The effect of aboveground cover on soil erosion and water management has been extensively studied. Canopy cover is known to prevent water and wind erosion (Cantón et al., 2011; Koudahe et al., 2022). A 30% vegetation cover can reduce runoff by 50% and erosion by 80% compared to bare soil (Kainz, 1989; Mills and Fey, 2004; White et al., 2016). The foliage can intercept the kinetic energy of raindrops (Dabney et al., 2001) and the physical obstacle of increased stem density can slow down overland flow, thereby decreasing the risk of water erosion (Elwell and Stocking, 1976; Durán and Rodríguez, 2008). However, stems can also encourage localised erosion by creating a horseshoe vortex of water around the stem (De Baets et al., 2007).

In addition to providing a physical obstacle, the accumulation and decomposition of aboveground cover crop biomass contributes organic matter that enhances soil aggregate stability, a key component of erosion resistance. The improvement of soil structure through input of cover crop-derived organic matter is a significant factor in reducing soil erosion (Dabney et al., 2010). The addition of soil organic matter not only benefits soil health and function chemically and biologically (*e.g.* by improving the storage and supply of nutrients) but also physically by improving soil structure. Soil organic matter binds soil particles together, encouraging the formation of aggregates. This process increases porosity, allowing faster water infiltration and greater water holding capacity, therefore improving plant growth conditions and resistance to soil erosion (Boyle et al., 1989).

Recent findings by Dai et al. (2024) highlight the positive effects of specific cover crop species on soil structure. In a three-year no-till field study, rye (*Secale cereale L.*) and winter field peas (*Lathyrus hirsutus*) significantly aggregate stability and reduced bulk density compared to bare soil. Rye showed the highest aggregate mean weight diameter (MWD) of 0.58 mm and reduced bulk density by up to 7.69%, while also promoting macro-aggregate formation (1–0.5 mm and 0.5–0.25 mm fractions). Pea treatments increased water-stable aggregates of >0.25 mm by 68.61% and reduced small aggregates (<0.25 mm) by 28.16%, with associated reductions in bulk density (up to 6.29%). These structural improvements are largely attributed to the contribution of organic inputs acting as binding agents for aggregation, further reinforcing the role of cover crops in enhancing soil resilience to erosion (Dai et al., 2024).

These soil structure improvements allow aboveground biomass to indirectly increase water infiltration, but cover crop canopies can directly influence soil water infiltration and storage through increasing evapotranspiration and providing surface cover. Numerous studies have reported increased infiltration rates under cover crops compared to bare soil (Ghafoor et al., 2012; Steele et al., 2012; Garcia-Gonzalez et al., 2018). Increased transpiration by cover crops can reduce soil water content, which in turn enhances the soil's water infiltration capacity during rainfall events, thereby reducing surface runoff (Jobbágy and Jackson, 2004). At the same time, the canopy provides surface cover that suppresses soil water evaporation, helping to retain moisture for the subsequent crop (Chen and Weil, 2011; Basche et al., 2016; Blanco-Canqui and Ruis, 2020).

However, the overall impact of cover crops on soil water availability is context-dependent. In water-limited environments, increased transpiration may lead to insufficient moisture reserves for the next crop (Nielsen et al., 2015). Conversely, in wetter climates, the reduced soil moisture following cover cropping can facilitate traffic operations and reduce the risk of soil compaction (Qi and Helmers, 2010). Thus, the effect of cover crop canopies on water availability varies with environmental conditions and management objectives.

Although the role of aboveground biomass in protecting and improving soil physical properties is clear (Fig. 1.1), roots have been found to be more influential in mitigating soil erosion (De Baets et al., 2006; Zhou and Shangguan, 2008; Burylo et al., 2012) and are essential for regulating soil water dynamics (George et al., 2024b).

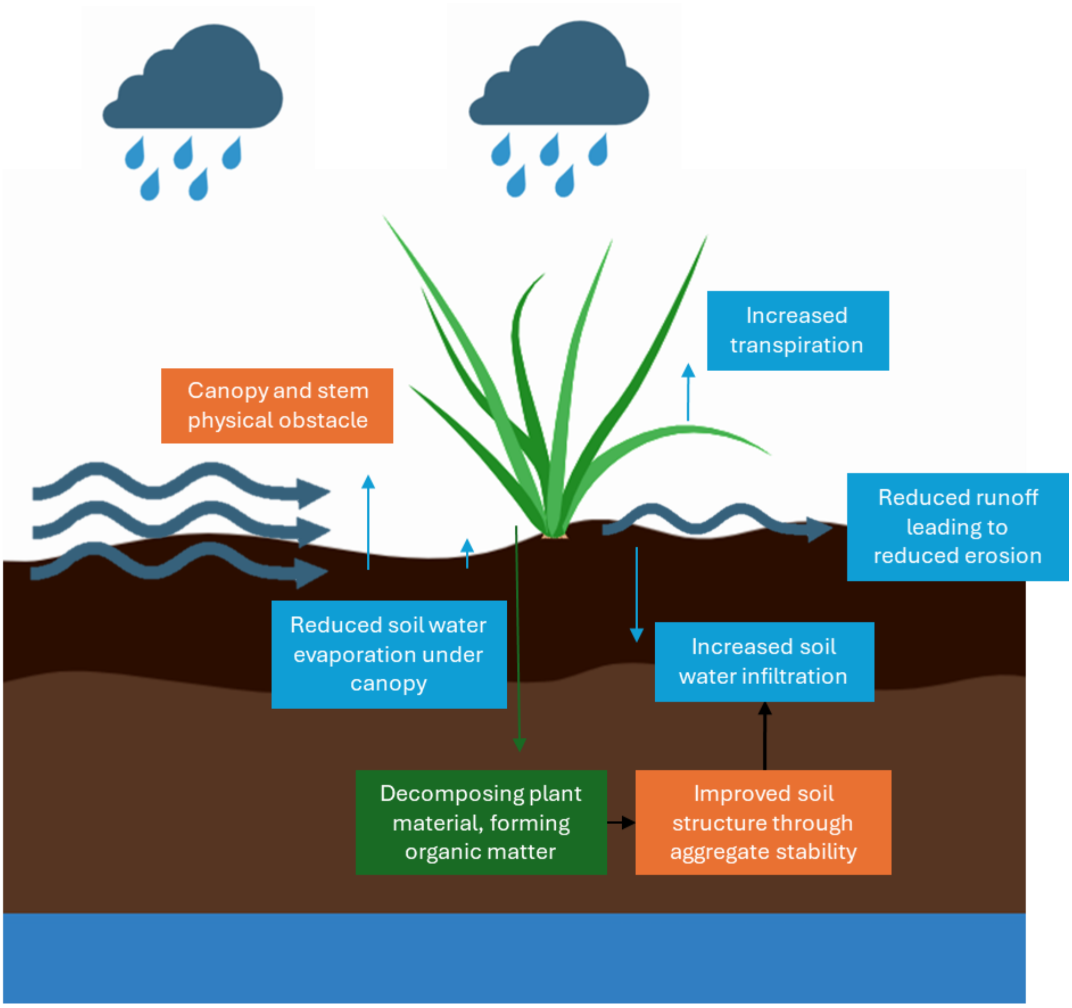

Figure 1.1 Summary diagram of the role of aboveground biomass in protecting and improving soil physical properties (blue = water processes, green = organic matter addition, orange = soil structure support).

1.2.2 How do roots mitigate erosion and improve water management?

The presence of roots and their architecture can mitigate and build resilience to soil erosion and water excess or deficit by improving soil structural and hydrological properties (Fig. 1.2). Griffiths et al. (2022) reviewed the effects of cover crop roots on ecosystem processes, and found they not only enhanced ecosystem services by capturing soil resources but also through providing physical anchorage and the addition of organic matter.

Firstly, through root growth and anchorage, roots reduce bulk density, bind soil, stabilise aggregates and provide biopores for water pathways, increasing soil water infiltration and saturated hydraulic conductivity (Bruce et al., 1991; Reeves et al., 1992). Root growth creates channels that prevent surface sealing and allow water to infiltrate instead of running over the soil surface (Liu et al., 2019). For example, one study found the coarse root axes of legumes reduced rainfall-induced runoff by up to 17% compared to thinner grass roots (Yu et al., 2016). In addition to the benefits of vertical root growth, the soil displacement by lateral root proliferation improves soil pore connectivity allowing increased soil water storage capacity and gas exchange (Helliwell et al., 2017). This enhancement of soil porosity by cover crops, specifically through increasing the proportion of macropores (Blanco-Canqui et al., 2011), is therefore critical for increasing water movement through the soil profile and mitigating soil erosion.

Secondly, roots add organic matter through decay and root exudation, known as rhizodeposition. Root exudates can act as a soil binding agent that changes the hydrophobicity of soil particles and improves soil aggregation, but also encourages microbial activity, therefore enhancing microbial breakdown of plant material leading to organic matter addition (Gyssels and Poesen, 2003). As mentioned in section 1.2.1, the addition of organic matter encourages the formation of stable aggregates, improving soils structure and its resilience to erosion. Root architectures vary between cover crop species and across time and space, meaning that roots can explore varied volumes of soil for water and nutrient pool access as well as provide a range of ecosystem functions (see Griffiths et al., 2022). For example, the dense and shallow rooting systems of grasses prevent soil water erosion more effectively than deep-taproot species due to higher root mass in the topsoil which improves the soil's resistance to water flow (Gyssels et al., 2005). Liu et al. (2024) found greater root length density and root surface area density of a fibrous rooting system (*Lolium perenne* L.) compared to a taproot (*Medicago sativa* L.) decreased soil detachment, with fibrous and taproot systems contributing up to 56% and 40%, respectively, to reducing soil detachment. Further, in an overland flow hydraulic flume experiment, species with a greater root diameter (e.g. *Sinapsis alba* L.) created greater localised overland flow erosion due to producing more turbulence than the thin and fibrous rooting system of grasses (e.g. *Secale cereale* L.) (De Baets et al., 2011). These findings highlight that the impact of root traits on erosion is context-dependent, underscoring the importance of selecting species based on specific land management goals.

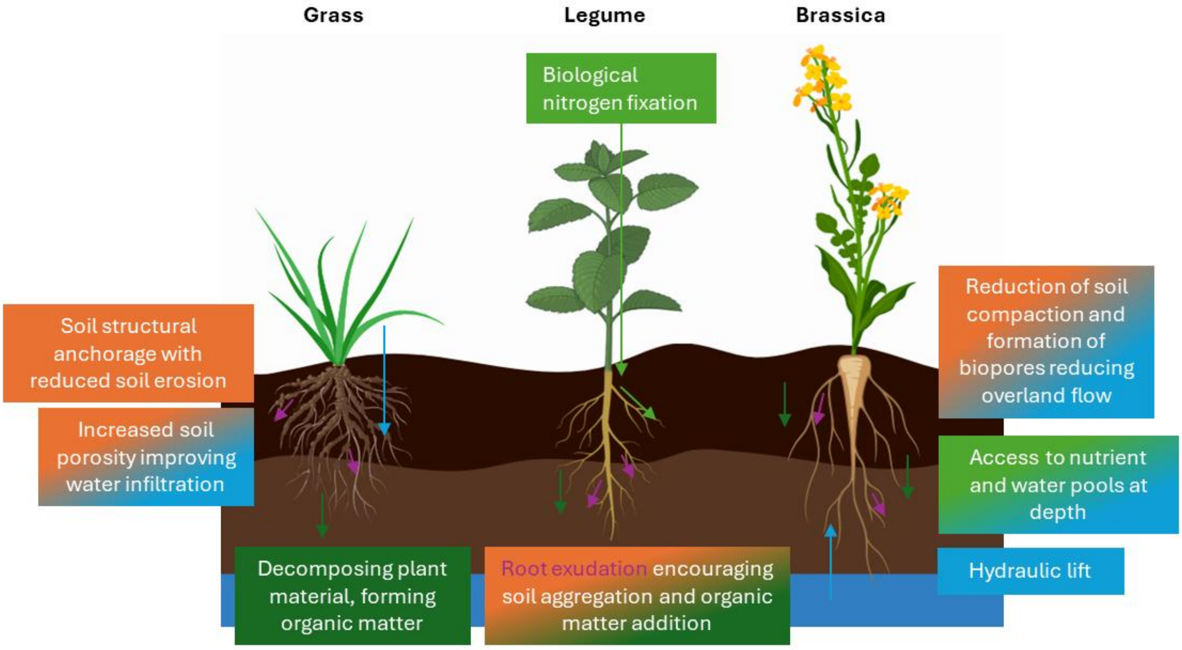

Figure 1.2 Summary diagram of the role of roots in protecting and improving soil physical properties with colours representing soil structure (orange), hydrology (blue), nutrient access (light green), organic matter addition (dark green), and root exudation (purple).

Understanding the impacts of different root traits across species is essential for informing sustainable land management and improving ecosystem resilience to abiotic stress. At the ecosystem level, roots play a vital role in shaping soil function, structure, and stability. A recent review by Gregory (2022) argues that roots influence soils so extensively that they are not "in" the soil but are fundamentally part of it, and therefore most soils in the environment are part of the rhizosphere.

The rhizosphere, coined by Hiltner (1904), is the soil that is influenced by root biological, physical, and chemical activity. Many of the abiotic stresses associated with climate change such as erosion, drought, and flooding will be primarily perceived by plants at this root–soil interface. Importantly, this interface plays a key role in mediating and potentially mitigating these stresses (George et al., 2024a). Despite its significance, the literature has largely ignored a key cover crop root trait for drought adaptation that indicates a plant's soil binding capacity: the rhizosheath, *i.e.* the layer of soil within the rhizosphere that adheres tightly to the roots. Though definitions can sometimes overlap, most studies treat the rhizosheath as a measurable proxy for rhizosphere processes (Pang et al., 2017).

1.3 The role of the rhizosheath

The rhizosheath, the soil layer that adheres to some plant roots (Fig. 1.3), is increasingly receiving attention for its potential for improving soil function and crop tolerance to abiotic stress. The rhizosheath was first observed by Volkens (1887) in desert graminoids and has since been identified in most angiosperm phyla (Brown et al., 2017). Rhizosheaths are richer in water, nutrients, labile carbon and microbial biomass than the surrounding rhizosphere (Hallett et al., 2022; Mo et al., 2023). The rhizosheath acts as a hotspot for plant-soil-microbe interactions and provides several functions including water and nutrient uptake (Rabbi et al., 2021), soil binding (Othman et al., 2004) and drought resistance (Basirat et al., 2019).

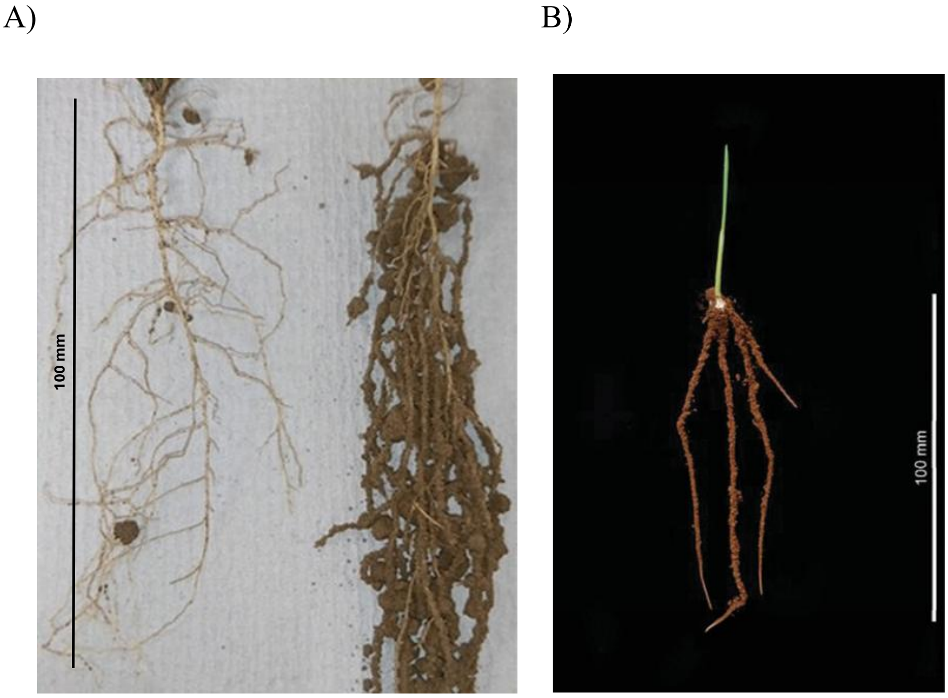

Figure 1.3 Examples of rhizosheath formation in A) *Lotus japonicus* with a hairless mutant genotype on the left and the wild type on the right (Burak et al., 2021b) and in B) roots of *Triticum aestivum* (Delhaize et al., 2012).

Multiple factors are involved in the formation of rhizosheaths including root hairs, root- and microbe-derived mucilage, mycorrhizal association (Peng et al., 2013; Ryan et al., 2016), and soil-related factors such as soil moisture content and texture. Several reviews identified the main factors affecting rhizosheath formation (Mo et al., 2023a; Aslam et al., 2022; Ndour et al., 2020; Pang et al., 2017) to be root hairs (length and density) and mucilage, however their relative contributions remain poorly understood, and results are variable across species and studies.

Mucilage, composed primarily of polysaccharides released from the root tip and produced by root-associated microbes (Vermeer and McCully, 1982; Read et al., 2003), is an adhesive substance that increases soil binding to roots and stabilises soil structure while facilitating plant water uptake (Sandhya et al., 2009; George et al., 2024b). During soil drying, the viscosity of mucilage increases and, due to its low surface tension and water adsorption capacity, mucilage helps maintain the continuity of the hydraulic connection to the soil matrix (Carminati, 2013; Benard et al., 2019). Dry-wet cycles affect rhizosheath formation by changing the viscosity and surface tension of mucilage influencing its expansion into the soil matrix and adhesiveness to soil particles (Aslam et al., 2022). This process is the reason why the rhizosheath is considered an adaptive trait to drought (Pang et al., 2017; Mo et al., 2023). However, some research using chia seed mucilage as an analogue of root mucilage (Rahim et al., 2023) has found dry-wet cycles may interrupt microbial mucilage production and therefore reduce rhizosheath formation compared to wet treatments with constant soil moisture conditions. Therefore, Naveed et al. (2017) suggest that the influence of dry-wet cycles on rhizosheath formation may depend on plant species mucilage composition and its adhesiveness.

Root hairs are tubular protrusions from root epidermal cells (Fig. 1.4) that are important for water and nutrient uptake (Parker et al., 2000). Numerous studies have found root hairs play a critical role in rhizosheath formation and its stability as they not only encourage mucilage production (Marasco et al., 2018; Galloway et al., 2022) but also increase the root-soil contact area (Peterson and Farquhar, 1996; Haling et al., 2010a; George et al., 2014; Brown et al., 2017; Burak et al., 2021b) and provide the physical structure to retain soil (Haling et al., 2010a). Longer and denser root hairs result in larger rhizosheaths in a variety of species such as *Solanum lycopersicum* (Karanja et al., 2021), *Hordeum vulgare* (Marin et al., 2021), *Triticum aestivum* (Delhaize et al., 2012) and *Vigna unguiculata* (Opoku et al., 2022). However, Brown et al. (2017), who analysed the rhizosheath formation capacity of 58 angiosperm species, found no correlation between root hair length and rhizosheath mass except for species with shorter root hairs. Further, Rongsawat et al. (2021) suggest shorter root hairs (up to 300 μm) are the primary driver of rhizosheath formation, and other root hair traits, such as morphology and density, combined with mucilage and other factors become dominant when root hairs are > 300 µm long. Some species naturally lacking root hairs, such as those from the *Allium* genus, are unable to form a rhizosheath (Brown et al., 2017). However, root-hairless mutants of *Hordeum vulgare* and *Zea mays* can still form a limited rhizosheath due to root exudation (Burak et al., 2021b). This suggests that factors affecting rhizosheath formation dominate or complement each other depending on species and genotype, highlighting the importance of understanding those of cover crop species for assessing the soil binding capacity of their root systems.

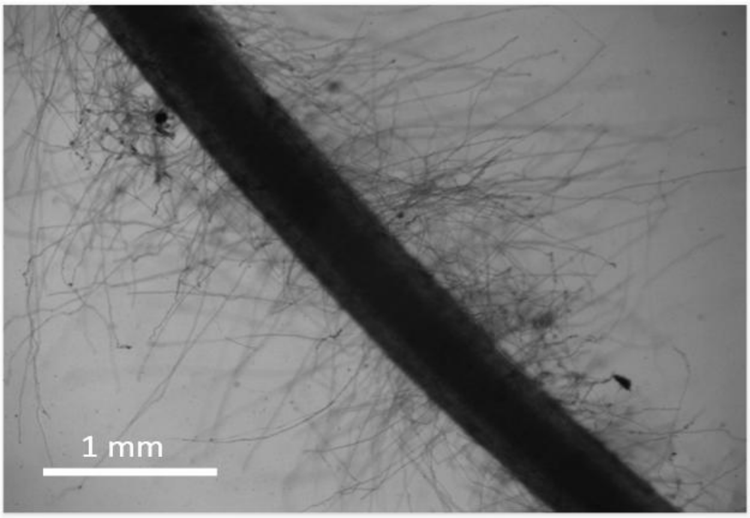

Figure 1.4 Example of root hairs on a *Secale cereale* root (from Chapter 4).

1.3.1 Role in abiotic stress

Rhizosheath formation is considered an adaptive trait to drought (Fig. 1.5) as it preserves root-soil contact, retains greater soil moisture than the bulk soil, and maintains water and nutrient uptake during soil drying (North and Nobel, 1997; Carminati et al., 2010; George et al., 2014; Pang et al., 2017; Cheraghi et al., 2023). Although the literature shows variable results (Hartnett et al., 2013; Marin et al., 2021), rhizosheaths are often larger under drought conditions and minimal or absent under wet conditions (Watt et al., 1993, 1994a; Zhang et al., 2020; Rabbi et al., 2021). Basirat et al. (2019) showed that a larger rhizosheath allowed plants to maintain a greater transpiration rate than a smaller rhizosheath which reached wilting point earlier, highlighting the importance of the rhizosheath to tolerate drought. Large rhizosheaths are common in arid and semi-arid environments (Hartnett et al., 2013), which is likely due to drought stress coupled with the soil texture as the lower root-soil contact and porosity of sandy soils, rather than loamy or clayey, often lead to larger rhizosheaths (Hallett et al., 2022). More recently, the rhizosheath has been emphasised as a key phenotypic component in plant-soil interactions research, specifically for drought adaptation, to achieve climate-resilient agricultural systems (Steiner et al., 2024). However, identifying the rhizosheath formation capacity of cover crop species as well as its response to variable water availability is still required.

Rhizosheaths also play a critical role in improving soil structure and protecting plant roots in challenging environments (Fig. 1.5). By forming a protective boundary, rhizosheaths help plants such as pioneer grasses in arid and semi-arid environments to anchor in loose, sandy soils and withstand environmental stresses such as erosion (Tian et al., 2019; T. Y. Liu et al., 2019; Wang et al., 2022). Compounds in rhizodeposits stabilise (Naveed et al., 2018) and strengthen the aggregates bound around roots (Czarnes et al., 2000). Therefore, rhizosheaths have a strong potential for improving soil aggregation and stabilising soil structure, which are key functions for improving soil health and resilience to a changing climate (Mo et al., 2023). Although the rhizosheath indicates the root system's capacity to bind soil, and therefore affect soil erosion (Burak et al., 2021a), there has been little research into the role of the rhizosheath in soil erosion control.

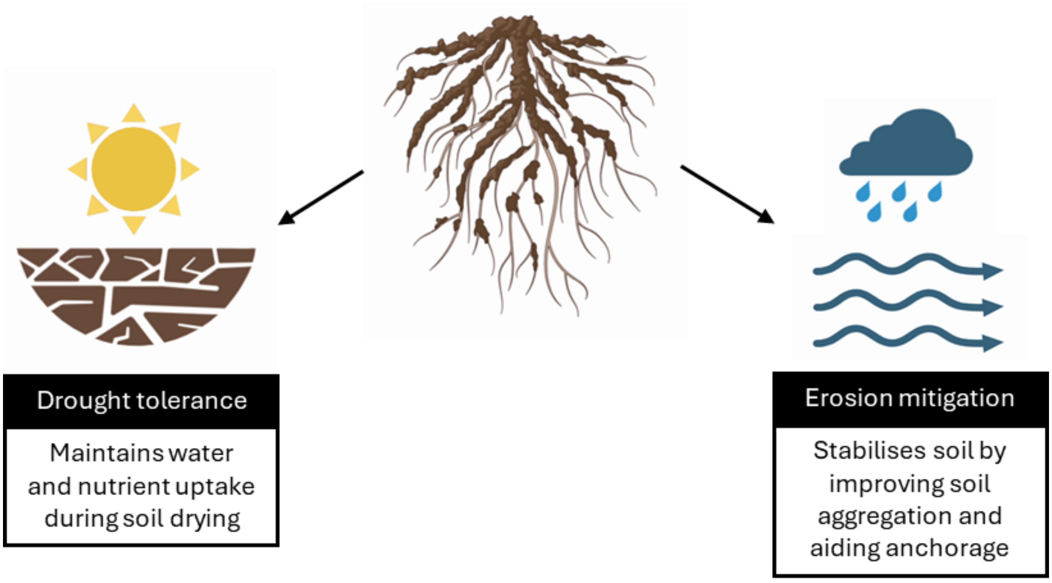

Figure 1.5 Summary diagram of the role of the rhizosheath in improving crop system tolerance to abiotic stress.

Despite the array of benefits the rhizosheath can bring to soil function and ecosystem services, little is known about its persistence over time. The rhizosheath is considered a plastic trait that is sensitive to rainfall patterns (Hartnett et al., 2013). Some short-term (approximately 1 week) experiments under controlled conditions found rhizosheath formation increases under dry conditions (Watt et al., 1994a; Haling et al., 2014; Liu et al., 2019), whereas rhizosheaths in *Hordeum vulgare* (Marin et al., 2021) and in semi-arid savanna grasses (Hartnett et al., 2013) decreased in mass during dry periods. This was attributed to better growing conditions over wetter periods that encourage root exudation and root hair development and therefore increase the overall mass of the root system and the associated rhizosheath mass. Therefore, understanding the plasticity of rhizosheath formation, a trait with great potential for improving system resilience, is important for selecting appropriate cover crops, especially during the winter months in temperate climates like the UK when soil stability is most needed.

Further, rhizosheath formation has been mainly investigated under controlled conditions with few field trials (Marin et al., 2021; Tian et al., 2022). Therefore, field experiments are needed to verify rhizosheath formation factors in crop species of diverse rooting systems (Hallett et al., 2022; Mo et al., 2023; George et al., 2024b). Moreover, since cover crops are sown to protect the soil over the winter months, complementary root traits between cover crop species could lead to synergistic or additive effects on soil binding capacity and soil stabilisation.

1.4 Can cover crop diversity enhance ecosystem function?

Increasing cover crop diversity has the potential to further improve ecosystem functioning through the complementary use of resources in time and space (Brooker et al., 2021; Homulle et al., 2022). A key ecological principal is that greater plant species diversity can benefit ecosystem function (Tilman et al., 1997), and in terms of resource capture, functional diversity is considered more influential than species richness (Tilman et al., 1997; Cardinale et al., 2012; Hernandez and Picon-Cochard, 2016; Bakker et al., 2016). Different cover crop functional traits within a mixture can provide benefits to various ecological niches, *e.g.* legumes fixing N_2 and brassicas reducing bulk density with their deeper, thicker rooting system, consequently facilitating resource acquisition and increasing biomass (Smith et al., 2014; Ruis et al., 2020). Therefore, investigating cover crop species functional traits and their complementary interactions is essential to select appropriate species combinations in the field to improve ecosystem service performance.

Most studies focus on above-ground traits (Roscher et al., 2012; Finegan et al., 2015), however resource complementarity is mainly expected to occur belowground (Bardgett et al., 2014; Yang et al., 2015). Farmers are advised to select diverse cover crop species combinations with various rooting depths and architectures to maximise the volume of soil exploited by roots and, therefore, for aggregate stability, increased organic matter, and improved water infiltration and soil structure (NRCS, 2024). Through vertical niche differentiation, root diversity could increase exploitation and decrease competition for resources (Bakker et al., 2016). However, few studies quantify the benefits of increasing plant diversity on soil-associated ecosystem services regulated by roots.

Cover crop mixture studies have primarily focused on direct benefits to the subsequent crop yield, or indirect ones such as weed suppression, pest control, and soil fertility (Florence and McGuire, 2020; Jian et al., 2020). However, cover crop mixtures also have the potential to improve soil properties through synergistic effects on a wide range of factors and processes (Chapagain et al., 2020). For instance, they can enhance root mass (Farney et al., 2018), water uptake (Hauggaard-Nielsen et al., 2012), microbial functional diversity (Drost et al., 2020), and soil carbon and nitrogen pools (Conant et al., 2005; Müller-Stöver et al., 2012).

Despite these known benefits, research on the impacts of belowground diversity on soil-associated ecosystem services, particularly its role in soil erosion control and water management, is limited. Koudahe et al. (2022) reviewed the effects of cover crops on aggregate stability (an indicator of soil structural development and erodibility), water infiltration and hydraulic conductivity (measures of water absorption and movement capacities). They found the extent to which cover crops improve these properties depends on cover crop species and mixtures, growth period, amount of biomass, and environmental conditions (Blanco-Canqui et al., 2011; Blanco-Canqui and Jasa, 2019; Darapuneni et al., 2021). This variability highlights the need to identify suitable cover crop combinations for soil and water conservation, especially under increasingly uncertain rainfall patterns.

The ability of cover crop mixtures to bind soil under varying levels of soil water availability remains largely unexplored. Cover crop diversity offers a range of root traits that may mitigate soil erosion and overland flow by filling canopy space more effectively and occupying a greater soil volume through increased above- and below-ground biomass (Liu et al., 2020). Although roots play a vital role in reducing sediment loss from overland flow erosion (De Baets et al., 2006, 2011; Ola et al., 2015; Kamchoom et al., 2022; Liu et al., 2024), no studies to date have examined the soil retention capacity of cover crop roots with varying diversity levels. Complementary root traits between cover crop species could lead to synergistic or additive effects on soil binding capacity and soil stabilisation, meaning that investigating rhizosheath development in cover crop mixtures has great potential to help improve the delivery of related ecosystem services.

This functional diversity not only has the potential to enhance soil retention but also improve multifunctionality in agricultural systems. As Griffiths et al. (2022) noted, the diversity of cover crop species currently available can provide an array of agroecosystem services, influencing the selection and utilization of cover crop mixtures tailored for specific ecosystem needs. Increasing agricultural plant diversity could be valuable for sustainable management decision-making in UK agricultural systems and restore agroecosystem functions, helping build resilience of cropping systems to abiotic stress.

1.5 Summary and research gaps

Here I summarise the current state of knowledge on how cover crops and their increasing diversity could help mitigate soil erosion and manage soil water. Specific attention is given to the roots and the rhizosheath, a critical trait of the root-soil interface that may hold significant importance for building resilience of agricultural systems to climate change and its associated extreme weather events. The rhizosheath helps plants mitigate the physiological impacts of water deficits, **but little is known about:**

- the rhizosheath formation capacity of cover crop species under varying levels of water availability, and whether increasing species diversity in cover cropping systems has synergistic or additive effects on their soil binding capacity.
- the effect of root functional diversity and the associated soil binding capacity on mitigating overland flow erosion, the frequency of which will increase with extreme weather events resulting from climate change.
- the persistence of rhizosheaths over time, specifically over winter months in temperate climates, when soil is subject to variable rainfall patterns.

- the mechanisms underlying rhizosheath formation in different cover crop species, and the relative contribution of each factor.

Addressing these research gaps (Fig. 1.6) will help understand the role the rhizosheath plays in binding soil in cover crop species grown in monocultures and mixtures and inform cover crop species selection in the field to help build soil resilience to abiotic stress.

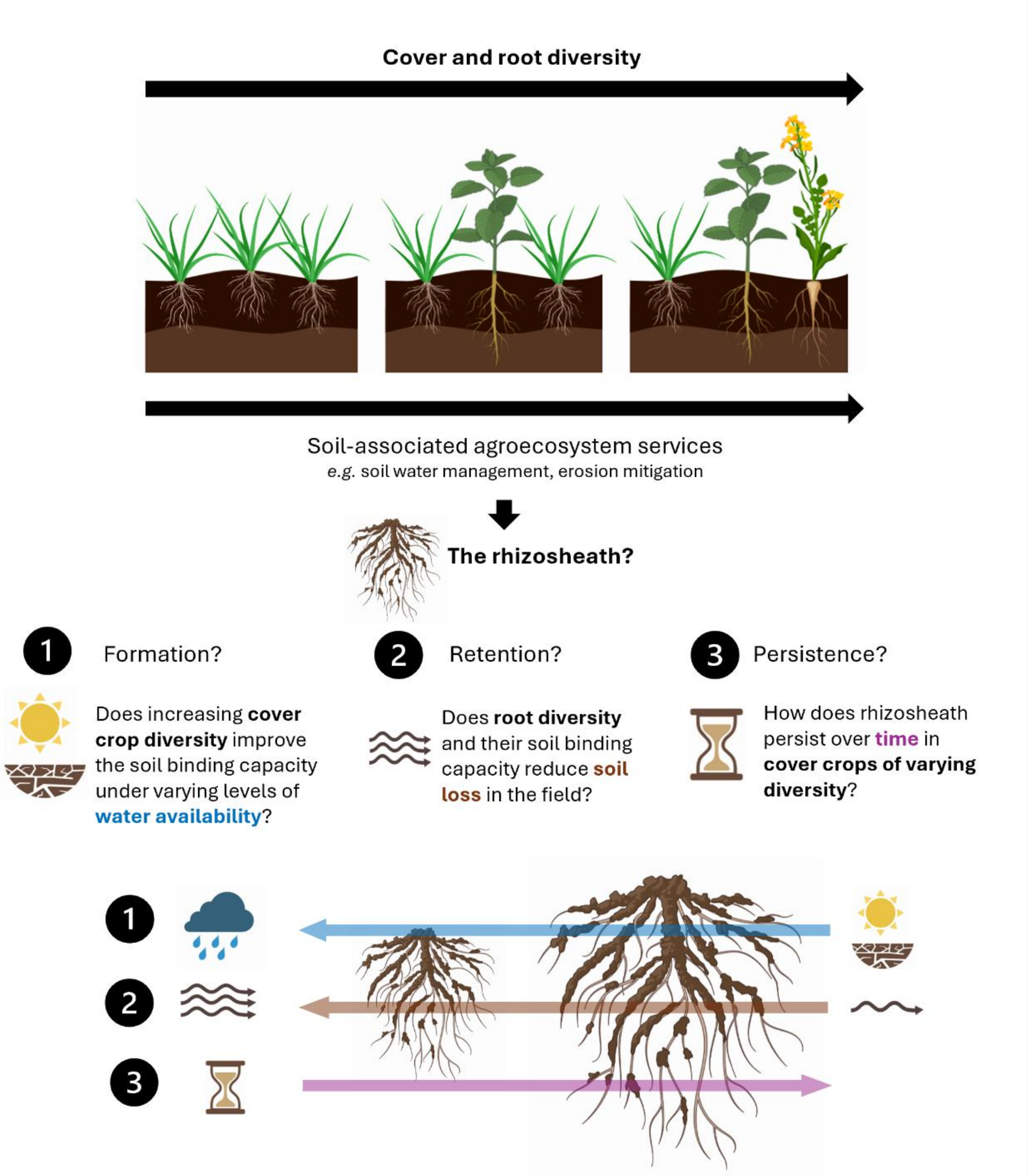

Figure 1.6 Summary diagram of key research gaps of the effects of increasing cover crop diversity on enhancing soil-associated ecosystem services through rhizosheath formation, i.e. soil binding capacity, as well as key hypotheses.

1.6 Thesis aims, objectives and structure

This thesis aims to test the hypothesis that:

- increased cover crop diversity improves soil binding capacity, thereby enhancing soil erosion control and resilience to water stress, by allowing complementary use of soil resources in time and space.

This will be achieved by carrying out a series of experiments in both mesocosm and field scales using different levels of cover crop diversity, ranging from individual species to cover crop mixtures of up to three functionally diverse species. The research quantifies how different levels of cover crop diversity affect rhizosheath formation, and whether there are additive or synergistic effects on soil binding under drought, soil water erosion control and rhizosheath persistence over time. Underpinning mechanisms are investigated and discussed. By providing scientific evidence for the effects of increased plant diversity on agroecosystem function, this project intends to inform land managers of cropping practices to conserve soil function and aid in delivering environmental policy targets for agriculture.

Chapter 2 aimed to determine rhizosheath formation capacities of cover crop species commonly used in UK agricultural systems under varying levels of water availability and species diversity at the mesocosm scale.

> H_1: Cover crop species under drought conditions will form a larger rhizosheath than under well-watered conditions.
>
> H_2: Grasses, due to their fibrous rooting system will form a larger rhizosheath than the tap roots of brassicas.
>
> H_3: Combining species may have an interactive effect on rhizosheath formation due to synergistic effects on *e.g.* root mass and therefore increasing cover crop diversity may be beneficial for soil and water management and strengthen the resilience to drought stress.

To test these hypotheses, two polytunnel mesocosm experiments were conducted where cover crop species of diverse functionality were grown under droughted vs well-watered conditions. Four species (*Secale cereale*, *Lolium westerwoldicum*, *Raphanus sativus*, *Brassica juncea*) were grown as monocultures in the first mesocosm experiment. In the second mesocosm experiment, *S. cereale* and *B. juncea* were grown as single or mixed species treatments due to their divergent rooting systems and soil binding capacities, to assess whether increased diversity led to greater rhizosheath formation.

Chapter 3 built on the findings on Chapter 2 by moving from the mesocosm scale to the field (plot scale) and aimed to assess the impact of cover crop diversity and associated root diversity on soil loss in the field. While Chapter 2 focused on how rhizosheath formation in single and mixed cover crops responded to drought in controlled mesocosms, Chapter 3 evaluated how the rhizosheath of single and mixed species functions in field conditions, particularly in its ability to mitigate erosion.

> H_1: Increased cover crop diversity increases root biomass and thus rhizosheath mass.
> H_2: Increased rhizosheath mass decreases sediment loss.
> H_3: Increased cover crop and root diversity reduces sediment loss by roots binding more soil.

To test these hypotheses, overland flow simulations were conducted in a winter cover crop field trial, established close to Dundee, Scotland, in autumn 2022, measuring soil loss as well as rhizosheath formation in treatments containing *Brassica juncea*, *Secale cereale* and *Vicia faba* grown in monocultures and in all possible combinations.

Chapter 4 aimed to investigate the underlying mechanisms of rhizosheath formation in the same cover crop species, adopting a more mechanistic approach to explore the drivers of rhizosheath development and rhizosheath persistence under field conditions across varying levels of species diversity. Building on previous chapters, it focused on how the rhizosheath changes over time, providing insight into the temporal dynamics of this important root-soil interface.

> H_1: Cover crop diversity will increase rhizosheath mass.
> H_2: Rhizosheath mass will change over time.
> H_3: Rhizosheath mass will increase with longer and denser root hairs.

To test these hypotheses, *B. juncea*, *S. cereale* and *V. faba* were sown as monocultures and mixtures in a winter cover crop field trial near Dundee, Scotland. Soil cores for rhizosheath and root trait measurements were collected three times during January-March 2023. This field trial was devised as a subset of the plots set up for the field trial used in Chapter 3.

Chapter 2. Rhizosheath formation of different cover crops at different soil water availability.

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Abstract

Cover crops can help increase the resilience of agricultural systems to drought by covering bare soil and inducing soil structural and hydrological changes. Increasing cover crop species diversity has been found to benefit ecosystem services aboveground, but quantifying the effects on ecosystem services delivered by belowground diversity have been largely ignored. How cover crops bind soil (rhizosheath development, an adaptive root trait to drought tolerance) has attracted little attention despite their potential for soil and water management under drought stress.

This work aimed at determining rhizosheath formation capacities of cover crop species commonly used in UK agricultural systems under varying levels of water availability and species diversity. To achieve this, two polytunnel mesocosm experiments were conducted to assess rhizosheath formation of different cover crop species under droughted vs. well-watered conditions. Four species (*Secale cereale L., Lolium westerwoldicum L., Raphanus sativus L., Brassica juncea L.*) were grown as monocultures in the first mesocosm experiment. In the second mesocosm experiment, *S. cereale* and *B. juncea* were grown as single or mixed species treatments due to their diverging rooting architectures and soil binding capacities to assess whether rhizosheath formation was interactive as diversity increased. Both mesocosm experiments consistently showed each individual species had a different soil binding capacity regardless of water availability. Grass species formed a greater specific rhizosheath mass than brassica species, likely due to their differing root traits. *S. cereale* outcompeted *B. juncea,* which maintained its specific rhizosheath mass whether grown in a monoculture or a mixture, therefore proving to be a good cover crop choice for binding soil. Further research is needed to assess rhizosheath persistence over time in the field as well as the mechanisms behind it, since here it decreased over time. Overall, assessing the soil binding capacities of different

cover crop species helps inform on-farm decisions and identifies cover crop combinations to build agricultural system resilience to the variable abiotic stresses.

2.1 Introduction

Cover crops can help build the resilience of our agricultural systems to rainfall uncertainty and frequent extreme weather events associated with climate change. Cover crops, often introduced into row crop production systems or during fallow periods, protect soil that would otherwise be left bare and exposed to the elements. By introducing above- and below-ground vegetation into bare soil, cover crops can reduce soil erosion and improve soil structure (De Baets et al., 2011), reduce soil evaporation, increase soil-water retention (Colla et al., 2000), and can also increase soil infiltration capacity when thick roots decay forming channels (Archer et al., 2002; Koudahe et al., 2022).

Increasing the diversity of cover crops by introducing cover crop mixtures instead of single species is thought to greatly improve soil properties (Chapagain et al., 2020), as combinations of different cover crop functional groups within a mixture can provide benefits to various ecological niches, *e.g.* legumes fixing N_2 and brassicas reducing bulk density with their deeper, thicker rooting system (Smith et al., 2014; Ruis et al., 2020). Growing cover crop mixtures can lead to synergistic effects on a wide variety of factors and processes essential to improving ecosystem function such as root mass (Farney et al., 2018), water uptake (Hauggaard-Nielsen et al., 2012), microbial functional diversity (Drost et al., 2020) and soil carbon and nitrogen pools (Conant et al., 2005; Müller-Stöver et al., 2012). Cover crop mixture studies mainly focus on soil fertility, weed suppression or pest management, with some studies finding that increasing the number of cover crop species is agronomically beneficial (Snapp et al., 2005; Chu et al., 2017; Hunter et al., 2019) while others not (Wortman et al., 2012; Finney et al., 2016). However, despite the pressing need to find suitable cover crop combinations for soil and water conservation under increasingly variable rainfall patterns, little is known about how cover crop mixtures influence root traits that contribute to soil binding under different levels of soil water availability. One such trait is rhizosheath formation, *i.e.* the layer of soil that adheres tightly to the root surface, which plays a critical role in mediating plant–soil interactions.

Rhizosheath formation, which reflects the soil binding capacity of the plant, is considered key for water stress tolerance (Ndour et al., 2020; Hallett et al., 2022). Under dry conditions, rhizosheaths of different plant species have been found to retain a greater moisture content than the bulk soil (Young, 1995; North and Nobel, 1997; Carminati et al., 2010). Basirat et al. (2019) found rhizosheath presence improved plant access to water and therefore enabled sustained transpiration and plant nutrient uptake. Under drought conditions, they found increased rhizosheath mass enhanced plant transpiration rate, whilst its absence led to an earlier wilting point. Rhizosheaths are therefore considered an adaptive trait, key for tolerating drought stress.

Drought stress has been found to encourage rhizosheath formation (Watt et al., 1994b; Rabbi et al., 2021). In maize, greater rhizosheath development was found in dry (5 times the volume of subtending roots) than in wet soil (1.2 times), and in dry soil, the rhizosheath was more resistant to removal from the root (Watt et al., 1994b). While larger rhizosheaths form in droughted conditions, wet conditions often lead to smaller rhizosheaths or their absence (Watt et al., 1993; Zhang et al., 2020). This is because the change in soil moisture can influence the key rhizosheath formation factors: mucilage and root hairs.

Root hairs are important as they are the physical structure that supports rhizosheath formation (Burak et al., 2021b). In addition, root hairs further aid plant drought tolerance by facilitating access to soil pore water and reducing evaporative water loss by improving soil particle connectivity and disrupting the continuous water film (Rabbi et al., 2021). Drought in maize plants encouraged root hair formation, causing 4.5-fold greater densities of root hairs in dry than wet soil (Watt et al., 1994b).

Mucilage is also a key factor influencing rhizosheath formation and it is formed primarily by polysaccharides exuded from plant roots and root-associated soil microbes (Vermeer and McCully, 1982; Read et al., 2003). Changes in soil moisture interact with the viscosity and adhesiveness of mucilage (Aslam et al., 2022). Dry-wet cycles can encourage rhizosheath formation (Pang et al., 2017) as wet cycles allow mucilage to diffuse into surrounding soil, while the loss of soil moisture can increase mucilage viscosity and therefore improve soil particle adhesion. The increase in mucilage viscosity leads to a reduction in its surface tension, allowing it to wet further soil particles (Pang et al., 2017;

Naveed et al., 2018; Aslam et al., 2022), and increase the rhizosheath water retention capacity (Zhang et al., 2021). However, dry-wet cycles do not always encourage rhizosheath development. Rahim et al. (2023) used chia seed mucilage on artificial roots (jute cords) to show that wet treatments enhanced rhizosheath development more than dry-wet cycles. They suggested that constant wet conditions may interfere less with microbial mucilage production, allowing uninterrupted rhizosheath accumulation (as opposed to it being interrupted by soil cracking and shrinkage during soil drying) as well as allowing mucilage to diffuse further into the bulk soil and consequently increase rhizosheath formation. Therefore, as suggested by Naveed et al. (2017), the influence of dry-wet cycles on rhizosheath formation may depend on mucilage adhesiveness and plant species.

Rhizosheath formation has been reported across a wide range of plant species (Brown et al., 2017), however no work has been found on rhizosheath development in cover crop species, particularly when grown in mixtures and under varying levels of water availability. Therefore, this research aims to determine rhizosheath formation capacities of cover crop species commonly used in UK agricultural systems under varying levels of water availability and species diversity. To achieve this, two mesocosm experiments were conducted in a polytunnel to assess rhizosheath formation of different cover crop species from two functional groups (grass and brassica) under droughted vs. well-watered conditions. Firstly, rhizosheath development under different watering regimes was assessed in single species stands of grass and brassica cover crop species: S*ecale cereale*, *Lolium westerwoldicum*, *Raphanus sativus* and *Brassica juncea.* A second mesocosm experiment assessed the effect of cover crop diversity on rhizosheath development in droughted *vs* well-watered conditions by mixing S. *cereale* and *B. juncea.* It is hypothesised that cover crop species under drought conditions will form a larger rhizosheath than under well-watered conditions, and that grasses, due to their fibrous rooting system will form a larger rhizosheath than the tap roots of brassicas. It is also hypothesised that combining species may have an interactive effect on rhizosheath formation due to synergistic effects on *e.g.* root mass and therefore increasing cover crop diversity may be beneficial for soil and water management and strengthen the resilience to drought stress.

2.2 Methods

Two mesocosm experiments were conducted in Hazelrigg Field Station, Lancaster University, UK (54°1’ N, 2°46’ W) in the summers of 2021 and 2022. The topsoil used was Upton Series, a silty clay loam (18.8 % sand, 57.2 % silt and 24 % clay) and was sieved to 1 cm to remove any debris carried over from its original site, resulting in a relatively uniform distribution of aggregate sizes up to 1 cm. Soil particle size was determined using a Mastersizer 2000 (Malvern instruments Ltd, UK). Before adding the topsoil, a 5 cm layer of pebbles was introduced into the bottom of each pot to facilitate drainage. The soil bulk density was determined at the end of each experiment as mass per volume of soil cores (5.8 cm diameter x 5.8 cm height) taken from the surface of each pot after oven-drying the soil at 105°C for 24 h.

2.2.1 Single species experiment

The first mesocosm experiment grew monocultures of cover crop species common to UK agricultural systems: Westerwold ryegrass (*Lolium westerwoldicum* L.), rye (*Secale cereale* L.), fodder radish (*Raphanus sativus* L.) and brown mustard (*Brassica juncea* L.). These species were chosen following an establishment trial conducted in the same location to ensure mesocosm success, and included 6 species of commonly grown cover crops in the UK: two grasses (*L. westerwoldicum* L. and *S. cereale* L.), two brassicas (*R. sativus* L. and *B. juncea* L.) and two legumes (*Vicia sativa* L. and *Trifolium repens* L.). The sowing densities were 3.7 g m^{-2}, 18.5 g m^{-2}, 1.5 g m^{-2}, 1.5 g m^{-2}, 8.4 g m^{-2}, 0.9 g m^{-2}, respectively. The legume species did not successfully establish and therefore the remaining 4 species were used for the first mesocosm experiment (Fig. 2.1).

Figure 2.1 Image of one of five blocks comprising the establishment trial using 1.5 L pots. From left to right, the top row pots contain mustard, vetch and white clover, and the bottom row pots contain radish, rye and ryegrass.

Based on this preliminary trial, the main experiment was designed as a randomised block experiment comprising 40 pots, which included four species, two watering regimes and 5 replicates. It was sown on 24th July 2021 in planter pots (36 cm length x 36 cm width x 38 cm height) at the same sowing densities as in the establishment trial. Cover crops were well-watered/rainfed and allowed to establish outside and reach 100 % surface coverage by the plant canopy before being placed in a polytunnel at week 5 to apply the two different watering regimes (droughted and well-watered) (Fig. 2.2).

Figure 2.2 Images of the day the mesocosm was sown (24th July 2021) (left) and the day the mesocosm was moved into the polytunnel (31st August 2021) (right). Pots were covered with aluminium foil to reduce soil water evaporation and maintain moisture required for germination.

Once in the polytunnel, stomatal conductance and soil moisture were measured daily to verify that drought conditions were achieved. Stomatal conductance (mmol m^{-2} s^{-1}) was measured at 10 am every day using a porometer (Model AP4, Delta-T Devices, Burwell, UK) (Appendix 1 Fig. 7.1). Two measurements were taken from leaves of two different plants per pot and averaged. Measurements were not taken on high temperature days due to equipment failure. Volumetric soil moisture content was measured after stomatal conductance using a FieldScout TDR 350 Soil Moisture Meter at a 7.6 cm depth in the centre of the top right quarter of each pot (Appendix 1 Fig. 7.3).

Well-watered pots were watered daily, whilst droughted pots were only watered if necessary for plant survival. This was assessed every day by visual checks. Watering regimes were applied from week 5 after sowing and soil cores were taken every subsequent two weeks (week 7, 9 and 11) to measure rhizosheath mass. The cores (15 cm diameter x 25 cm height) were taken from different corners of the pot each time and backfilled with Upton soil to maintain integrity of the mesocosm.

The number of plants within the cored diameter was recorded; the shoots were harvested by cutting at the stem base and oven dried at 70°C for four days to record aboveground dry biomass. Cores were stored in a cold room at 4°C and processed within three weeks. Rhizosheath mass was measured by carefully breaking the core open, removing the roots from the core and gently shaking them by hand until the bulk soil detached. Using deionised water, the rhizosheath of each species was washed off the roots into a pre-weighed aluminium tray. Rhizosheath soil and roots were oven-dried at 105°C overnight and at 70°C for four days, respectively, and the dry mass was recorded. Specific rhizosheath mass was calculated by dividing the rhizosheath soil dry mass by the corresponding root dry biomass. After the last sampling time (week 11), the total number of plants per pot was recorded and all aboveground biomass was harvested by cutting at the base of the shoots, then oven dried at 70°C for seven days and weighed.

2.2.2 Mixed species experiment

The second mesocosm experiment grew rye and brown mustard as single and mixed species to assess rhizosheath development under varying watering regimes and species diversity. These species were chosen due to their differing rooting architectures and functionalities (Griffiths et al., 2022). This experiment was designed as a randomised block experiment containing a total of 30 pots, which included two species grown as monocultures and mixed (three treatments), two watering regimes (well-watered and drought) and 5 replicates. It was sown on 2nd June 2022 at the same sowing rates as described above, and at 50:50 % of the monoculture sowing densities in the mixed treatment (Fig. 2.3).

Figure 2.3 Close-up of a rye and brown mustard mixture. Cover crops were well-watered/rainfed, allowed to establish outside, and moved into the polytunnel on week 3. The experiment was not moved into the polytunnel earlier due to a heatwave causing high temperatures inside the polytunnel to which the plants were not acclimatised.

The plants were allowed to establish outside the polytunnel until week 3, when they were moved into the polytunnel. Once in the polytunnel, watering regimes were applied and stomatal conductance was measured every second day, while soil moisture was measured daily to verify that drought conditions were achieved as in the previous experiment (Appendix 1 Fig. 7.5 and Fig. 7.7, respectively). In the mixed treatment pots, stomatal conductance was measured on leaves from two separate individuals of each species.

The watering regime was established according to the moisture retention curve of this soil at the bulk density of the pots in the first mesocosm experiment (Appendix 1 Fig. 7.9). The moisture retention curve was measured using the HYPROP evaporation method for the wet range (between 0 and −100 kPa) and the WP4C psychrometer method for the dry range (METER Group AG, München, Germany). Well-watered plants were watered to a target soil moisture of 25 % VWC (75 - 100 % field capacity) and droughted plants were allowed to drop down to a soil moisture level of 7 % VWC (~ 25 % field capacity). Daily soil moisture measurements using the FieldScout TDR 350 Soil Moisture Meter, which was calibrated against air-dried soil cores, helped inform watering amounts.

Soil cores (6.4 cm diameter x 20 cm height) were collected weekly from each pot in weeks 4 to 7. The size of the core was reduced for practical and time-saving purposes compared to that of the first experiment, where larger cores were needed to accommodate the size of the radish roots. Plant density, aboveground dry biomass, rhizosheath dry mass and root dry biomass were recorded as described above. After the last sampling time (week 7), the total number of plants per pot was recorded and all aboveground biomass was harvested by cutting shoots at the stem base, then oven dried at 70°C for seven days and weighed.

2.2.3 Data analysis

Statistical analysis was performed in R (R Core Team, 2024). For both mesocosm trials, aboveground dry mass (g plant^{-1}), root dry mass (g plant^{-1}) and specific rhizosheath mass (rhizosheath mass per unit of root dry mass) were logarithmically transformed to normalise the data, while rhizosheath dry mass (g plant^{-1}) was logarithmically transformed for the first experiment and square root transformed for the second. They were all tested for normality using the D'Agostino's K^2 test.

Linear regressions were performed to identify relationships between stomatal conductance and soil moisture content; and between aboveground biomass and soil moisture content. Factorial repeated measures ANOVAs were performed to assess the main effects and interactions for the factors of species, watering regime and sampling time on soil moisture, stomatal conductance, aboveground dry mass, root dry mass, rhizosheath dry mass and specific rhizosheath mass in experiment 1. The same was tested in experiment 2, but including the effect of diversity. In both experiments, block was set as random factor and pot was included as a nested factor to account for repeated measures on the same pots at different time points. Pairwise comparisons using a post hoc Tukey's HSD test were conducted to identify treatment groups showing significant differences following a significant ANOVA main effect or interaction. Data graphically presented in the results section include all sampling dates. All results presented are mean ± standard error and significance is at a 95% confidence level. Packages used were ggplot2, stats, dplyr and emmeans.

2.3 Results

2.3.1. Single species experiment

Soil bulk density was 0.96 g cm^{-3} for experiment 1. Soil moisture and stomatal conductance were significantly less in droughted than well-watered treatments (F(1, 91) = 296.46, $p < 0.001$ and F(1, 91) = 157.85, $p < 0.001$, respectively) (Appendix 1 Fig. 7.2 and Fig. 7.4) and had a significant and weakly positive relation ($p < 0.001$, $r^2 = 0.18$, $y = 112 + 4.97x$) (Fig. 2.4).

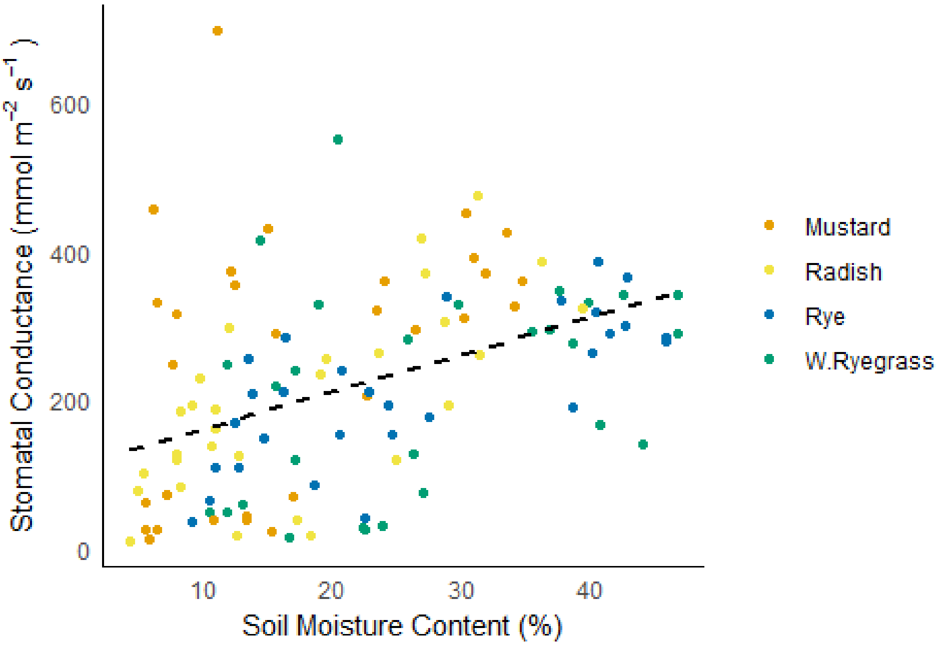


Figure 2.4 Overall relationship between soil moisture content (%) and stomatal conductance (mmol m^{-2} s^{-1}) for four species belonging to two functionally diverse families (brassicas and grasses), where orange represents mustard, yellow radish, blue rye and green W. ryegrass (n = 117). Line represents the slope of the relationship.

Aboveground dry mass per plant was not significantly different between watering regimes (F(1, 91) = 1.88, p = 0.17) but was between species (F(3, 91) = 104.04, $p < 0.001$). Aboveground dry mass per plant was larger in radish than mustard (T(91) = 14.41, $p < 0.001$), W. ryegrass (T(91) = 15.63, $p < 0.001$) and rye (T(91) = 11.8, $p < 0.001$), with the latter being significantly larger than W. ryegrass (T(91) = 4.02, $p < 0.001$). Further, aboveground dry mass per plant was significantly smaller in week 7 than week 9 (T(91) = -3.31, $p < 0.001$) and week 11 (T(91) = -4.72, $p < 0.001$), but did not differ between week 9 and week 11 (T(91) = 1.34, p = 0.37), resulting in a significant effect of time (F(2, 91) = 11.91, p < 0.001). Thus, aboveground dry mass per plant was not affected by watering regimes, was largest in radish and rye and smallest in mustard, and increased from week 7 to week 9, remaining stable in week 11 (Appendix 1 Table 7.1).

Root dry mass per plant did not differ between watering regimes (F(1, 91) = 0.01, p = 0.89) (Fig. 2.5a) but varied significantly between species (F(3, 91) = 45.47, p < 0.001). Rye and radish had a significantly larger individual plant root dry mass than W. ryegrass (T(91) = 4.68, p < 0.001 and T(91) = 7.03, p < 0.001, respectively) and mustard (T(91) = -8.27, p < 0.001 and T(91) = -10.63, p < 0.001, respectively), the latter being significantly smaller than W. ryegrass (T(91) = -3.41, p = 0.005) (Fig. 2.5b). Further, root dry mass per plant was significantly smaller in week 7 than week 11 (T(91) = 2.67, p = 0.02), resulting in a significant effect of time (F(2, 91) = 3.64, p = 0.03). Thus, root dry mass per plant did not differ between watering regimes, was larger for rye and radish than W. ryegrass, and smallest for mustard, and increased with time.

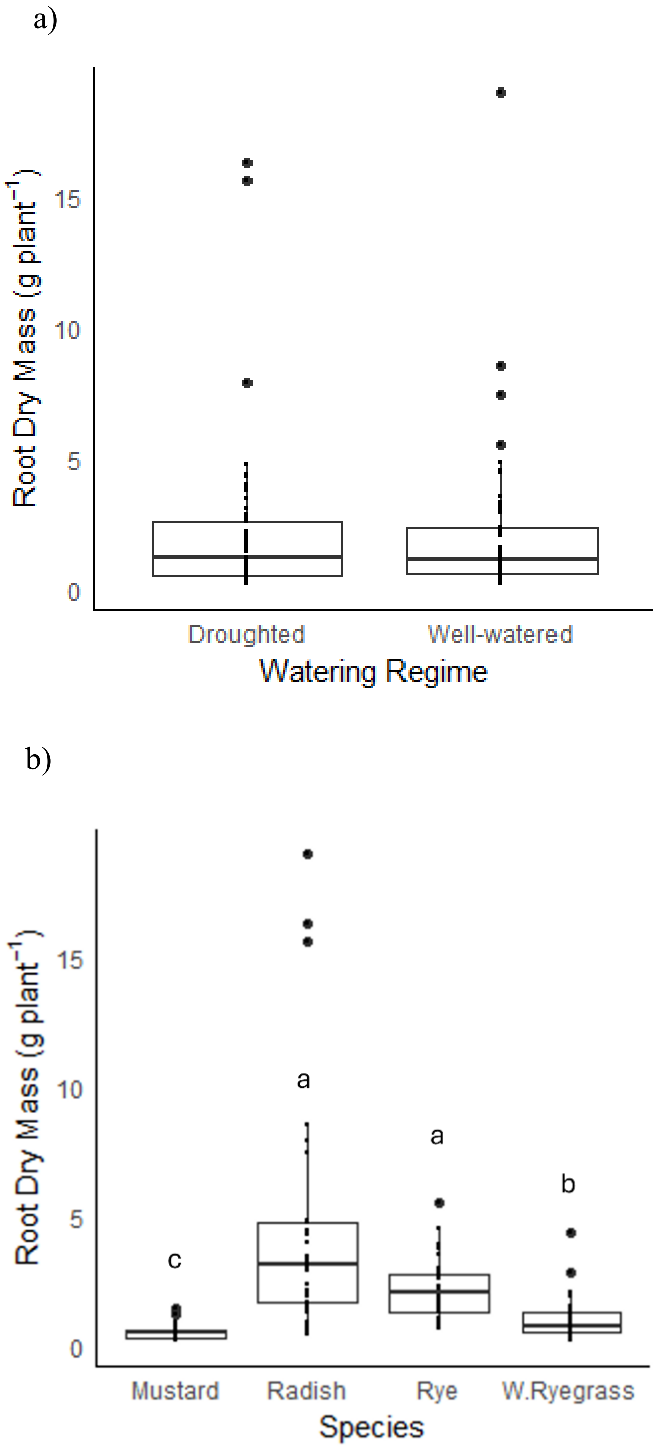


Figure 2.5 Root dry mass (g plant^{-1}) across watering regimes (droughted n = 59, well-watered n = 58) (A) and species (n = 30 per boxplot except radish n = 29 and W. ryegrass n= 28) (B). Data presented include all sampling dates. Boxes indicate the 25 & 75% quartile and lines the median, with points indicating individual samples. Treatments are significantly different ($p < 0.05$) from each other if they do not share a letter.

Rhizosheath dry mass per plant was not significantly different between watering regimes (F(1, 91) = 0.09, p = 0.75) (Fig. 2.6a) but was significantly different between species (F(3, 91) = 21.59, p < 0.001) (Fig. 2.6b). Mustard had a significantly smaller rhizosheath dry mas per plant than radish (T(91) = -6.21, p < 0.001), rye (T(91) = -7.47, p < 0.001) and W. ryegrass (T(91) = -4.28, p < 0.001), and these did not differ between each other, except rye having significantly larger values than W. ryegrass (T(91) = 3.04, p = 0.01). Rhizosheath dry mass per plant significantly decreased from week 7 to week 9 (T(91) = 4.14, p < 0.001) and week 11 (T(91) = -3.01, p = 0.009), but remained stable between week 9 and week 11 (T(91) = 1.14, p = 0.48). Thus, rhizosheath dry mass was not affected by drought but decreased and then stabilised with time, and mustard plants adhered the smallest rhizosheath dry mass of all species.

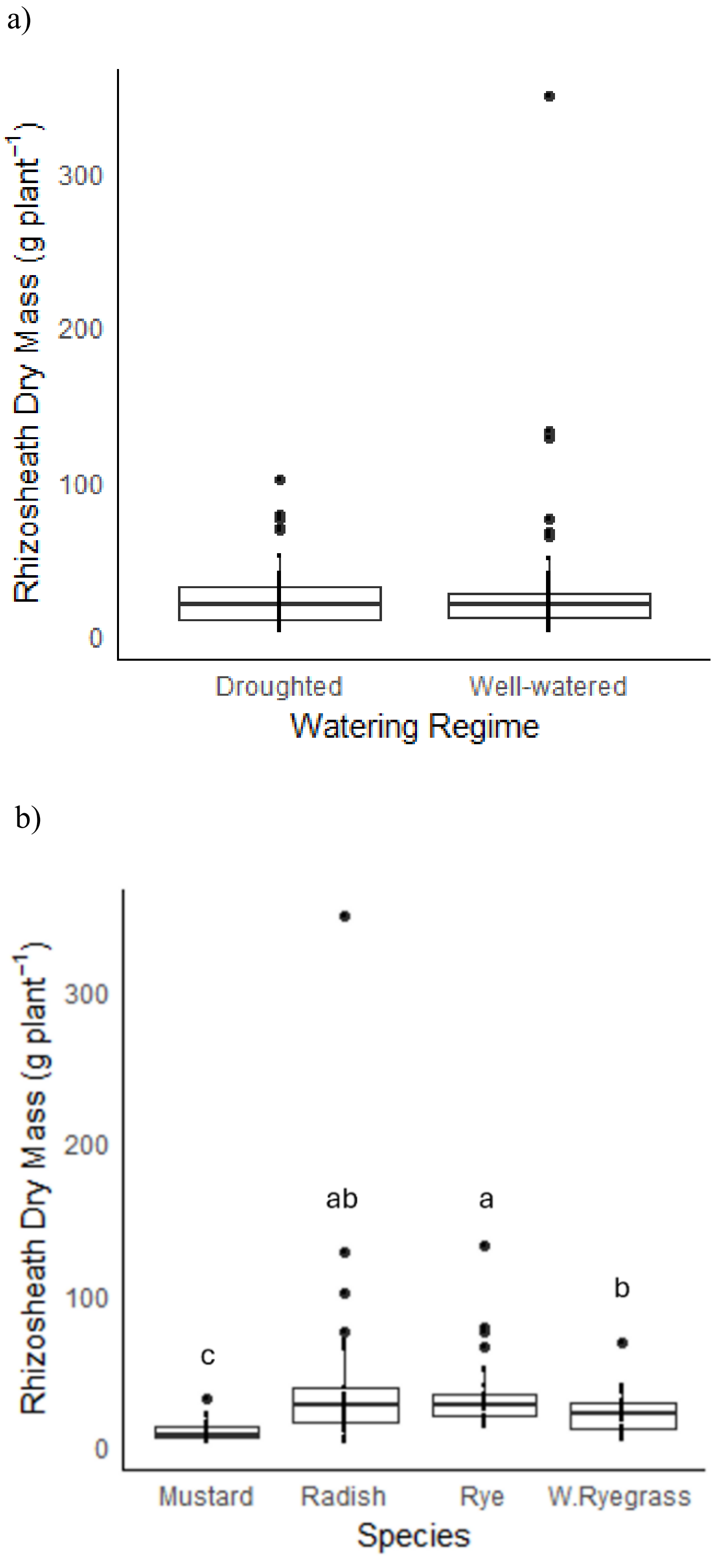
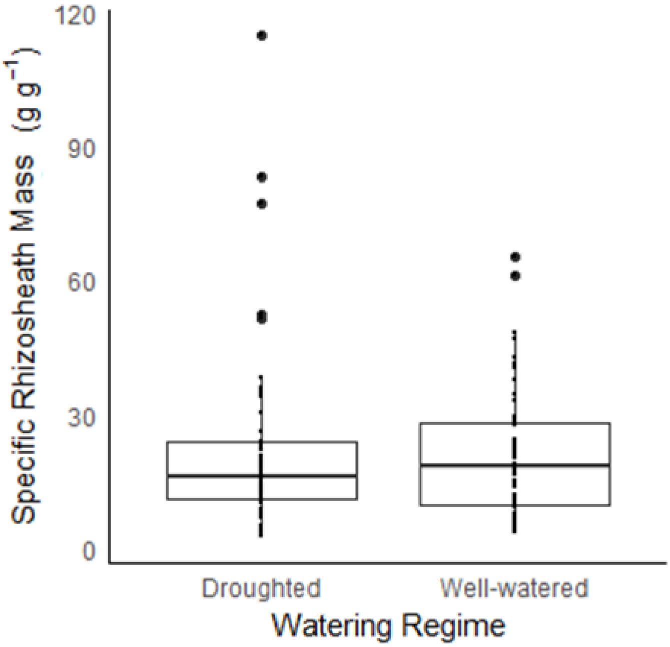


Figure 2.6 Rhizosheath dry mass (g plant^{-1}) across watering regimes (droughted n = 59, well-watered n = 58) (A) and species (n = 30 per boxplot except radish n = 29 and W. ryegrass n= 28) (B). Data presented include all sampling dates. Boxes indicate the 25 & 75% quartile and lines the median, with points indicating individual samples. Treatments are significantly different ($p < 0.05$) from each other if they do not share a letter.

Specific rhizosheath dry mass was not significantly different between watering regimes (F(1, 91) = 0.22, p = 0.63) (Fig. 2.7a), but was significantly different between species (F(3, 91) = 22.23, p < 0.001) (Fig. 2.7b). Values for radish were significantly smaller than mustard (T(91) = -6.42, p < 0.001), rye (T(91) = -5.51, p < 0.001) and W. ryegrass (T(91) = -7.56, p < 0.001), with no significant differences between the latter three species. Further, there were significant effects of time (F(2, 91) = 42.61, p < 0.001) and the interaction between species, watering regime and time (F(6, 91) = 0.63, p = 0.01). Overall, specific rhizosheath mass decreased by 61 % from start to finish of the experiment, reducing significantly from week 7 to 9 (T(91) = 7.8, p < 0.001) and remaining stable between week 9 and 11 (T(91) =0.2, p = 0.96). While rye remained unchanged between watering regimes and time points, specific rhizosheath mass of droughted W. ryegrass, well-watered mustard and well-watered radish decreased significantly from week 7 to 11 (T(91) = -4.3, p = 0.008; T(91) = 4.41, p < 0.001; T(91) = 4.51, p = 0.004; respectively) (Appendix 1 Fig. 7.10). Thus, specific rhizosheath mass was less in radish than the other three species, was not different between watering regimes, and decreased and then stabilised with time specifically for droughted W. ryegrass, well-watered mustard and well-watered radish.

a)



b)

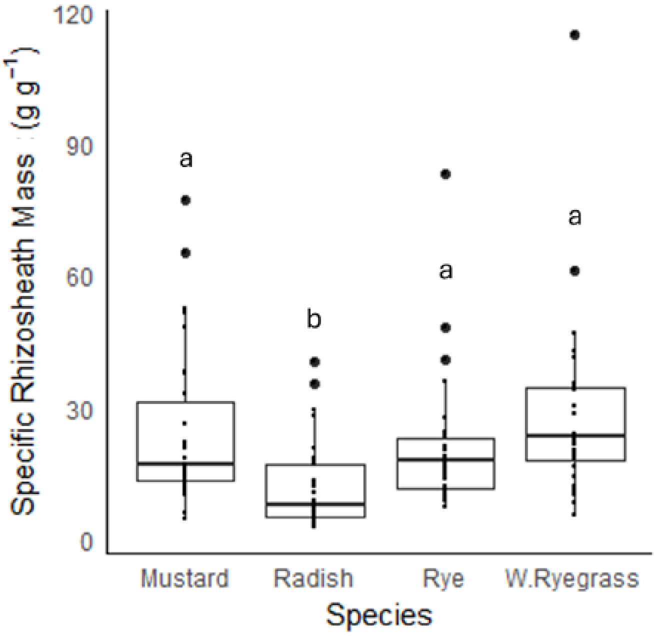


Figure 2.7 Specific rhizosheath mass (rhizosheath dry mass per unit of root dry mass) across watering regimes (droughted n = 59, well-watered n = 58) (A) and species (n = 30 per boxplot except radish n = 29 and W. ryegrass n = 28) (B). Data presented include all sampling dates. Boxes indicate the 25 & 75% quartile and lines the median, with points indicating individual samples. Treatments are significantly different ($p < 0.05$) from each other if they do not share a letter.

2.3.2. Mixed species experiment

Soil bulk density was 0.93 g cm^{-3} for experiment 2. Soil moisture and stomatal conductance were significantly less in droughted than well-watered treatments (F(1, 94) = 347.76, $p < 0.001$ and F(1, 126) = 372.45, $p < 0.001$, respectively) (Appendix 1 Fig. 7.6 and Fig. 7.8) and had a significant and moderately strong positive relation ($p < 0.001$, $r^2 = 0.35$, $y = 3.28 + 0.67x$) (Fig. 2.8).

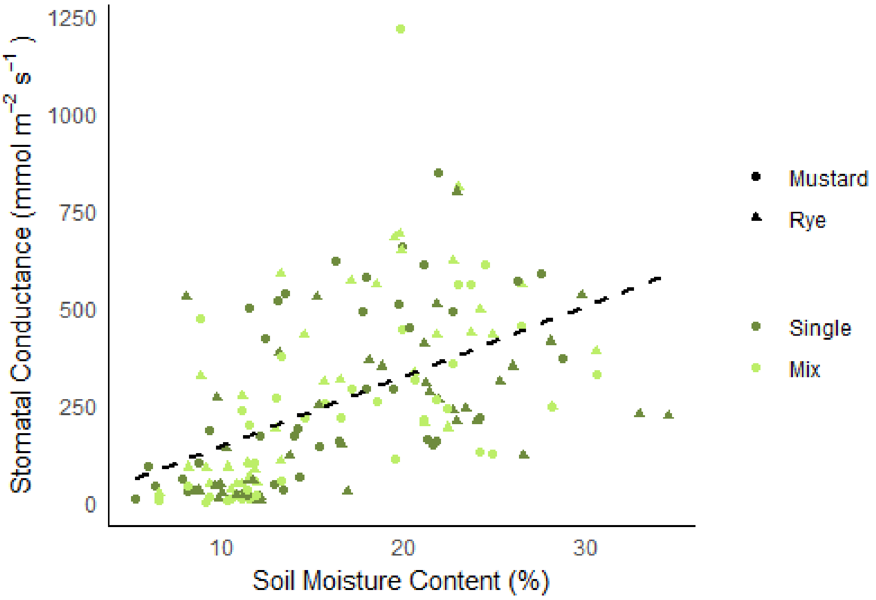


Figure 2.8 Overall relationship between soil moisture content (%) and stomatal conductance (mmol m^{-2} s^{-1}) for two functionally diverse species, where circles represent mustard, triangles rye, and colour represents whether they are grown in a monoculture (dark green) or mixed (light green) (n = 160). Line represents the slope of the relationship.

Aboveground dry mass per plant was significantly smaller in droughted than well-watered treatments (T(123) = -7.725, p <.0001) and had a weakly positive significant relationship with soil moisture ($p < 0.001$, $r^2 = 0.12$, $y = -0.97 + 0.04x$) (Fig. 2.9). Aboveground dry mass per plant of mustard grown in a mix was significantly smaller than in a monoculture (T(123) = -3.28, p = 0.007), rye in a mix (T(123) = -6.28, $p < 0.001$) and rye in a monoculture (T(123) = -4.87, $p < 0.001$), and values for rye in a mix was significantly larger than mustard in a monoculture (T(123) = 3.12, $p < 0.01$), resulting in a significant effect of species (F(1, 123) = 31.25, $p < 0.001$) and the interaction between species and diversity (F(1, 123) = 13.31, $p < 0.001$). Further, aboveground dry mass per plant increased from week 4 to week 7 (F(3,123) = 11.15, p < 0.001) (Appendix 1 Table 7.2). Thus, aboveground dry mass per plant was smaller in droughted than well-watered treatments, was larger for rye than mustard, with only mustard being smaller in a mix than a monoculture and increased with time.

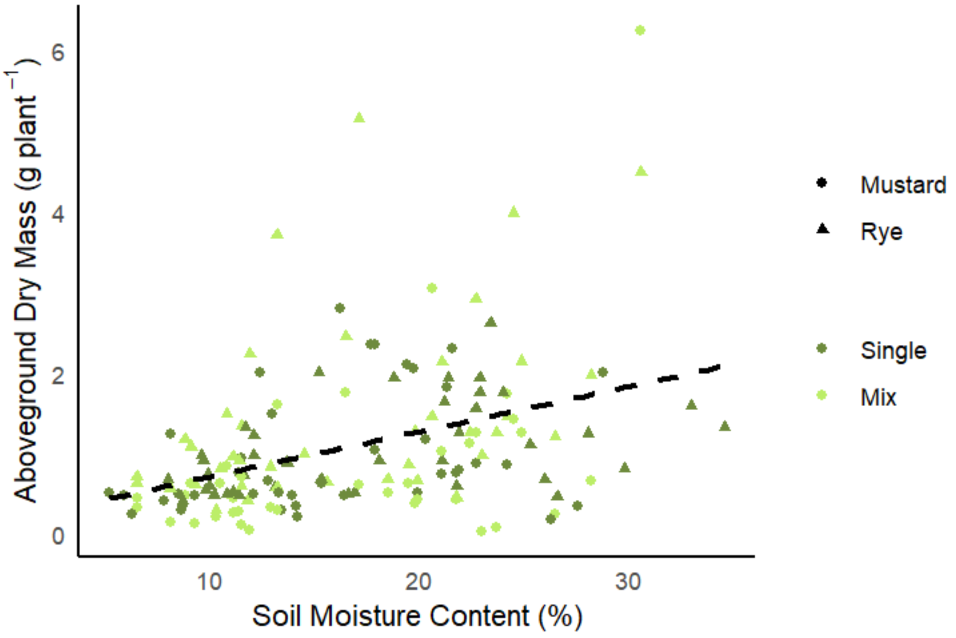


Figure 2.9 Overall relationship between soil moisture content (%) and aboveground dry mass (g plant^{-1}) for two functionally diverse species, where circles represent mustard, triangles rye, and colour represents whether they are grown in a monoculture (dark green) or mixed (light green) (n = 160). Line represents the slope of the relationship.

Well-watered treatments had a significantly larger root dry mass per plant than droughted treatments (F(1, 126) = 63.49, p < 0.001) (Fig. 2.10a). Root dry mass per plant in droughted treatments did not significantly differ over time (p > 0.05), but well-watered treatments showed a significantly smaller root dry mass per plant in week 4 than week 5 (T(126) = -5.74, p < 0.001), week 6 (T(126) = -7.62, p < 0.001) and week 7 (T(126) = -7.8, p < 0.001). This resulted in significant effects of time (F(3, 126) = 18.74, p < 0.001) and the interaction between watering regime and time (F(3, 126) = 8.93, p < 0.001). Further, root dry mass per plant in a rye monoculture was not significantly different to that of rye in a mix (T(126) = 0.87, p = 0.81), however it was significantly smaller for mustard in a mix than mustard in a monoculture (T(126) = -3.48, p = 0.003) and smaller for both mustard in a mix and in a monoculture compared to their equivalent in rye (T(126) = -12.08, $p < 0.001$ in mixes, T(126) = -7.71, $p < 0.001$ in monoculture). Therefore, root dry mass per plant was found to have a significant species effect (F(1, 126) = 197.27, $p < 0.001$) and a significant interaction between species and diversity (F(1, 126) = 9.47, $p = 0.002$) (Fig. 2.10b). Thus, root dry mass per plant was overall smaller in droughted than well-watered treatments, increased with time for well-watered treatments, but not for droughted treatments, and was smaller for mustard than rye, being smaller for mustard in a mix than in a monoculture but not differing for rye between a mix and monoculture.

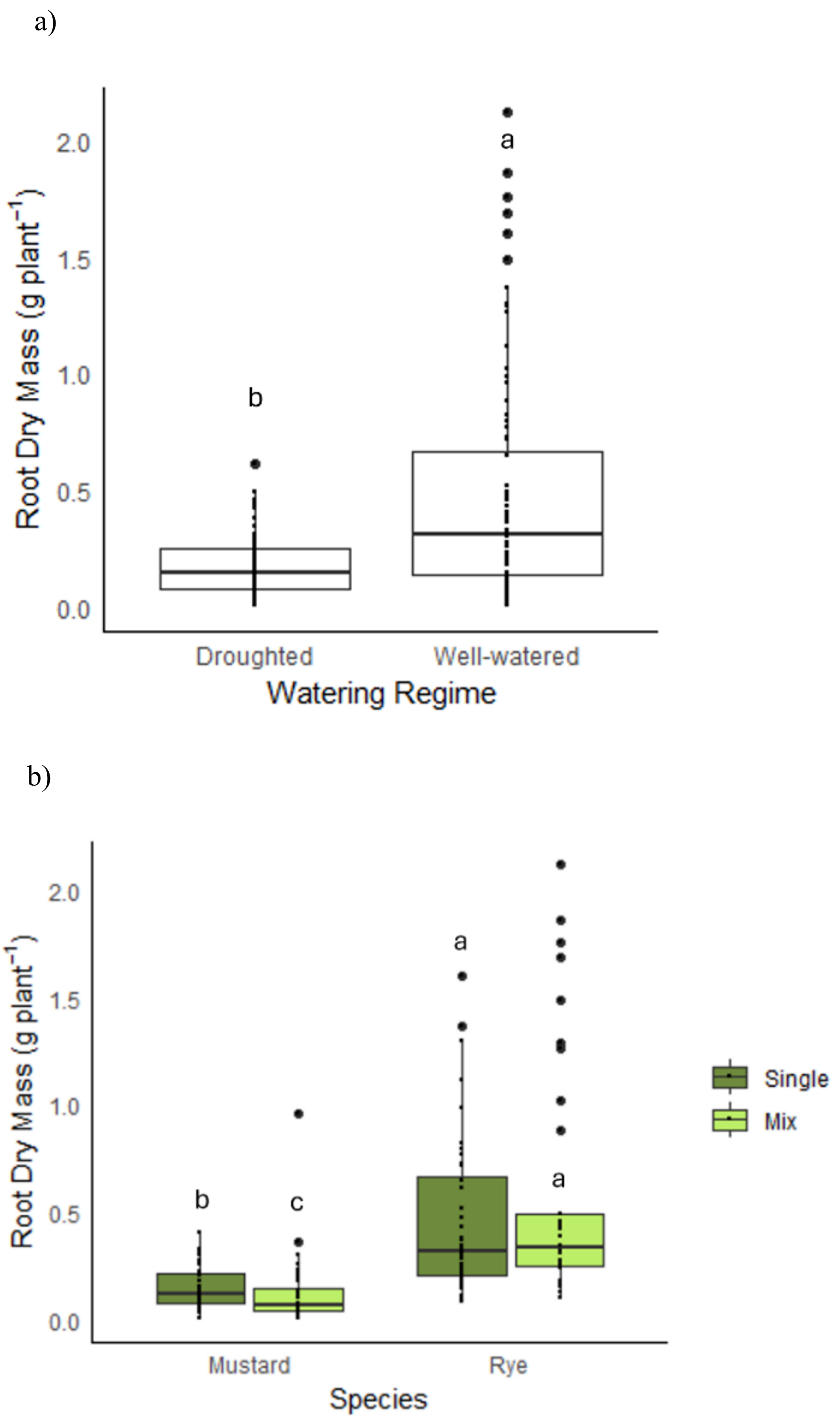


Figure 2.10 Root dry mass (g plant^{-1}) across watering regimes (n = 80 per boxplot) (A) and species (n = 40 per boxplot) (B), where colour distinguishes plants grown in a monoculture or mixed. Data presented include all sampling dates. Boxes indicate the 25 & 75 % quartile and lines the median, with points indicating individual samples. Treatments are significantly different ($p < 0.05$) from each other if they do not share a letter.

Well-watered treatments had a significantly greater rhizosheath dry mass per plant than droughted treatments (F(1, 126) = 24.02), $p < 0.001$) (Fig. 2.11a). Rhizosheath dry mass per plant in droughted treatments was significantly greater in week 4 than week 6 and week 7 (T(126) = 3.5, p = 0.01 and T(126) = 3.77, p = 0.005, respectively), whilst in well-watered treatments it was smaller in week 4 for than week 5 (T(126) = -3.9, p = 0.003) and remained stable in week 6 and 7. This resulted in significant effects of time (F(3, 126) = 6.86, $p < 0.001$) and the interaction between watering regime and time (F(3, 126) = 6.86, $p < 0.001$).

Rhizosheath dry mass per plant in monocultures was significantly greater in week 5 than week 6 (T(126) = 4.1, p = 0.001) and week 7 (T(126) = 4.13, p = 0.001) and greater than that of mixtures in week 7 (T(126) = 3.24, p = 0.03), resulting in a significant time effect (F(3, 126) = 4.89, p = 0.002) and a significant interaction between species diversity and time (F(3, 126) = 4.27, p = 0.006).

Rhizosheath dry mass per plant in droughted mustard was not significantly different to that of well-watered mustard (T(126) = -1.42, p = 0.48), however it was significantly less in droughted rye than well-watered rye (T(126) = -5.55, $p < 0.001$) and smaller for both watering regimes in mustard compared to their equivalent in rye (T(126) = -10.30, $p < 0.001$ in droughted, T(126) = -14.48, $p < 0.001$ in well-watered), resulting in significant effects of species F(1, 126) = 308.36, $p < 0.001$), watering regimes (F(1, 126) = 24.02, $p < 0.001$), and their interaction (F(1, 126) = 8.662, p = 0.003).

Further, although no significant difference was found between rye grown in a monoculture and in a mix (T(126) = 2.19, p = 0.12), rhizosheath dry mass per plant was significantly less for mustard grown in a mix than in a monoculture (T(126) = -3.11, p = 0.01), resulting in a significant interaction between species and diversity (F(1, 126) = 14.04, $p < 0.001$) (Fig. 2.11b).

Thus, rhizosheath dry mass per plant was larger in well-watered treatments than droughted treatments for rye but not different for mustard and was always greater for rye than mustard. Growing in a mix reduced individual plant rhizosheath mass for mustard but not for rye, and rhizosheath mass per plant did not change over time in mixes but decreased in monocultures and droughted treatments, while for well-watered treatments it increased from week 4 to 5 and then remained stable on week 6 and 7.

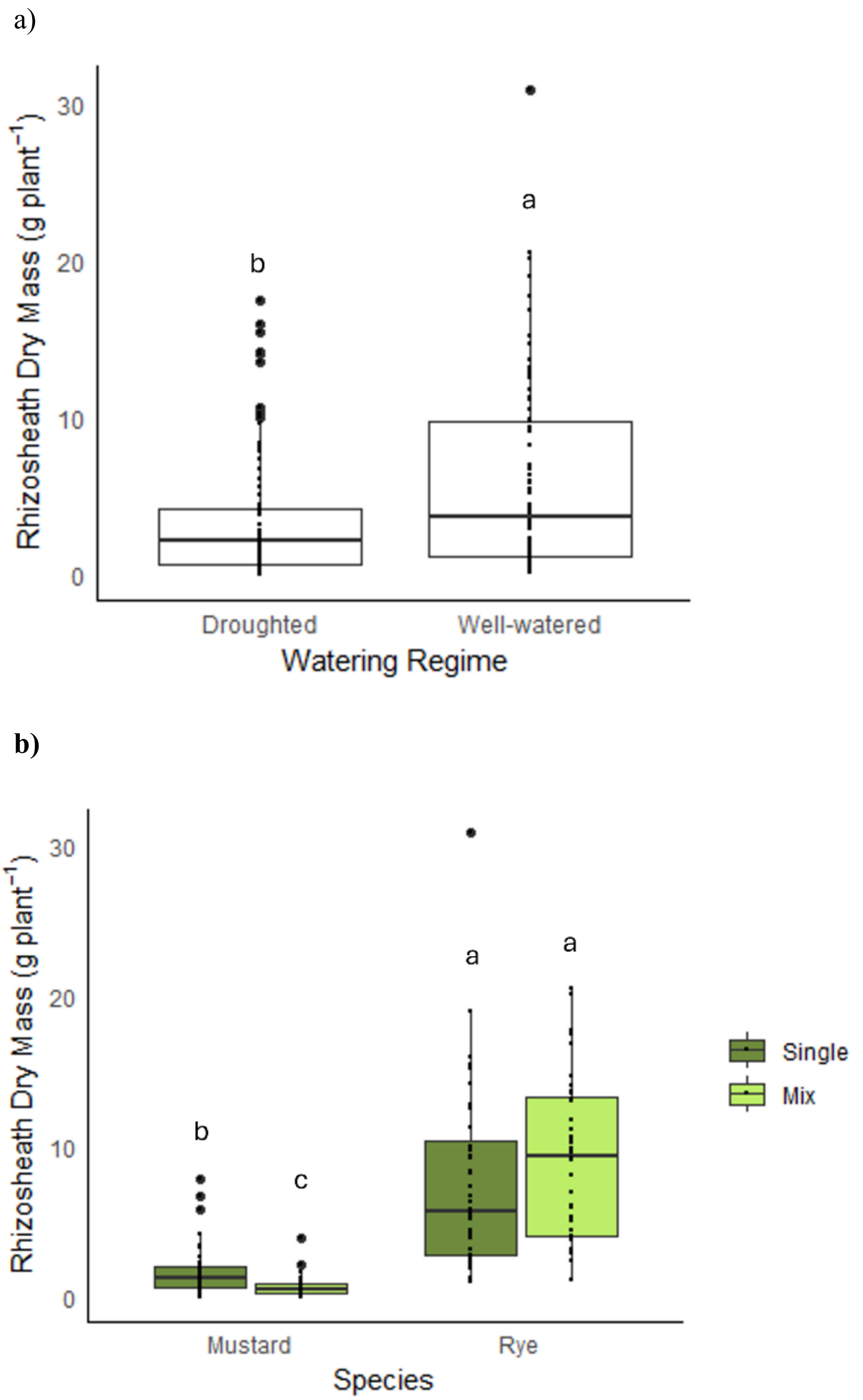


Figure 2.11 Rhizosheath dry mass (g plant^{-1}) across watering regimes (n = 80 per boxplot) (A) and species (n = 40 per boxplot) (B), where colour distinguishes plants grown in a monoculture or mixed. Data presented include all sampling dates. Boxes indicate the 25 & 75 % quartile and lines the median, with points indicating individual samples. Treatments are significantly different ($p < 0.05$) from each other if they do not share a letter.

Specific rhizosheath mass per plant did not differ between watering regimes (F(1, 126) = 2.53, p = 0.11) (Fig. 2.12a) and was significantly greater (approx. 2-fold) in rye than mustard (F(1, 126) = 66.73, p < 0.001). For mustard, specific rhizosheath mass per plant was less when grown in a mix than in a monoculture (T(126) = -3.56, p = 0.002), remaining unchanged between rye in a mix and in a monoculture (T(126) = 1.01, p = 0.74). This resulted in a significant interaction between species and species diversity (F(1, 126) = 10.49, p = 0.001) (Fig. 2.12b). Further, specific rhizosheath mass per plant was found to decrease by 70 % from start to finish of the experiment, significantly from week 4 to week 5 (T(126) = 2.92, p = 0.02), week 5 to 6 (T(126) = 4.58, p < 0.001), and remained stable from week 6 to 7 (T(126) = 0.14, p = 0.99). Thus, specific rhizosheath mass per plant did not differ between watering regimes, was greater in rye than mustard, lower when grown in a mix than in a monoculture for mustard but not for rye and decreased and then stabilised with time.

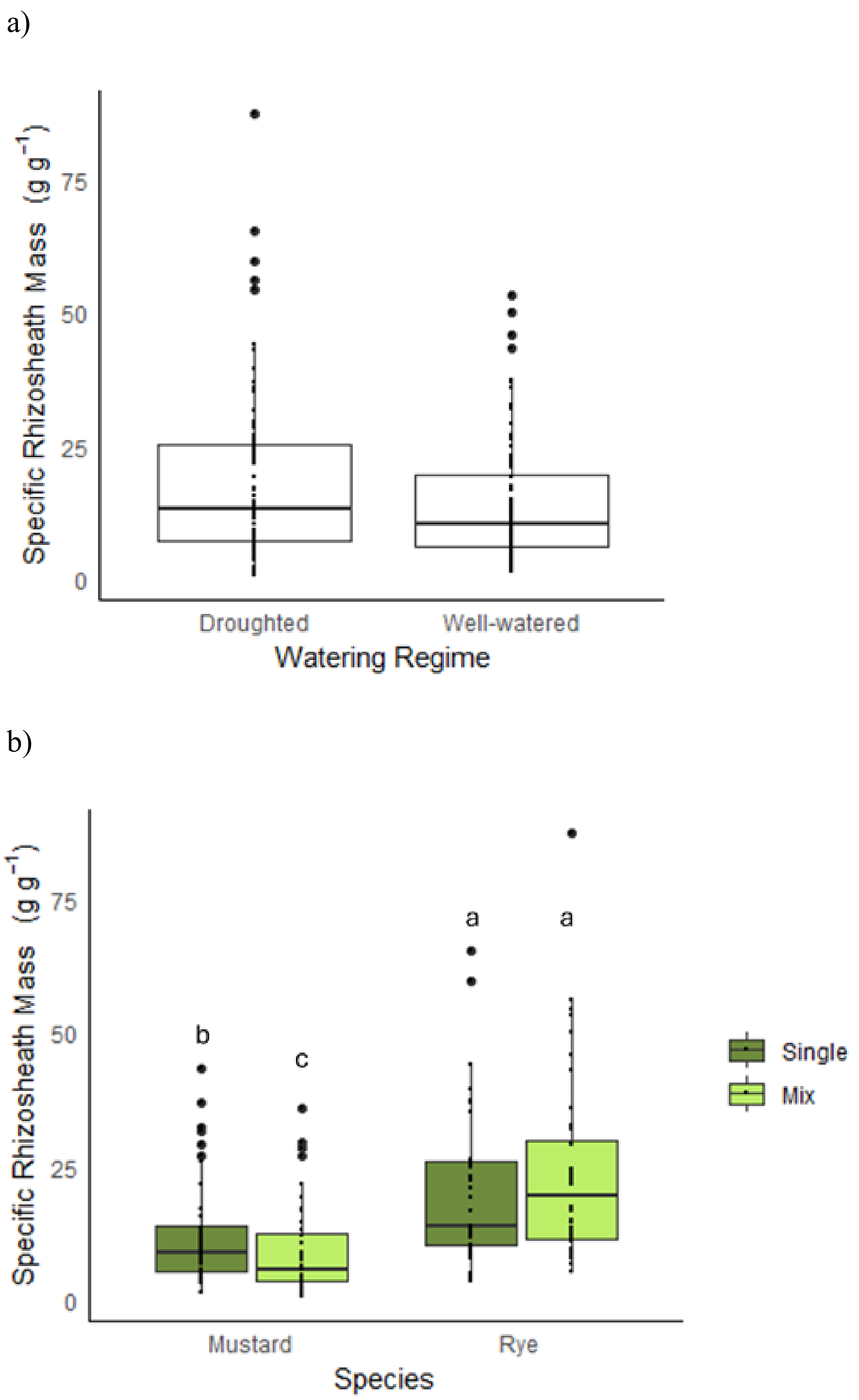


Figure 2.12 Specific rhizosheath mass (rhizosheath dry mass per unit of root dry mass) across watering regimes (n = 80 per boxplot) (A) and species (n = 40 per boxplot) (B), where colour distinguishes plants grown in a monoculture or mixed. Data presented include all sampling dates. Boxes indicate the 25 & 75 % quartile and lines the median, with points indicating individual samples. Treatments are significantly different ($p < 0.05$) from each other if they do not share a letter.

2.4 Discussion

To our knowledge, our study is the first to assess the rhizosheath development of different cover crop species under varying levels of soil water availability and their interactive effect in mixtures. The evidence presented shows that water availability had no effect on rhizosheath development except in rye, which bound a greater rhizosheath mass under well-watered conditions. Furthermore, grasses bound more soil than brassicas, with rye maintaining its soil binding capacity when grown in a mixture, suppressing the soil binding capacity of mustard. Identifying rhizosheath development capacities of cover crop species and their combinations under abiotic stress is key for maintaining or enhancing the soil binding capacity of the system.

Consistent across both mesocosm experiments, cover crop species did not form a larger rhizosheath under droughted conditions than under well-watered conditions (Fig. 2.6a, 2.7a, 2.11a and 2.12a), contradicting our hypothesis. This is surprising as the literature considers the rhizosheath an adaptive trait to drought stress (Hartnett et al., 2013; Basirat et al., 2019) and it is recognised to develop in response to limited soil water availability (Watt et al., 1993, 1994b; Zhang et al., 2020; Rabbi et al., 2021). Droughted conditions were achieved in both mesocosm experiments (see Appendix 1 Fig. 7.1 and 7.2 for stomatal conductance differences and Fig. 7.3 and 7.4 for soil moisture differences in the first mesocosm experiment), particularly in the second mesocosm experiment (see Appendix 1 Fig. 7.5 and 7.6 for stomatal conductance differences and Fig. 7.7 and 7.8 for soil moisture differences in the second mesocosm experiment), leading to greater aboveground and root dry masses in well-watered than droughted conditions (Fig. 2.9 and 2.10a).

Rhizosheath dry mass was greater under well-watered conditions than droughted for rye but not different for mustard, which bound a small rhizosheath mass regardless of watering regime. The difference in rhizosheath dry mass for rye may be due to the overall better growing conditions provided by greater water availability, resulting in larger roots and therefore larger rhizosheaths, as found by Hartnett et al. (2013) in savanna grasses in the field. Another aspect to consider is the impact of species and the exudates they produce. Barley produces exudates that disperse soil, while the polysaccharides exuded by maize bind soil (Naveed et al., 2017). Since many observations of greater soil binding under drought are for maize (Morel et al., 1991; Watt et al., 1993, 1994), smaller rhizosheaths under drought may be expected in grasses like barley or rye. Further, differences in rhizosheath formation induced by soil moisture might only be observed in the early stages of plant growth. Generally, studies that observe changes in rhizosheath formation under varying levels of water availability are conducted in controlled glasshouse conditions on young plants, harvested approximately 1 week after sowing (Watt et al., 1994b; Haling et al., 2014). Few studies assess rhizosheath formation under varying levels of water availability over time in older plants and are usually across seasons (Hartnett et al., 2013; Marin et al., 2021). Therefore, further research is required to understand the plasticity of rhizosheath development in response to drought, and whether water availability only affects rhizosheath formation in early root development stages, and the mechanisms involved.

The fibrous rooting systems of the grass species were more effective at binding soil to develop a rhizosheath than the tap root systems of the brassica species, matching our hypothesis. Although drought did not affect rhizosheath formation, cover crop species showed differing soil binding capacities (Fig. 2.7b and 2.12b). This difference was not particularly clear in the first mesocosm experiment. Radish was found to form the smallest specific rhizosheath mass due to its large tap root, while mustard adhered the smallest rhizosheath mass, along with having the smallest root dry mass, therefore resulting in a similar specific rhizosheath mass to both grass species. The difference was clearer in the second mesocosm experiment, where the specific rhizosheath mass of rye was approximately twice that of mustard. Aboveground, root and rhizosheath dry masses were smaller in mustard than rye, therefore, overall, mustard was a smaller plant and its tap root had a smaller surface area to adhere rhizosheath than the fibrous rooting system of rye. Fibrous rooting systems in grasses provides a good physical structure to retain soil (De Baets et al., 2006), and are generally known to be better at retaining soil than the taproots of brassicas (McGourty and Reganold, 2005). Grasses also produce longer and denser root hairs than brassicas, which are considered to provide the physical framework for rhizosheath formation (Brown et al., 2017; see Chapter 4). Therefore, choosing cover crop species with fibrous rooting systems such as rye seems essential for ensuring soil retention and further research should identify the root traits (*e.g.* root hair length and density) that lead to greater rhizosheath mass in different cover crop species to improve the soil binding capacity in these systems.

Interestingly, species in the first mesocosm experiment responded to soil moisture availability by functional group, with grasses maintaining a larger soil moisture content in both watering regimes than their brassica counterparts (Appendix 1 Fig 7.3). This suggests grasses were better at retaining soil water than brassicas, probably due to the canopy in grasses being denser and closer to the ground, reducing soil water evaporation, and the higher water uptake demand of brassicas (Thomson, 2005). Although water availability did not affect rhizosheath formation, plants experienced heat stress associated with growing inside the polytunnel which might have influenced soil binding capacities by altering mucilage production or affecting root hair presence. Brassica species bolted (i.e. heat avoidance) and redirected their efforts towards flowering, potentially reducing their soil binding capacity. On the other hand, despite the limited soil binding capacity of brassicas, their large water demand and their ability to remove soil moisture from their surroundings could prove them to be a good companion to grass species under high soil moisture conditions.

Mixing cover crop species could have an interactive effect on rhizosheath formation as specific rhizosheath mass of rye remained unchanged whether grown in a mix or a monoculture, while for mustard it decreased when grown in a mix (Fig. 2.12b), confirming our hypothesis. Mustard aboveground, root (Fig. 2.10b) and rhizosheath dry masses per plant (Fig. 2.11b) were all smaller when grown in a mix than a monoculture, showing its rhizosheath formation capacity and plant growth in general were negatively affected when grown with rye. Conversely, no differences were found in rye aboveground, root or rhizosheath dry masses per plant grown in a mix or a monoculture. Grass species have been shown previously to dominate in mixed systems (Hauggaard-Nielsen et al., 2012; Farney et al., 2018). Therefore, the dominant nature of rye over mustard may have supressed the growth and soil binding capacity of mustard, making rye a good cover crop candidate for covering and binding soil.

Interestingly, the rhizosheath binding capacity overall decreased with plant age across both mesocosm experiments (approximately 61 % from week 7 to 11 in the single species mesocosm and 70 % from week 4 to 7 in the mixed species mesocosm, see Appendix 1 Table 7.1 and 7.2). In some cases (i.e. well-watered and grass treatments), this was due to an increase in root dry mass while rhizosheath dry mass remained stable, while in other cases (i.e. droughted and brassica treatments), this was due to loss of bound soil. Favourable growing conditions have been previously suggested to lead to a greater rhizosheath mass due to root exudation and growth of root hairs being dependent on plant carbon gain during its growth (Hartnett et al., 2013). Here, rhizosheath mass did not increase but remained stable in healthy well-watered plants while diminishing in drought-stressed plants, suggesting two hypotheses: 1) rhizosheath soil detachment with time is due to root hairs dying as the plants age, therefore losing the physical framework for rhizosheath attachment, and this is exacerbated under stress and 2) rhizosheath mass remaining stable while root dry mass increases is due to rhizosheath formation occurring in the early stages of plant development, *i.e.* actively growing root tips, and does not keep expanding with time. Some other studies have also found rhizosheath decreases with time in a range of plant species and lengths of time (George et al., 2014; Liu et al., 2024), but further research on rhizosheath persistence over time in cover crops is required, especially in the field, to assess their soil binding capacity and inform cover crop species choices.

2.5 Conclusion

This work shows that each cover crop species has a different rhizosheath formation capacity regardless of soil moisture availability. Drought had no overall effect on rhizosheath formation, except in rye, which bound more soil under well-watered conditions. Further research is required to understand whether water deficit only affects rhizosheath development in early stages of plant growth. Grasses were better than brassicas at forming a rhizosheath, which was likely due to their differing root traits and exudate production and requires further research to discern the mechanisms. Rye was found to outcompete mustard, maintaining its large soil binding capacity regardless of being grown in a monoculture or a mixture, therefore rye, and potentially species with similar root traits, is a good cover crop choice for binding soil. Further research is required to assess rhizosheath persistence over time in the field and the mechanisms behind it as the overall decrease identified here, especially under drought stress, could affect cover crop choices for different environmental conditions. Overall, assessing the soil binding capacities of different cover crop species helps identify cover crop combinations to build agricultural system resilience to the variable abiotic stresses induced by climate change.

2.6 Acknowledgements

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Chapter 3. Can increased cover crop root diversity retain more soil and mitigate overland flow erosion?

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Abstract

Although cover crops can potentially improve on-farm soil and water management to reduce erosion, how they bind soil (through rhizosheath development) and whether multi-species cover crops offer additional benefits has attracted little attention. Cover crop treatments (*Secale cereale*, *Brassica juncea*, *Vicia faba* as monocultures and in all possible species combinations) were established in plots within a randomised block trial in a silt loam field near Dundee, Scotland, and shoots were removed from half of the plots prior to measurements to isolate root diversity effects. Overland flow simulations were applied to all plots to investigate the impact of cover crop diversity on rhizosheath development and soil erodibility. Low flow rate (8 L min^{-1}) simulations were performed using a header tank and portable purpose-built aluminium plot borders, and lasted 25 minutes from runoff generation, with subsampling at 5-minute intervals. Soil cores were collected for rhizosheath and root measurements, and crop cover and soil erodibility were quantified. Individual species identity, rather than species diversity, determined root and rhizosheath mass, with treatments containing *V. faba* having 64% more root mass than the other species, and treatments containing *S. cereale* having 50% more rhizosheath mass per unit of root dry mass than others. Shoot presence delayed runoff, but neither rhizosheath mass nor species diversity affected soil erodibility. Intra-field variability of soil structural and hydraulic properties had a greater influence on runoff and erodibility. This work highlights the importance of the variation in soil hydraulic properties in adopting effective soil and water management strategies aiming to conserve soil function.

3.1 Introduction

Over 4 million tonnes of soil are lost to erosion annually in the UK alone (Rickson et al., 2019; Graves et al., 2015). Vegetation cover is important for controlling soil erosion by protecting the soil surface from the force of rainfall and intercepting runoff (Durán and Rodríguez, 2008; Elwell and Stocking, 1976). In agricultural systems, using cover crop mixtures over the autumn and winter months, when the soil would otherwise be left bare and exposed to the elements, can be an effective erosion mitigation strategy (Hudek et al., 2022; Yu et al., 2016) and is a cornerstone of agricultural policy in the UK (DEFRA, 2024) and the EU (CAP Strategic Plan Regulation, 2021).

A key ecological principle underpinning the use of cover crop mixtures is that plant diversity improves ecosystem function by more efficiently using resources in time and space (Homulle et al., 2022). Several reviews have assessed the effect of cover crop mixtures *vs.* monocultures on different ecosystem services, such as weed suppression, pest control and nitrogen scavenging (Florence and McGuire, 2020) and soil carbon (Jian et al., 2020). Koudahe et al. (2022) reviewed the extent to which mixtures altered soil properties such as soil structure and hydraulic properties, with effects varying according to growing period length, species combinations, total biomass produced, and environmental conditions. Most studies on the effect of cover crops on soil erodibility focus on above-ground cover of single species (Gómez et al., 2018; Sastre et al., 2017). Fewer studies have focused on how diverse mixes affect erosion particularly below-ground, despite the essential role of roots in reducing sediment loss from overland flow erosion (Liu et al., 2024; Kamchoom et al., 2022; Ola et al., 2015; De Baets et al., 2011). One of the few grassland studies on root diversity (De Baets et al., 2006) showed that root density is the trait that most effectively reduces soil erosion in grass mixtures. To our knowledge, the impact of increasing diversity of cover crops root traits on soil erodibility has not been investigated.

The presence of roots and their architecture can reduce soil erosion by increasing aggregate stability and infiltration rate, amongst other soil properties (Bryan, 2000). Several rainfall simulations on laboratory-grown plots and overland flow hydraulic flume experiments using soil monoliths extracted from the field have found that root system characteristics can outweigh the contribution of the above-ground biomass to controlling soil erosion, whereas shoots and stems play a greater role in reducing surface runoff (Liu et al., 2021; Burylo et al., 2012; De Baets et al., 2011; Zhou and Shangguan, 2008; De

Baets et al., 2006). Cover crop roots, specifically the coarse root axes of legumes, can improve soil hydraulic conductivity, leading to a decrease in rainfall-induced runoff of up to 17% (Yu et al., 2016). However, cover crop species with thin, fibrous roots (*e.g. Secale cereale* L.) have been shown to reduce sediment loss more effectively than species with thicker roots (*e.g. Sinapsis alba* L.), which was attributed to increased turbulence caused by a greater root diameter leading to increased localised erosion (De Baets et al., 2011). Similarly, in an overland flow flume simulation, the greater root length density and root surface area density of a fibrous rooting system (*Lolium perenne* L.) compared to a taproot (*Medicago sativa* L.) decreased soil detachment, with fibrous and taproot systems contributing up to 56% and 40%, respectively, to reducing soil detachment (Liu et al. 2024). Considering the range of root traits in different species that can influence soil erosion, increasing cover crop diversity might alleviate overland flow and soil loss as mixtures can fill canopy space more effectively and potentially occupy greater soil volume through greater above- and below-ground biomass (Liu et al., 2020).

Studies of soil erosion have often ignored the rhizosheath as an indicator of the soil binding capacity of roots despite its potential benefits for erosion control (Burak et al., 2021a). Rhizosheath formation is determined by root hair traits (density and length) and the production of mucilage derived from roots and microbes (Mo et al., 2023; Burak et al., 2021b), which create a favourable environment for water and nutrient uptake and retention (Marasco et al., 2022; Benard et al., 2019). Greater root hair length and density provide a larger root-soil interface area for soil particles to attach (George et al., 2014; Haling et al., 2010a). In some species, such as *Zea mays* L., the high viscosity and low surface tension of mucilage prevents soil particles connected to it from breaking up when drying, thereby enhancing water retention and reducing local drying rates (Benard et al., 2019). Rhizosheath formation of cereal species has been shown to depend more on root hair development than root exudate adhesiveness, although exudate adhesiveness facilitates rhizosheath formation if no root hairs are present (Burak et al., 2021b). Rhizosheath presence and adhesion strength varies across species when grown *ex situ* (Brown et al., 2017) but further research is needed to characterise species differences in field conditions (Mo et al., 2023c). Despite surveys of rhizosheath presence and adhesion strength across a range of plant taxa (Brown et al., 2017), rhizosheath development of plants specifically selected as cover crops does not appear to have been investigated. Moreover, our literature search revealed no field trials examining the effect of increasing cover crop and root diversity on soil erosion control, or investigating the role of the rhizosheath.

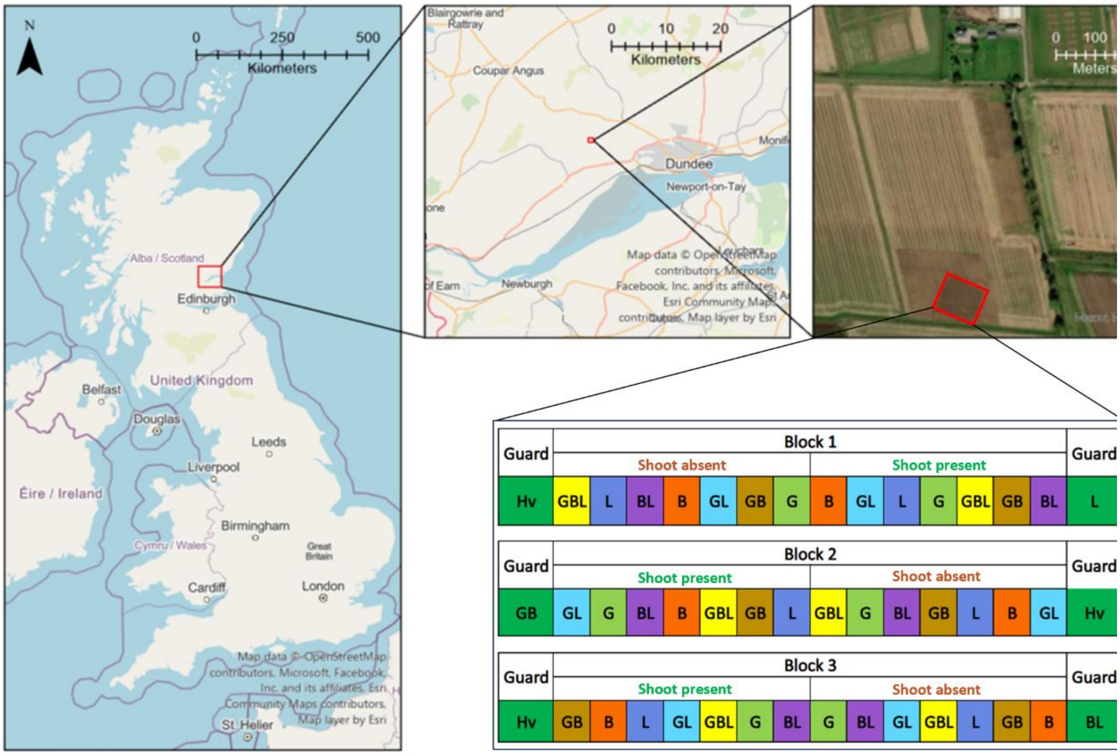
This work aims to fill this gap by assessing the impact of cover crop diversity and associated root diversity on soil loss by testing the following hypotheses: (1) increased cover crop diversity increases root biomass and thus rhizosheath mass, (2) increased rhizosheath mass decreases sediment loss, (3) increased cover crop and root diversity reduces sediment loss by roots binding more soil. To test these hypotheses, overland flow simulations were conducted in a field trial, established close to Dundee, Scotland, in autumn 2022, measuring soil loss as well as rhizosheath formation in treatments with a range of cover crop species diversity.

3.2 Methods

3.2.1 Field site

Winter cover-crop mixtures were sown on the 1st September 2022 at Balruddery Farm, Dundee, Scotland (56°28'46.6"N 3°07'53.1"W). Three cover crops representing grass (G), brassica (B) and legume (L) species were chosen due to their suitability to climatic conditions and their differing functional groups (Tilman et al., 1997). Specifically, *Secale cereale*, *Brassica juncea* and *Vicia faba* were sown individually at the typical commercial sowing rates of 18.5 g m^{-2}, 1.5 g $m^{-2,}$ and 20 g m^{-2} respectively, together with all possible combinations in equal fractions. This gave seven treatments: grass (G), brassica (B), legume (L), grass and brassica (GB), grass and legume (GL), brassica and legume (BL), and grass, brassica and legume (GBL).

The experimental layout was a randomised split-block design of three blocks. All treatments were duplicated within each block and the shoots of the plants (stems and leaves) were removed from half of the plots prior to overland flow simulation: this was done to allow the effects of aboveground material on the measured variables to be isolated from the effects of root material alone. These treatments are referred to as ‘shoots present’ and ‘shoots absent’. Guard strips were sown at the ends of each block to limit edge effects on outside plots (Fig. 3.1). The experimental plots (2 m long x 1.5 m wide) were established on a 5° slope. Each plot comprised a runoff area (2 x 1 m) and a surrounding area which was reserved for rhizosheath sampling. The soil texture was silt loam (28.47 % sand, 51.35 % silt, 20.18 % clay). The bulk density was 1.14 g cm^{-3}.



Guard	Block 1														Guard
	Shoot absent							Shoot present							
Hv	GBL	L	BL	B	GL	GB	G	B	GL	L	G	GBL	GB	BL	L

Guard	Block 2														Guard
	Shoot present							Shoot absent							
GB	GL	G	BL	B	GBL	GB	L	GBL	G	BL	GB	L	B	GL	Hv

Guard	Block 3														Guard
	Shoot present							Shoot absent							
Hv	GB	B	L	GL	GBL	G	BL	G	BL	GL	GBL	L	GB	B	BL

Figure 3.1 Layout of field trial in House field, Balruddery Farm, UK, comprising plots of *Secale cereale* (G), *Brassica juncea* (B), *Vicia faba* (L) as single species and in all possible combinations, including guard plots composed of *Hordeum vulgare* (Hv), L, GB, and BL (in dark green). Basemaps: Esri, OpenStreetMap and Microsoft.

3.2.2 Field campaign

The field campaign (18th October - 11th November 2022) started one month after sowing to allow cover crop species establishment. This was also assumed to be the time of peak root activity for rhizosheath formation (Liu et al., 2024; George et al., 2014). Three to five plots were measured each day and blocks were sampled sequentially, with the plots in each block taking a period of 8 days. Total rainfall was 191 mm between the sowing date and the end of the campaign (Cosmos weather station at the institute farm, Dundee; data not presented).

Overland flow simulations

The equipment to conduct overland flow simulations was adapted to the field from Habibiandehkordi et al. (2015). A 3 mm aluminium overland flow piece (header tank), Gerlach trough, two barriers (side planks) (Figure 3.2) and stainless-steel levelling platform (Figure 3.2b,c) were manufactured for this trial.

Figure 3.2 Photographs of overland flow simulations equipment where A shows the full set up, B shows the water flowing evenly out of the header tank and over the capillary matting, and C shows the header tank on the levelling platform with capillary matting held in place by bricks.

For each simulation, the header tank was placed upslope and levelled using the levelling platform. Capillary matting (1 m wide) was draped over the side of the tank to rest on top of the slope and held in place inside the tank using bricks to ensure an even water flow over the lip of the tank and onto the soil surface at the top of the plot (Figure 3.2b, c). The Gerlach trough was dug into the ground at the downslope end of the plot. The area below and near the Gerlach trough lip was compacted by hand and with a mallet to restrict water seeping underneath. A plastic sheet was placed above the lip and over the compacted area. The side borders were placed >5 cm into the ground and, along with the plastic sheet, edges were sealed with soil paste made by hand on site using soil extracted for the Gerlach trough. The plastic sheet was cleaned prior to starting each simulation (Figure 3.2a).

A 1000 L bowser was used as the water source. A flow meter (GARDENA Water Smart Flow Meter 8188-20, Germany) was placed on the hose system and three readings taken during the simulation averaging 8 L min^{-1}. Each plot required an application of approximately 250 litres of water. A flow rate of 8 L min^{-1} (0.0001 m^3 s^{-1}) was selected because higher rates could not be achieved with the available bowser equipment, and lower rates did not start producing runoff within a practical 30-minute window—required to complete at least three simulations per day. In this trial, the maximum peak discharge generated by applying 8 L min^{-1} to 2 m^2 plots was 6.8 L min^{-1} (0.0001 m^3 s^{-1} or 0.1 L s^{-1}). This is comparable to a low flow (< 1 m^3 s^{-1}) in a given catchment according to Segond et al. (2007), and an average flow discharge used in other overland flow experiments (Liu et al., 2024).

Time was recorded from the moment water flowed onto the plot until it started pouring out of the runoff piece pipe to quantify the effect of treatments on speed of water flow over the plots. From this point, the overland flow simulations continued for 25 minutes to allow runoff water and sediment to be collected for a standardised period of time. Subsamples were collected every 5 mins for 1 min or until the 1 L pre-weighed bottle was full and the time they took to fill was recorded. The rest of the runoff was collected and poured into buckets of known volume to estimate total discharge. Subsurface flow was also collected and quantified in the cases where it was generated (22 out of 42 plots).

Soil moisture

Three soil moisture measurements at a 7.6 cm depth were taken in a zig-zag shape across each plot immediately before each overland flow simulation using a FieldScout TDR 350 Soil Moisture Meter.

Plant cover and above-ground biomass

Photographs were taken of a 0.5 m x 0.5 m quadrat and uploaded to Canopeo (Patrignani and Ochsner, 2015) to estimate crop coverage (percentage of ground area covered). The number of individuals of each species was recorded to calculate stem density. Above-ground biomass of the quadrat was harvested before (in shoot-absent plots) and after (in shoot-present plots) overland flow simulations, oven-dried at 70°C for four days and weighed.

Rhizosheath

Before the simulation, an intact soil core (6.4 cm diameter x 20 cm height) was taken from within each plot but avoiding the portion of the plot subjected to runoff to avoid disturbing the plot surface. Before taking the core, the number of plants per species present within the cored diameter was recorded, harvested, oven-dried at 70°C for four days and weighed. Cores were stored in a cold room at 4°C and processed after all overland flow simulations had been completed (approx. 1.5 months). Rhizosheath development was measured by carefully breaking the core open, removing the roots from the core and gently shaking them by hand until the bulk soil detached. Using deionised water, the rhizosheath of each species was washed off the roots into a pre-weighed aluminium tray. Rhizosheath trays and roots were oven-dried at 105°C overnight and 70°C for four days respectively and the dry mass was recorded. Specific rhizosheath was calculated by dividing the rhizosheath soil dry mass by the corresponding root dry biomass. Root length was not calculated in this experiment due to time constraints, as scanning is time-intensive. However, it was measured in the experiment presented in Chapter 4.

Sediment loss

The runoff samples in 1 L bottles were brought to the laboratory and weighed after each field day. The bottle contents were poured into a pre-weighed aluminium tray and oven-dried at 105°C overnight to evaporate the liquid and record sediment dry mass. Total sediment loss (g), total runoff (L) and sediment concentration loss (g L^{-1}) over the 25 min simulation was calculated by using the trapezium rule.

3.2.3 Data analysis

Statistical analysis was performed in R (R Core Team, 2023). Data were checked for test assumptions and subsequently analysis of variance (ANOVA), post-hoc tests (Tukey's HSD), and linear regressions were performed. Mean sediment concentration was logarithmically transformed to achieve a normal distribution. Analyses of variance were performed to test: the effect of species combinations on crop coverage, above-ground dry mass, stem density, root dry mass, rhizosheath mass, specific rhizosheath mass, and total sediment; the effect of species diversity on root dry mass, mean sediment concentration, time taken to generate runoff and total runoff volume; and the effect of above-ground vegetation presence on total sediment, mean sediment concentration, time taken to generate runoff and total runoff produced. Blocks were included as a random effect to account for spatial variation within the field and temporal variation in the time of sampling. Linear regressions were performed to identify relationships between rhizosheath mass and root biomass (forced through 0), between total runoff and mean sediment concentration, total sediment and time until runoff collection and between mean sediment concentration and time until runoff collection. All results presented are mean ± standard error of the mean and significance is at a 95% confidence level. R packages used were ggplot2, stats, and dplyr.

3.3 Results

3.3.1 Plant measurements

Above-ground coverage did not show significant (F (6, 35) = 0.78, p = 0.71) treatment differences, averaging 51 ± 2 %. Across all species treatments, above-ground dry biomass (g) was not significantly different (F (6, 35) = 0.97, p = 0.45) and averaged 42.55 ± 2.07 g dry mass m^{-2}. Following sowing densities (see Methods), treatments containing field bean (L) had significantly (F (6,35) = 27.02, $p < 0.001$) lower stem density (stems m^{-2}), 85% less than the mustard (B) and rye (G) treatments (Appendix 2 Table 8.1).

Root dry mass did not significantly increase with cover crop diversity (F (2, 39) = 1.93, p = 0.09)(Fig. 3.3a). Species composition significantly (F (6, 35) = 5.82, $p < 0.001$) affected root dry mass, with treatments containing field bean having 64% more root dry biomass than treatments without field bean (Fig. 3.3b). Rhizosheath mass increased significantly with root dry mass (F (1, 40) = 223.8, $p<0.001$; $r^2 = 0.84$), with mustard showing the smallest root dry mass and rhizosheath mass (Fig. 3.4; see Appendix 2 Table 8.2 for individual treatment relationships). Treatments containing rye formed 50 % more rhizosheath per unit root dry mass (F (6, 35) = 7.84, $p < 0.001$) than other treatments (Fig. 3.5).

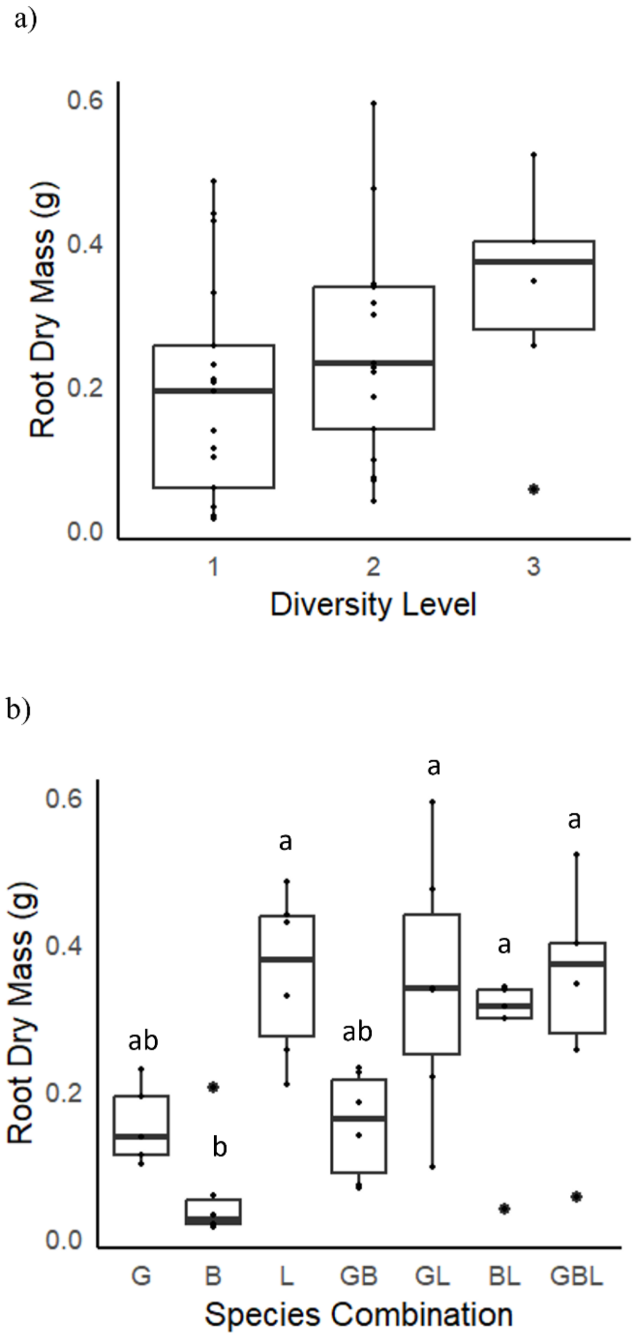


Figure 3.3 Root dry biomass across different levels of cover crop diversity where 1 = single species (n = 18), 2 = two species (n = 18), and 3 = three species (n = 6) (A) and across species combinations (n = 6) (B) comprising *Secale cereale* (G), *Brassica juncea* (B), *Vicia faba* (L) as single species and in all possible combinations. Boxes indicate the 25% and 75% quartile and lines the median, with smaller points indicating individual plots. Treatments significantly ($p < 0.05$) differ from each other if they do not share a letter.

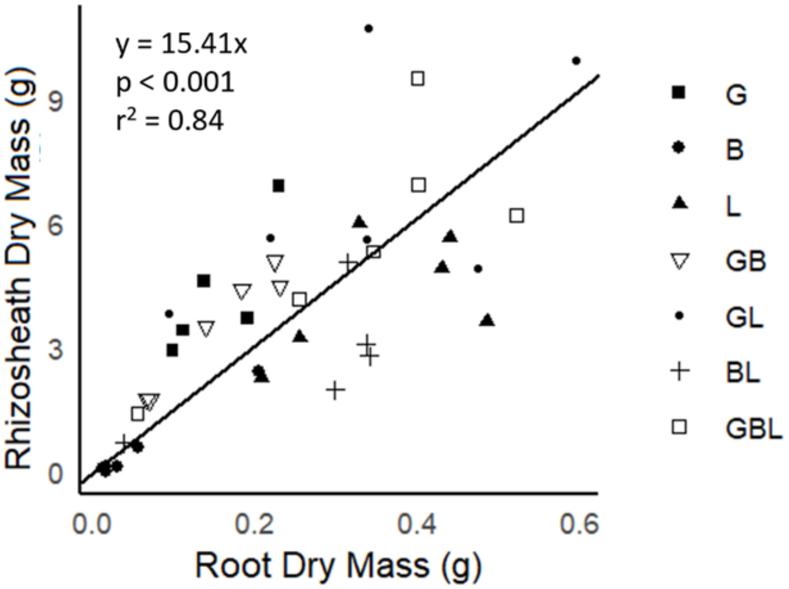


Figure 3.4 Rhizosheath dry mass plotted against the associated root dry mass with species combinations distinguished by symbol (n = 42) comprising *Secale cereale* (G), *Brassica juncea* (B), *Vicia faba* (L) as single species and in all possible combinations.

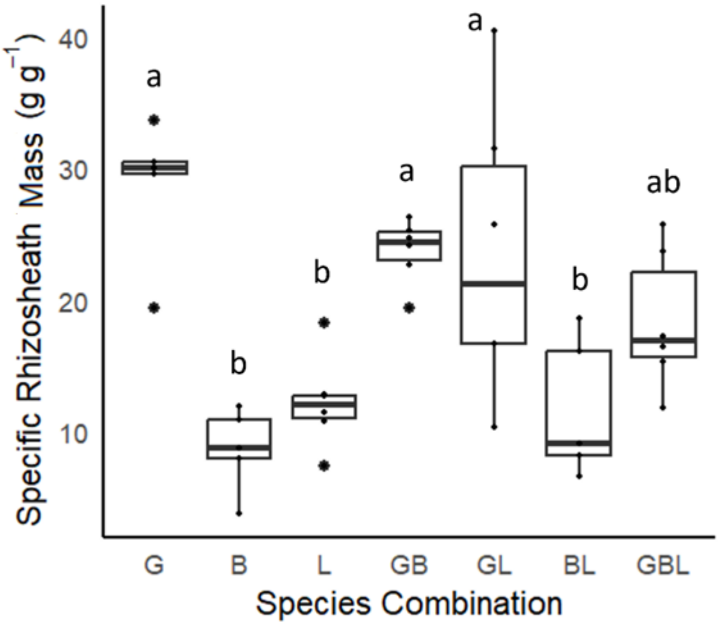


Figure 3.5 Specific rhizosheath mass per unit of root dry mass across species combinations (n = 6) comprising *Secale cereale* (G), *Brassica juncea* (B), *Vicia faba* (L) as single species and in all possible combinations. Boxes indicate the 25% and 75% quartile and lines the median, with smaller points indicating individual plots. Treatments significantly ($p < 0.05$) differ from each other if they do not share a letter.

3.3.2 Overland flow simulations

Two data points were removed from the overland flow data set (G and BL shoots present plots from block 2) as the time for the overland flow to reach the runoff collection point was disproportionally long and additional water was manually added to create runoff, thereby increasing the flow rate beyond that experienced by the other treatments. Soil moisture before the simulations averaged 20.1 % (± 0.3) across all plots and had no significant effect on any measured variable across all treatments ($p > 0.05$) (see Appendix 2 Table 8.3).

Total sediment loss (g) was similar (F (6, 33) = 0.61, p = 0.27) between treatments (Fig. 3.6), averaging 47 ± 2 g across all plots. Overall, the presence of shoots led to more sediment being lost from plots (F (1, 38) = 9.24, p = 0.007) than where shoots were absent (respectively, 53 ± 2 g vs 40 ± 3 g). Rhizosheath mass was not significantly correlated (F (1, 38) = 2.17, p = 0.14) with total sediment mass.

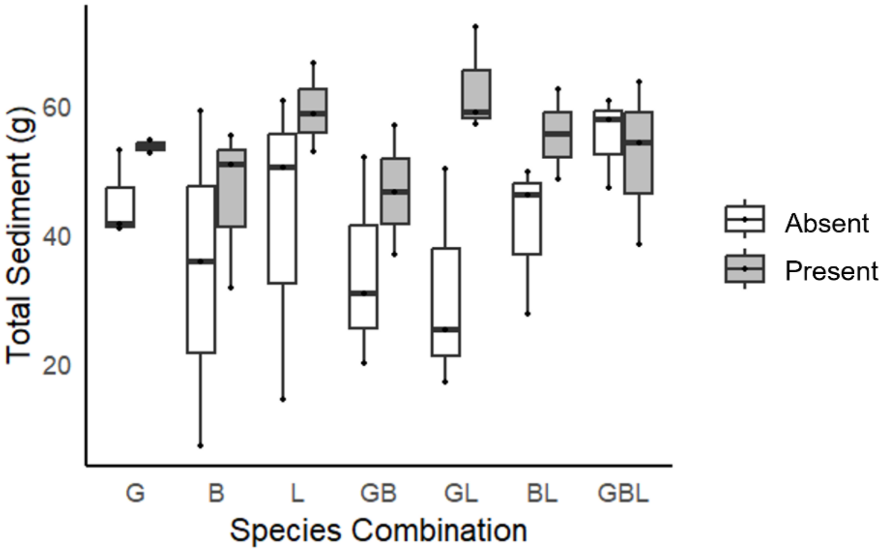


Figure 3.6 Total sediment lost (g) across species combinations in plots with and without plant shoots present (all treatments n =3, except n = 2 in BL and G where shoots were present) over the entire simulation. Species combinations comprise *Secale cereale* (G), *Brassica juncea* (B), *Vicia faba* (L) as single species and in all possible combinations. Boxes indicate the 25% and 75% quartile and lines the median, with smaller points indicating individual plots.

Increasing cover crop diversity did not affect (F (2, 37) = 0.66, p = 0.52) mean sediment concentration (Fig. 3.7a). Overall, the presence of shoots led to a significantly (F (1, 38)

= 8.37, p = 0.01) greater sediment concentration in the runoff than where shoots were absent (Fig. 3.7b).

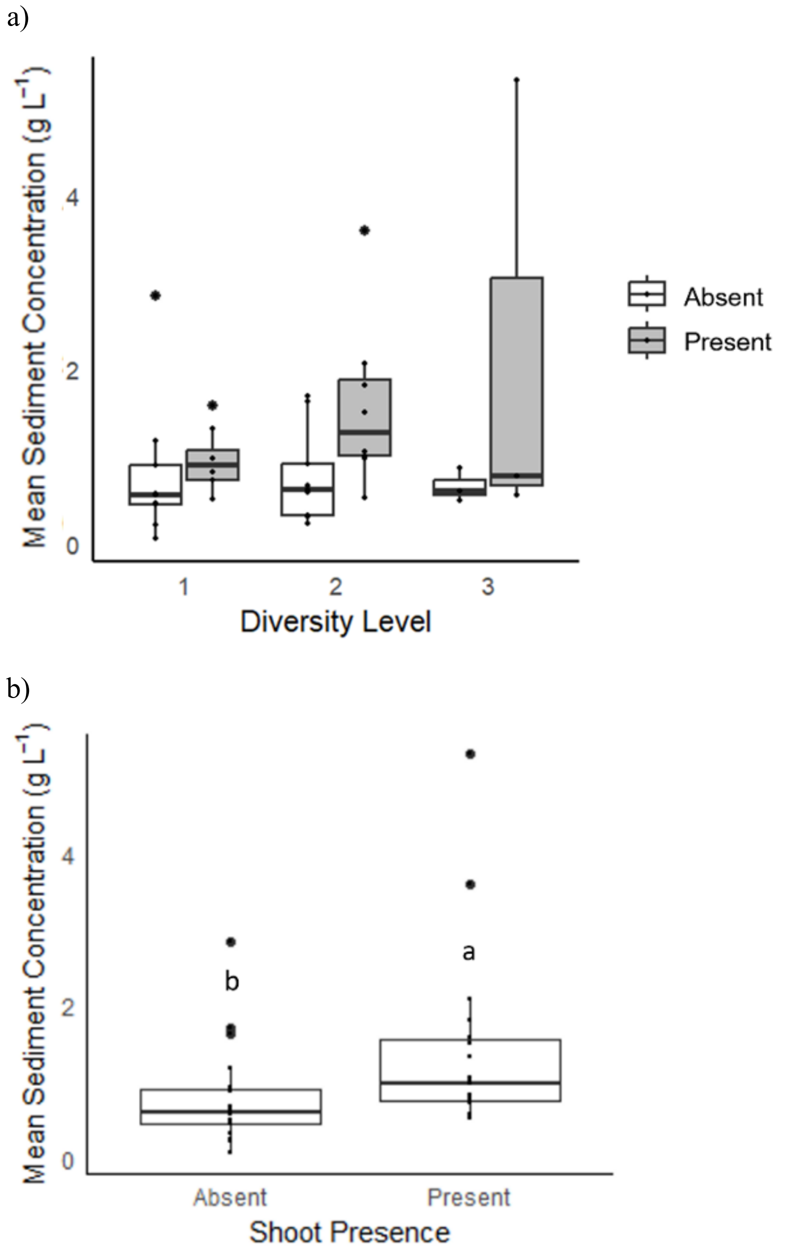
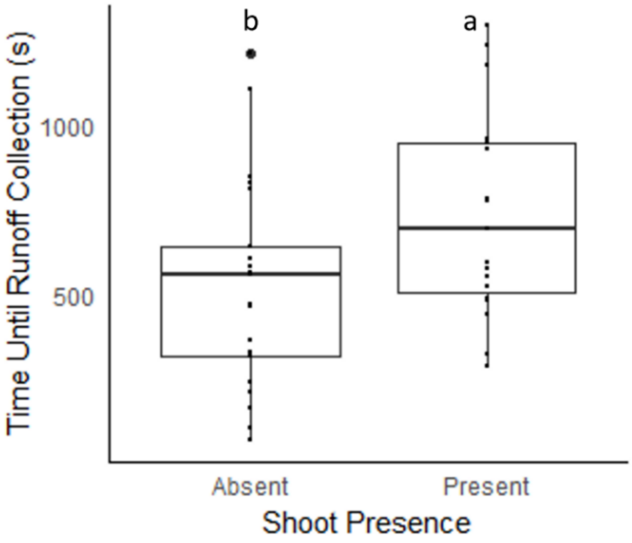


Figure 3.7 Mean sediment concentration in runoff from plots of varying levels of diversity including those with shoots present or absent (A) and mean concentration of sediment collected during overland flow in plots with shoots present (n = 19) or absent (n = 21) (B). In graph A: single species and two species treatments where shoots were absent, n = 9 each; single and two species treatments where shoots were present, n = 8 and three species in both cover presence treatments, n = 3. Mean Boxes indicate the 25% and 75% quartile and lines the median, with smaller points indicating individual plots. Treatments significantly ($p < 0.05$) differ from each other if they do not share a letter.

The presence of shoots significantly (F (1, 38) = 4.64, p = 0.03) increased the time taken to generate runoff and produced significantly (F (1, 38) = 4.57, p = 0.03) less runoff water than where shoots were absent (Fig. 3.8). The number of species did not affect the time taken to generate runoff (F (2, 37) = 0.2, p = 0.56) or runoff volume (F (2, 37) = 0.77, p = 0.24). Runoff flow rates (L min^{-1}) where shoots were absent were significantly higher than where shoots were present (F (1, 38) = 4.7, p < 0.001), being 3 L min^{-1} and 2.25 L min^{-1} respectively.

a)



b)

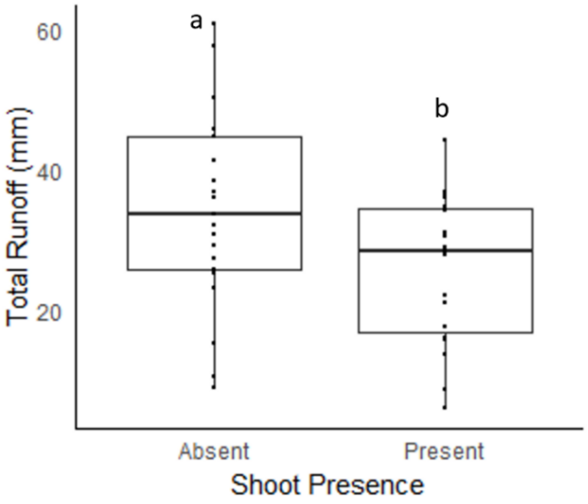
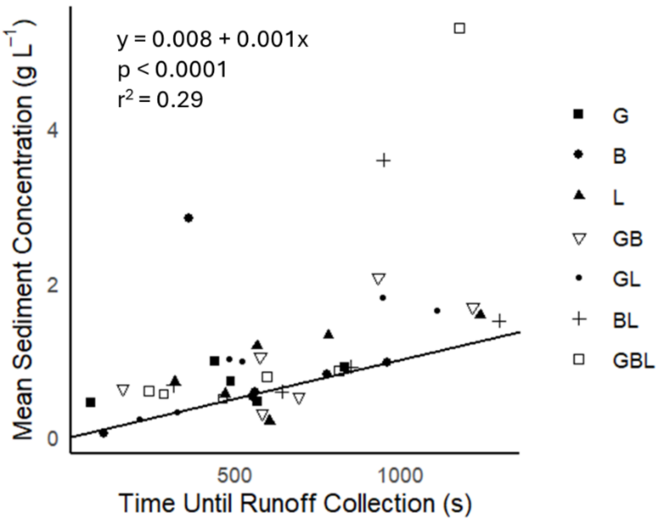


Figure 3.8 Time taken to generate runoff (A) and total runoff generated (B) where shoots were absent (n = 21) and present (n = 19). Boxes indicate the 25% and 75% quartile and lines the median, with smaller points indicating individual plots. Treatments significantly (p < 0.05) differ from each other if they do not share a letter.

Overall, the time taken to generate runoff was significantly positively related to (F (1, 38) = 17.61, p = 0.001; $r^2 = 0.29$) sediment concentration, but significantly negatively related to (F (1, 38) = 46.68, p < 0.001; $r^2 = 0.51$) total runoff volume (Fig. 3.9).

a)



b)

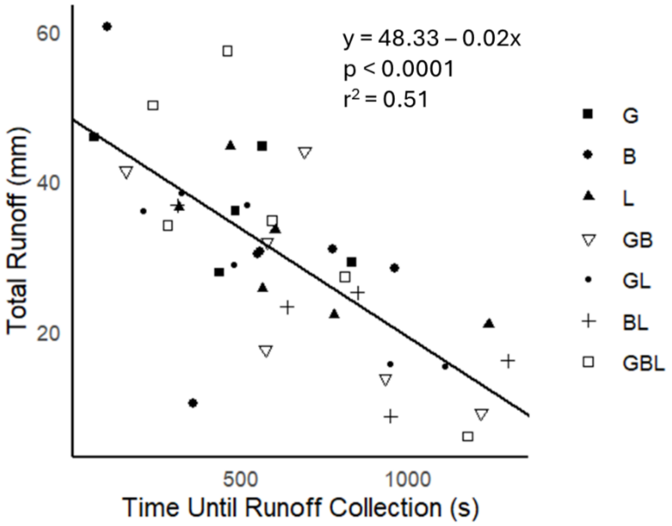


Figure 3.9 Time taken to generate runoff *vs.* mean sediment concentration in runoff (a), and *vs.* total amount of runoff generated (b) (n = 40) with species combinations distinguished by symbol comprising *Secale cereale* (G), *Brassica juncea* (B), *Vicia faba* (L) as single species and in all possible combinations.

3.4 Discussion

To our knowledge, this is the first field study of whether root diversity and associated rhizosheath development affects overland flow erosion. We provide evidence that species identity, rather than overall species diversity, determined the amount of rhizosheath formed, with cover crop combinations including rye having the largest specific rhizosheath. However, differences in this indicator of soil binding capacity did not affect sediment loss.

Root biomass was greater when treatments included field beans, due to the thick and robust taproot system of field beans. In contrast, other studies have found that root biomass increases with diversity e.g. in Yang et al. (2019) where grassland species (grasses, legumes and nonlegume herbaceous forbs) produced a 100% more root biomass in mixtures of 16 species than the best performing monoculture. Rhizosheath mass increased with root biomass (Figure 4) as previously demonstrated (Brown et al., 2017; Moreno-Espíndola et al., 2007), but as seen in Chapter 2, rye presence determined specific rhizosheath mass rather than number of species. These findings reject our hypothesis that increased cover crop diversity increase root biomass and thus rhizosheath mass, and suggest that, as with other grasses (Brown et al., 2017), the longer and denser root hairs of rye formed a larger specific rhizosheath.

Although rye formed a larger rhizosheath than the species from other functional groups, this did not lead to a differential reduction in sediment loss (Fig. 3.6). This does not support our hypothesis and contrasts with other studies that suggest a greater mass of rhizosheath, or greater soil binding capacity of the cover crops, could diminish soil erosion. For example, the presence of root hairs enhanced rhizosheath development in barley, and significantly decreased soil detachment in a mesocosm-scale rainfall simulation on 35-day old plants (similar to the plant age at the start of the overland flow simulation campaign here) (Burak et al. 2021b). This rejection of our hypothesis is most likely due to the sediment lost during overland flow being detached from the soil surface and not from the root zone meaning that the rhizosheath interacted little with overland flow.

Plant diversity did not affect overland or measured soil erosion. However, as expected, and in accordance with other studies (Wang et al., 2015; Ludwig et al., 2005), shoot presence influenced the erosion process by slowing down overland flow (Fig. 3.8). Once water had reached the bottom of the plot, the total amount of sediment lost over a fixed period of 25 minutes did not differ across species or species combinations. This contrasts with other studies that have shown that increasing plant diversity does have the potential to improve soil retention. For example, Berendse et al. (2015) found that certain species such as red fescue, when in multi-species plots, quickly spread to reduce sediment loss within a simulated river embankment. At the species level, fibrous root systems (*e.g.* red fescue and rye) better control soil erosion than thicker taproot systems (*e.g.* mustard and field bean) due to the erosion caused by the horseshoe vortex that forms when concentrated flow meets a root obstacle (De Baets et al., 2011, 2007).

Although more runoff water was collected when shoots were absent, due to faster flow over the soil surface, the quantity of soil loss was less than when shoots were present. As particles were more likely to detach from the soil surface rather than the root zone, shoot absence might have avoided localised horseshoe vortex erosion associated with stems (De Baets et al., 2011, 2007). Additionally, the 25 min simulations started from the moment the overland flow reached the collecting trough, rather than when the water was added to the upslope boundary. As plots with shoots took longer to start runoff collection (Fig. 3.8a), they were exposed to overland flow at the top of the plots for longer than plots where shoots were absent. This means that erosion occurred in the upper part of the plot, producing sediment which was transferred to the outlet once the overland flow had reached the trough. Other studies also found a longer time for overland flow connection in plots with intact shoots (Liu et al., 2021; Zhang et al., 2019), where the obstacle provided by plant presence decreased runoff generation. In real-world terms, this suggests that in the event of a storm, the presence of above-ground biomass will delay runoff and its associated soil erosion reaching the edge of the field and its further transportation.

Cover crops are being promoted worldwide as a soil conserving measure, and species mixtures are attracting attention for their potential for fine tuning erosion control (Corsi and Muminjanov, 2019). Rye's enhanced rhizosheath development seems to have an important role to play as a cover a crop where soil conservation is paramount. Although field bean and mustard seem to have little effect on rhizosheath formation and soil binding capacity, they provide other ecosystem services (*e.g.* nitrogen fixation, pest management) of economic importance to farmers. Field trials are important to help farmers manage their agricultural systems sustainably, although discerning clear hydrological and sedimentological effects is often difficult due to the variability of the data (Deasy et al., 2014). In the present study, soil structural and hydrological properties that underpin that variation were likely more important for sediment loss than the plant diversity treatments imposed experimentally. Conducting smaller scale trials under more controlled conditions could provide a clearer understanding of the effect of cover crop diversity on sediment loss by bypassing the field variability encountered here, but would lack the real-world applicability that farmers are keen to observe.

3.5 Conclusion

Cover crop diversity did not affect sediment loss, but the presence of shoots did delay runoff generation. That cover crop diversity did not affect sediment loss does not exclude the possibility that cover crop mixtures can enhance other ecosystem services (e.g. soil fertility). The presence of individual species, rather than diversity *per se*, determined root biomass mass and rhizosheath formation. Root biomass was greatest in field bean, whereas rye produced the largest rhizosheath mass per unit root mass. Although the denser, fibrous root system and greater rhizosheath mass of grasses should better retain soil, rye did not decrease sediment loss any more than the other species and species combinations, with field variability obscuring potential effects of cover crop diversity on sediment loss. However, experimenting in real-world conditions provides understanding of the importance of species choice in cover crop mixtures for erosion control practices. Future work should clarify the effect of increasing crop and root diversity on sediment loss by further investigating the soil binding capacity of a greater range of species combinations that can complementarily use resources in time and space.

3.6 Acknowledgements

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Chapter 4. Rhizosheath formation and persistence in winter cover crop mixtures in the field.

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Abstract

Background and Aims

While much research has focused on the benefits of cover crop diversity for crop productivity, there is limited evidence on how root diversity and species selection stabilise soil. Although cover crops can potentially improve on-farm soil and water management, how they bind soil (through rhizosheath development) and whether multi-species cover crops offer additional benefits has attracted little attention. This study aimed to assess rhizosheath persistence in field-grown cover crops and their mixtures to understand the impact of species diversity on soil binding capacity.

Methods

Brassica juncea, *Secale cereale* and *Vicia faba* were sown as monocultures and mixtures in a winter cover crop field trial near Dundee, Scotland. Soil cores were collected three times during January-March 2023. Measurements included rhizosheath mass, root length, and root hair length and density.

Results

While overall rhizosheath mass decreased by 27% with plant age, *S. cereale* maintained the largest rhizosheath mass per unit root length (1.75 and 5.60 times greater than *V. faba* and *B. juncea,* respectively) regardless of time, diversity or species combination. The fibrous rooting system and long, dense root hairs of *S. cereale* made this cover crop exceptionally effective at binding soil. Root hair length and density partially explained (42%) the variation in rhizosheath mass.

Conclusion

This field trial highlights that species selection is more important than diversity *per se* and indicates that *S. cereale* is particularly effective at binding soil to the roots, suggesting it is a particularly valuable cover crop for farmers to include in their mixture over winter months.

4.1 Introduction

The resilience of agroecosystems to climate stress can be strengthened by planting cover crop mixtures over the winter months. Cover crop root systems can enhance biogeochemical interactions in the rhizosphere (Hallama et al., 2019) that can improve soil health and functioning during a time of the year where field soils would otherwise be left bare and more vulnerable to the effects of extreme weather conditions. Increasing cover crop diversity has the potential to further improve ecosystem functioning through the complementary use of resources in time and space (Brooker et al., 2021; Homulle et al., 2022).

By binding soil to the roots, rhizosheath formation has the potential to promote soil structure and stabilisation (Mo et al., 2023) by enhancing soil aggregation around root systems (Marasco et al., 2018) that is critical for mitigating soil erosion. Several factors influence rhizosheath formation and its stability such as root traits, microbial activity, soil characteristics and moisture content (Mo et al., 2023). Primarily, rhizodeposition (from root decay and root-derived exudates, including mucilage) and drying-wetting cycles facilitate soil aggregation (Carminati et al., 2010; Aslam et al., 2022; Rahim et al., 2023), and root hairs can expand rhizosheath mass (Brown et al., 2017; Burak et al., 2021). Compounds in rhizodeposits stabilise (Naveed et al., 2018) and strengthen the aggregates bound around the roots (Czarnes et al., 2000). Therefore, investigating the rhizosheath formation capacity of crops used in our agricultural systems is essential to optimise soil structure and stabilisation in our fields.

Rhizosheath formation has been mostly studied in crop species grown under controlled conditions. For example, a comprehensive glasshouse screening study confirmed rhizosheath presence in most angiosperm orders tested, but with varying specific rhizosheath masses, *i.e.* rhizosheath mass per unit of root mass, across species (Brown et al., 2017). The few field trials that exist show that specific rhizosheath mass varied across grassland species in China, becoming larger with increasing nitrogen availability (Mo et al., 2022; Tian et al., 2022). The relative lack of field data highlights the need for additional experiments to verify the factors influencing rhizosheath formation in species with contrasting rooting systems (Hallett et al., 2022; Mo et al., 2023). Since cover crops are planted to protect the soil over the winter months, complementary root traits between cover crop species could lead to synergistic or additive effects on soil binding capacity and soil stabilisation.

Although rhizosheaths can improve soil stability by aggregating soil, their persistence over time is sensitive to environmental conditions. Rhizosheath formation by grasses in a semi-arid savanna was a plastic trait that varied annually depending on rainfall patterns, where rhizosheath thickness was greater in wetter than drier years, attributed to better growing conditions encouraging exudate production and root hair development (Hartnett et al. 2013). Similarly, Marin et al. (2021) found *Hordeum vulgare* specific rhizosheath mass (rhizosheath mass per unit of root length) decreased during the growing season in an exceptionally dry summer in the UK, whereas research in controlled conditions found that dry soils stimulated rhizosheath formation (Watt et al., 1994; Haling et al., 2014; Liu et al., 2019). Thus, the plasticity of root traits and their dynamic relationship requires further investigation to assess whether the rhizosheath persists in cover crop species over the winter months in temperate climates such as in the UK.

Root hairs, tubular-shaped outgrowths of root epidermal cells, are considered the key root trait for rhizosheath formation as they provide a physical framework to adhere to soil particles and increase the root-soil surface area contact to facilitate rhizosheath formation (Haling et al., 2010). Species without root hairs (*e.g. Allium schoenoprasum* and *A. cepa aggregatum*) did not form rhizosheaths (Brown et al. 2017), and although root exudation from root-hairless mutants of *Hordeum vulgare* and *Zea mays* allowed limited rhizosheath formation, soil binding was significantly greater when root hairs were present (Burak et al., 2021). Further, root hairs can promote mucilage production (Galloway et al., 2022; Marasco et al., 2018), enhancing rhizosheath formation. Therefore, understanding the root hair characteristics of cover crop species is important for assessing the soil binding capacity of their root systems.

Longer and denser root hairs can be important for rhizosheath formation, although the literature contains variable results. Tomato rhizosheath mass was positively correlated with root hair length under dry conditions, and with root hair density under well-watered conditions (Karanja et al., 2021). Specific rhizosheath mass and root hair length and density were strongly correlated in *Hordeum vulgare* (Marin et al., 2021), *Triticum aestivum* (Delhaize et al., 2012) and *Vigna unguiculata* (Opoku et al., 2022) while in different *Hordeum vulgare* genotypes (Brown et al., 2017; George et al., 2014; Haling et al., 2010) and sorghum (Adu et al., 2023) they were weakly or not correlated. Shorter root hairs have been found to correlate positively with specific rhizosheath mass (Brown et al., 2017); with root hairs up to 300 μm long suggested to be the primary driver of rhizosheath formation (Rongsawat et al., 2021). Alternatively, when root hairs are absent or their length is > 300 μm, mucilage and other root hair traits (e.g. morphology and density) become dominant. This suggests that factors affecting rhizosheath formation dominate or complement each other depending on species and genotype.

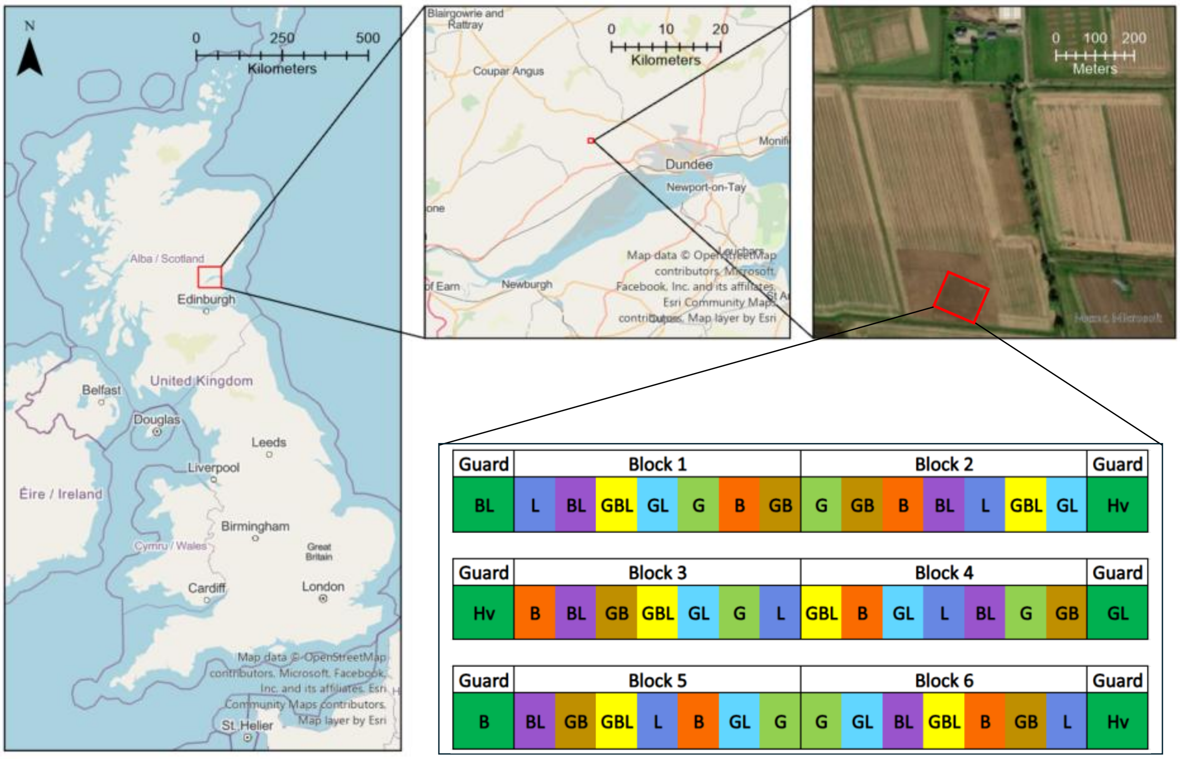
This study investigated rhizosheath persistence in a winter cover crop mixtures field trial, assessing whether root traits such as root length, root hair length and root hair density affected rhizosheath dynamics of different cover crop combinations and whether rhizosheath mass changed over time. We hypothesised that: 1) cover crop diversity will increase rhizosheath mass; 2) rhizosheath mass will change over time; and 3) rhizosheath mass will increase with longer and denser root hairs.

4.2 Methods

4.2.1 Field site

A winter cover-crop mixtures field trial was sown on 1st September 2022 at the James Hutton Institute's Balruddery Farm, Dundee, Scotland (56°28'46.6"N 3°07'53.1"W, Fig. 4.1). *Secale cereale* (G), *Brassica juncea* (B) and *Vicia faba* (L) were sown individually at a rate of 18.5 g m^{-2}, 1.5 g $m^{-2,}$ and 20 g m^{-2} respectively, and in all possible combinations in equal fractions, *i.e.* sowing rates for each species were halved or reduced to a third in the mixtures. The rates were recommended by the seed merchant, DLF Seeds A/S. These grass, brassica and legume species were chosen due to their suitability to climatic conditions and their contrasting plant functional groups (Tilman et al., 1997). A total of seven plant treatments were tested: grass (G), brassica (B), legume (L), grass and brassica (GB), grass and legume (GL), brassica and legume (BL), and grass, brassica and legume (GBL).

The plots were a subset of the field trial set up for measurements collected in Chapter 3, and were not sampled in Chapter 3. The experimental layout was a randomised design of six blocks. Guard strips of barley (cv. Laureate) were sown at the ends of each block to limit edge effects on outside plots. The experimental plots (2 m long x 1 m wide) were established on a 5° slope. The soil texture was silt loam (28.47 % sand, 51.35 % silt, 20.18 % clay). The bulk density was 1.14 g cm^{-3}.



Guard	Block 1							Block 2							Guard
BL	L	BL	GBL	GL	G	B	GB	G	GB	B	BL	L	GBL	GL	Hv

Guard	Block 3							Block 4							Guard
Hv	B	BL	GB	GBL	GL	G	L	GBL	B	GL	L	BL	G	GB	GL

Guard	Block 5							Block 6							Guard
B	BL	GB	GBL	L	B	GL	G	G	GL	BL	GBL	B	GB	L	Hv

Figure 4.1 Layout of field trial at Balruddery Farm, UK, comprising plots of *Secale cereale* (G), *Brassica juncea* (B), *Vicia faba* (L) as single species and in all possible combinations, including guard plots composed of BL, *Hordeum vulgare* (Hv), GL, and B (in dark green). Basemaps: Esri, OpenStreetMap and Microsoft. Figure adapted from Chapter 3 showing a different set of experimental plots (a subset of the trial) sown at the same time and adjacent to those used in that chapter.

4.2.2 Field measurements

Plots were sampled three times: in January (30th - 31st January), February (14th - 15th February) and March (1st - 2nd March) 2023. Total rainfall during the sampling period was 32.3 mm, and 514.1 mm between the sowing date and the end of the sampling period. At each time point, three soil moisture measurements were taken at a 7.6 cm depth in a zig-zag shape across each plot during using a FieldScout TDR 350 Soil Moisture Meter.

At each sampling, photographs were taken of a 0.5 m x 0.5 m quadrat and uploaded to Canopeo (Patrignani and Ochsner, 2015) to estimate canopy coverage percentage. On the last sampling time point, the number of plant individuals of each species was recorded to calculate stem density, and aboveground biomass was harvested by cutting at the stem base, then oven-dried at 70 °C for four days and weighed.

One soil core (6.4 cm diameter x 20 cm height) was taken per plot at each sampling time point. Before taking the core, the number of plants per species present within the cored diameter was recorded, then the plants were harvested by cutting at the stem base and oven-dried at 70 °C for four days after which the dry mass was recorded. Cores were stored in a cold room at 4 °C while they were processed over the next 4-5 days. Rhizosheath development was measured by carefully breaking the core open, removing the roots from the core and gently shaking them by hand until the bulk soil detached. Using deionised water, the rhizosheath of each species was washed off the roots into a pre-weighed aluminium tray. Roots from each species were easily differentiated due to their contrasting root architectures and colouring. Rhizosheath trays were oven-dried at 105°C overnight and the dry mass was recorded and expressed per unit area.

Roots were stored in 50% ethanol at 4 °C until all were processed together following the last sampling time point. Roots were suspended in water and an image scanned using an Epson Expression 10000XL scanner (Epson UK, London, UK). Background colour settings were adjusted for the differing root colour of each species *i.e.* a white background for the dark *V. faba* roots and a black background for the light *S. cereale* and *B. juncea* roots. Images were analysed for average diameter and root length using WinRHIZO (Regent Instruments, Quebec, Canada), with the latter expressed per unit area.

Root sections were then photographed using a compound light microscope (Leica MZF111, Leica Microsystems, Bannockburn, IL, USA) at 3.2x (for grass) and 5× (for brassica and legume) magnification with a Leica DC480 camera attachment (Leica Microsystems (UK) Ltd, Milton Keynes, UK). Photographs were taken of the root hair zones of all species within all treatments and time points. Since brassica tap roots were hairless, images were taken of the finer lateral roots where hairs were present. Representative root hair length of each species within all treatments was estimated by measuring 10 individual root hairs per image using ImageJ software (U.S. National Institutes of Health, Bethesda, Maryland, USA) providing an average value for each sample which was then taken forward for further data analysis. Root hair density was calculated by counting the number of root hairs within a 1 mm^2 area of root surface using ImageJ. Root hair length and density were multiplied to calculate total root hair length per unit of root length, a single variable to represent the combination of both root traits.

Once the images were taken, roots were oven-dried at 70°C for four days and the dry mass recorded. Specific rhizosheath mass was calculated by dividing the rhizosheath soil dry mass by the corresponding root length. Total rhizosheath of each species was calculated by estimating the average rhizosheath mass per plant in a core sample and then extrapolating to rhizosheath mass per m^2 by using the plant density determined from the number of plants in a quadrat.

4.2.3 Data analysis

Statistical analysis was performed in R (R Core Team, 2023). To normalise the data, canopy coverage (% ground cover), root hair density (number of hairs mm^{-2}), root length (cm m^{-2}) and specific rhizosheath mass (g cm^{-1}, rhizosheath dry mass per unit of root length) were square root transformed, while root hair length (μm), rhizosheath dry mass (g m^{-2}), total root hair length per unit of root length, and aboveground plant dry mass (g m^{-2}) were logarithmically transformed.

Factorial repeated measures ANOVAs were performed to assess the main effects and interactions of the factors of soil moisture, sampling time, diversity, species and treatment (*i.e.* species combination) on canopy coverage, aboveground dry mass, root diameter, rhizosheath mass, root length, specific rhizosheath mass, root hair length and density, and total root hair length per unit of root length. Block was set as random factor and plot was included as a nested factor to account for repeated measures on the same plots at different time points. Pairwise comparisons using a post hoc Tukey's HSD test were conducted to identify significant differences within groups following a significant ANOVA result.

Linear regressions were performed to identify relationships between rhizosheath mass and volumetric soil moisture content; and between specific rhizosheath mass and root hair length, root hair density, and total root hair length per unit of root length. A multiple linear regression was performed to assess the combined effect of root hair length and density on specific rhizosheath mass. All results presented are mean ± standard error and significance is at a 95% confidence level. Packages used were ggplot2, stats, dplyr and emmeans.

4.3 Results

Volumetric soil moisture content did not differ (F (6, 105) = 1.51, p = 0.19) between species treatments, but decreased significantly between sampling times (F (2, 105) = 76.67, $p < 0.001$), averaging 22.1 ± 0.3% at time point 1, 20.8 ± 0.2% at time point 2, and 16.6 ± 0.2% at time point 3 (n = 42 per time point).

Canopy coverage differed significantly (F (6, 98) = 6.63, $p < 0.001$) across species treatments. In the three species mix (GBL) coverage averaged 33 ± 3% and was significantly greater than coverage in B (23 ± 2%; t (98) = 3.92, p = 0.005), GL (22 ± 2%; t (98) = 4, p = 0.002) and L (19 ± 2%; t (98) = 5.66, $p < 0.001$). The field bean monoculture coverage was significantly less than in G (28 ± 2%; t (98) = 3.74, p = 0.005), GB (26 ± 2%; t (98) = 3.21, p = 0.02) and BL (26 ± 2%; t (98) = 3.14, p = 0.03). Although not significantly different between time points overall (F (2, 98) = 1.34, p = 0.25), there was an interactive effect between treatments and time (F (12, 98) = 1.71, p = 0.04). Table 4.1 shows coverage significantly decreased between time point 2 and 3 for brown mustard (B) and field bean (L) in monocultures, but no other treatments varied significantly over time. At time point 1, field bean monoculture coverage was significantly less than that of the three species mix, but neither differed significantly from other treatments. At time point 2, both GB and GL coverage were significantly less than GBL coverage, and at time point 3, field bean monoculture coverage was significantly less than that of rye monoculture, GB, and GBL coverage. Therefore, the three species mix consistently showed the most coverage and field bean monoculture generally the least.

Table 4.1 Coverage (%) and standard error of the mean of all treatments per sample time (n = 6). Values not sharing a letter are significantly different from each other ($p < 0.05$). Significant differences between treatments within each time point (columns) are shown by lower case letters. Significant differences between time point within each treatment (rows) are shown by upper case letters.

	Time point 1	***Time point 2***	***Time point 3***
Grass (G)	26.5 (±2.3) ^{ab}	24.6 (± 1.6) ^{ab}	32.0 (± 2.4) ^{a}
Brassica (B)	22.1 (± 3.0) $^{ab\ AB}$	29.8 (± 1.8) $^{ab\ A}$	19.4 (± 3.9) $^{ab\ B}$
Legume (L)	19.3 (± 2.7) $^{b\ AB}$	24.2 (± 2.2) $^{ab\ A}$	13.5 (± 1.7) $^{b\ B}$
BL	25.3 (± 1.8) ^{ab}	29.1 (± 2.0) ^{ab}	24.1 (± 4.1) ^{ab}
GB	25.8 (± 2.6) ^{ab}	23.6 (± 1.1) ^{b}	29.6 (± 2.8) ^{a}
GL	21.1 (± 2.4) ^{ab}	21.6 (± 1.5) ^{b}	25.9 (± 2.9) ^{ab}
GBL	32.2 (± 4.3) ^{a}	35.5 (± 5.7) ^{a}	31.0 (± 4.7) ^{a}

Aboveground plant dry biomass (g m^{-2}) differed significantly across species (F (2, 185) = 170.14, $p < 0.001$), diversity levels (F (2, 185) = 29.88, $p < 0.001$) and time (F (2, 185) = 10.16, $p < 0.001$) with significant interactions occurring between species and diversity (F (4, 185) = 8.51, $p < 0.001$) and species and time (F (4, 185) = 3.56, p = 0.007). Overall, rye aboveground biomass was approximately 3.5 times greater (55 ± 6 g m^{-2}) than brown mustard (16 ± 2 g m^{-2}), and brown mustard was 1.5 times greater than field bean (11 ± 1 g m^{-2}). Species in monoculture treatments produced significantly more aboveground biomass than when grown in mixes of two (t (185) = 3.72, $p < 0.001$) and three species (t (185) = 7.25, $p < 0.001$), as well as being greater in mixes of two rather than three species (t (185) = 4.65, p = 0.001). Field bean followed this decreasing pattern with increasing diversity, while brown mustard decreased from a monoculture to two (t (185) = 3.47, p = 0.01) and three species mixes (t (185) = 5.46, $p < 0.001$), but not between two and three species mixes (t (185) = 2.88, p = 0.09). Rye aboveground biomass did not differ significantly between diversity levels (Table 4.2). Overall, aboveground biomass increased significantly from time point 1 to 2 (t (185) = 4.18, p = 0.001) by 31% and did not significantly change from time point 2 to 3 (t (185) = 2.2, p = 0.07) or time point 1 to

3 (t (185) = 0.84, p = 0.67). The increase in mass from time point 1 to 2 was driven largely by an increase in dry mass of brown mustard, specifically when grown in the three species mix (GBL) (Table 2). Thus, rye consistently maintained the greatest aboveground dry mass regardless of species combination and time point, while field bean and brown mustard aboveground dry mass decreased with increasing diversity.

Table 4.2 Aboveground plant dry mass (g m^{-2}) and standard error of the mean of all treatments per sample time (n = 6). Values not sharing a letter are significantly different from each other ($p < 0.05$). Significant differences between treatments within each time point (columns) are shown by lower case letters. Significant differences between time point within each treatment (rows) are shown by upper case letters.

	T1	***T2***	***T3***
Grass (G)	56.62 (±13.37) ^{a}	63.11 (±7.66) ^{a}	30.48 (±2.65) ^{abc}
Brassica (B)	14.70 (±2.73) ^{ab}	26.60 (±5.00) ^{abc}	37.21 (±14.37) ^{ab}
Legume (L)	19.21 (±2.96) ^{ab}	23.37 (±3.15) ^{abc}	16.27 (±3.37) ^{b}
BL - B	11.04 (±3.67) ^{bcd}	21.76 (±3.16) ^{abc}	24.36 (±5.88) ^{abc}
BL - L	12.32 (±3.54) ^{bc}	13.74 (±2.26) ^{bcd}	11.91 (±2.03) ^{cd}
GB - G	50.31 (±6.69) ^{a}	72.64 (±2.63) ^{a}	56.76 (±11.18) ^{a}
GB - B	7.37 (±2.42) ^{bcd}	11.83 (±2.63) ^{cd}	7.93 (±1.89) ^{cd}
GL - G	61.59 (±20.82) ^{a}	50.09 (±7.42) ^{ab}	70.51 (±14.33) ^{a}
GL - L	7.51 (±1.50) ^{bcd}	8.95 (±2.03) ^{cd}	6.96 (±1.11) ^{cd}
GBL - G	33.03 (±4.00) ^{a}	60.68 (±8.53) ^{a}	51.39 (±6.06) ^{ab}
GBL - B	3.40 (±1.19) $^{d\ B}$	10.86 (±1.76) $^{cd\ A}$	10.7 (±2.08) $^{cd\ AB}$
GBL - L	3.97 (±1.28) ^{cd}	5.82 (±2.20) ^{d}	4.83 (±1.40) ^{d}

Root diameter was significantly (F (2, 185) = 525.76, p < 0.001) greater in field bean, averaging 1.12 ± 0.03 mm, whereas rye and brown mustard averaged 0.38 ± 0.01 mm and 0.38 ± 0.01 mm respectively. Average root diameter (mm) did not change significantly with time (F (2, 185) = 0.65, p = 0.52) or diversity level (F (2, 185) = 2.4, p = 0.09).

Total root length (cm m^{-2}) differed significantly between species (F (2, 185) = 224.9, $p < 0.001$). On average, rye total root length (8,807 ± 345 cm m^{-2}) was greater than that of brown mustard (3,678 ± 255 cm m^{-2}), while field bean had the shortest total root length (2,042 ± 149 cm m^{-2}). Overall total root length (cm m^{-2}) was significantly greater in monocultures than in the two (t (185) = 3.64, p = 0.001) and three species mixes (t (185) = 4.67, $p < 0.001$), but did not differ significantly (t (185) = 1.8, p = 0.17) between the two and three species mixes. This pattern was found in brown mustard, where total root length in a monoculture was greater than in the two (t (185) = 3.22, p = 0.03) and three species mixes (t (185) = 3.78, p = 0.006), but similar (t (185) = 1.2, p = 0.95) between two and three species mixes. Brown mustard total root length was greater in a monoculture than in GB (t (185) = 3.87, p = 0.008) and GBL (t (185) = 3.89, p = 0.007) but was not significantly different to BL (t (185) = 1.76, p = 0.83), and the mixes did not differ between each other. Field bean total root length in a monoculture was significantly greater than in the three species mix (t (185) = 4.2, p = 0.001), but was not significantly different between the monoculture and the two (t (185) = 2.86, p = 0.10) or the two and three species mixes (t (185) = 2, p = 0.54). Rye total root length did not differ significantly between diversity levels (Fig. 4.2). Although the species with greatest total root length (rye) maintained it across diversity levels, growing brown mustard and field bean in mixes decreased their total root lengths compared to their growth in monocultures.

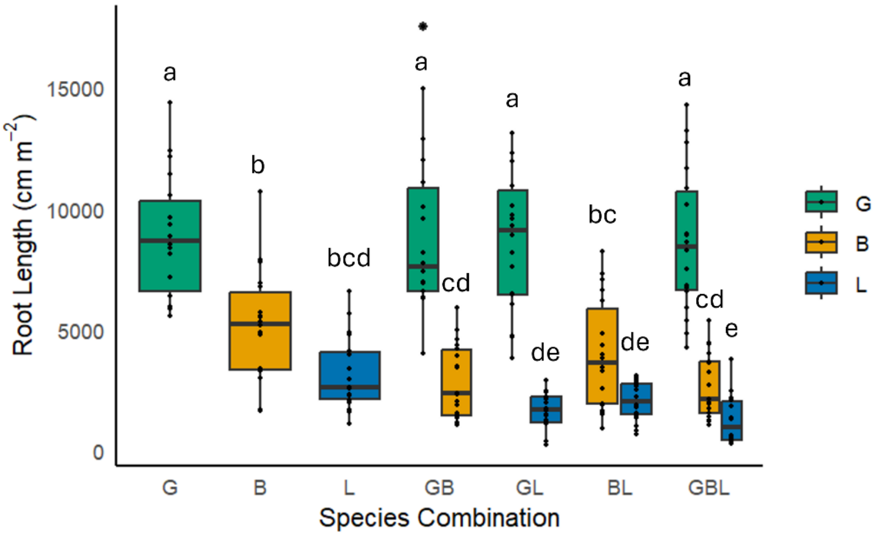


Figure 4.2 Root length (cm m^{-2}) across species treatments distinguishing the species within treatments by colour, where green is *Secale cereale* (G), yellow is *Brassica juncea* (B) and blue is *Vicia faba* (L), (n = 18 per boxplot). Boxes indicate the 25 & 75% quartile and lines the median, with smaller points indicating individual samples. Species significantly ($p < 0.05$) differ from each other if they do not share a letter.

Cover crop diversity significantly decreased (F (2, 185) = 11.09, $p < 0.001$) total rhizosheath mass per plant species (g m^{-2}). Overall, monocultures had a significantly larger rhizosheath mass than two (t (185) = 2.83, p = 0.01) and three species (t (185) = 4.57, $p < 0.001$) mixes, being significantly smaller in the three species than the two species mixes (t (185) = 2.47, p = 0.03) (Fig. 4.3).

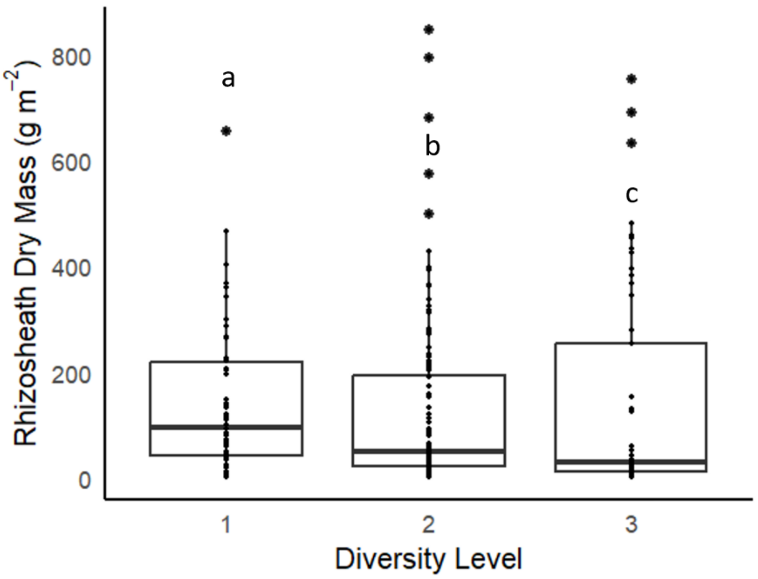


Figure 4.3 Rhizosheath dry mass per plant species (g m^{-2}) across diversity levels (level 1 n = 54; level 2 n = 108; level 3 n = 54). Boxes indicate the 25 & 75% quartile and lines the median, with smaller points representing individual species per plot. Diversity levels significantly differ ($p < 0.05$) from each other if they do not share a letter.

Rhizosheath mass (g m^{-2}) differed significantly (F (2, 185) = 228.02, $p < 0.001$) between species. On average, rye bound 7 times more soil (325.0 ± 20.3 g m^{-2}) than field bean (49.0 ± 4.6 g m^{-2}) and 11 times more than brown mustard (28.7 ± 3.2 g m^{-2}). There was a significant interaction (F (4, 185) = 7.58, $p < 0.001$) between diversity level and species driven by field bean rhizosheath mass, which was overall significantly larger in a monoculture than in the two species (t (185) = 3.85, p = 0.005) and the three species mixes (t (185) = 6.67, $p < 0.001$), and significantly larger in two than three species mixes (t (185) = 3.87, p = 0.004). At the treatment level, field bean rhizosheath mass was significantly larger in a monoculture than in GL and in GBL (t (200) = 3.53, p = 0.02 and t (200) = 7.09, $p < 0.001$, respectively), but not significantly different to BL (t (200) = 3.23, p = 0.06). Brown mustard rhizosheath mass was significantly smaller in GBL than BL (t (200) = 3.84, p = 0.008), but remained similar across all other treatments. For rye, rhizosheath mass did not differ across diversity levels or treatments. Thus, rye bound the largest amount of soil per m^2 regardless of diversity and species combination, brown mustard bound the least amount of soil regardless of diversity and species combination except when grown in a three species mix (GBL), and field bean bound more soil in a monoculture and when grown with brown mustard (BL) than when grown with rye (GL, GBL) (Fig. 4.4).

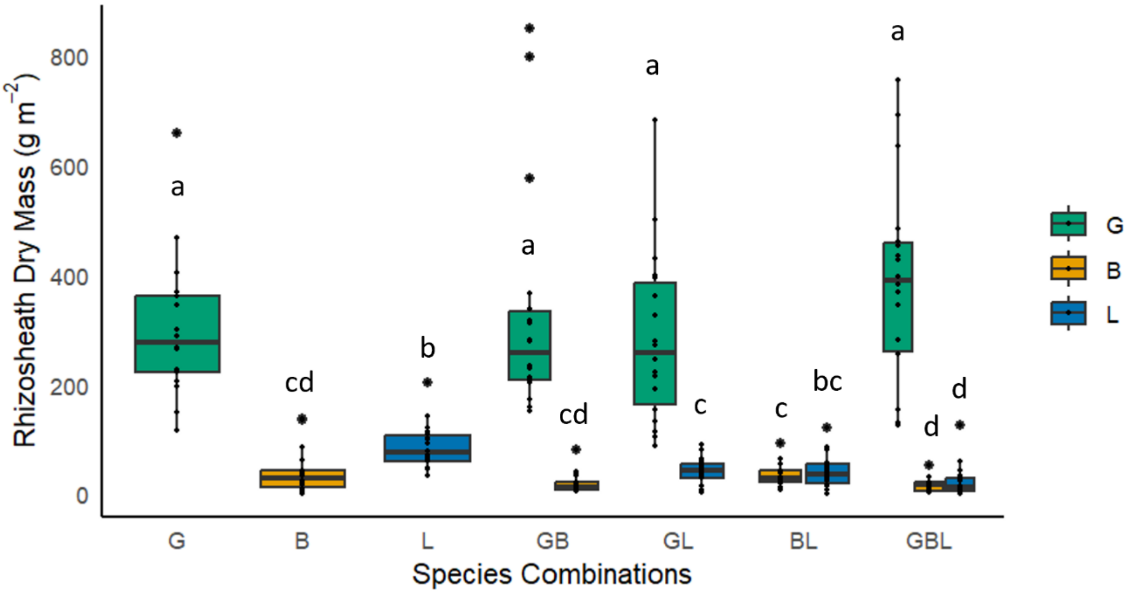


Figure 4.4 Rhizosheath dry mass (g m^{-2}) across species treatments distinguishing the species within treatments by colour, where green is *Secale cereale* (G), yellow is *Brassica juncea* (B) and blue is *Vicia faba* (L), (n = 18 per boxplot). Boxes indicate the 25 & 75% quartile and lines the median, with smaller points indicating individual samples. Species significantly ($p < 0.05$) differ from each other if they do not share a letter.

Rhizosheath mass (g m^{-2}) decreased with time (F (2, 185) = 4.41, p = 0.01), specifically between time point 1 and 3 (t (185) = 2.62, p = 0.02) by 27 % (Fig. 4.5). Rhizosheath dry mass increased significantly (F (1, 213) = 4.35, p = 0.03) with soil moisture, although moisture explained little of the variation ($r^2 = 0.01$).

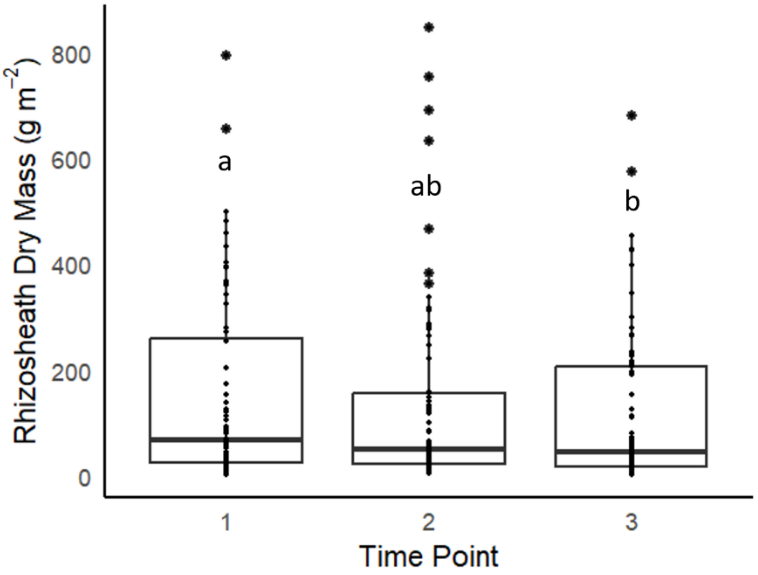


Figure 4.5 Rhizosheath dry mass (g m^{-2}) across time points ($n = 72$ per boxplot). Boxes indicate the 25 & 75% quartile and lines the median, with points indicating individual samples. Time points are significantly different ($p < 0.05$) from each other if they do not share a letter.

Root length (cm m^{-2}) did not change significantly over time ($F(2, 185) = 0.09$, $p = 0.9$), however specific rhizosheath mass (rhizosheath dry mass per unit of root length) did ($F(2, 185) = 6.71$, $p = 0.01$). Overall, specific rhizosheath mass decreased by 22% from time point 1 to time point 3, and did not change ($F(4, 185) = 0.34$, $p = 0.84$) within species over time (Fig. 4.6a) but differed between species ($F(2, 185) = 157.33$, $p < 0.001$). Specific rhizosheath mass of rye was 1.75 times greater than field bean and 5.60 times greater than brown mustard, while that of field bean was 3 times greater than brown mustard. Specific rhizosheath mass does not change within species across species combinations (Fig. 4.6b), and was greater in rye particularly when combined with field bean and brown mustard (GBL).

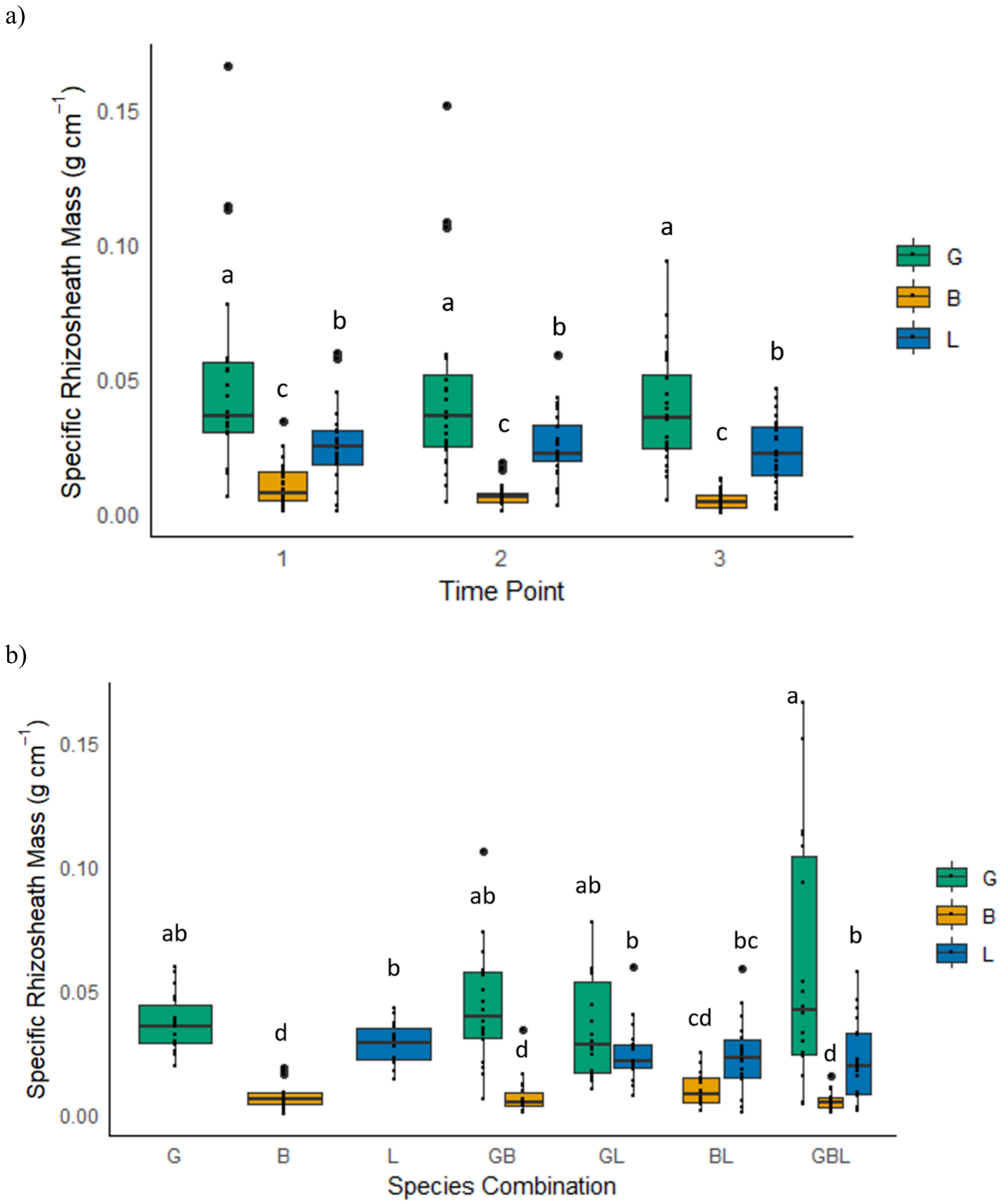
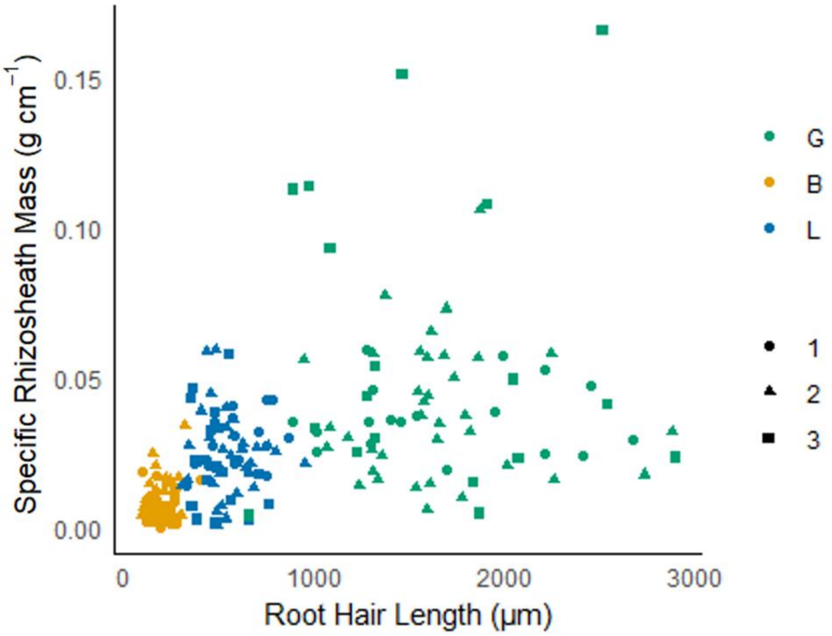


Figure 4.6 Specific rhizosheath mass (rhizosheath dry mass per unit of root length, g cm^{-1}) across time points (n = 24 per boxplot) (A) and species treatments (n = 18 per boxplot) (B) distinguishing the species within time points or treatments by colour, where green is *Secale cereale* (G), yellow *Brassica juncea* (B) and blue is *Vicia faba* (L), (n = 24 per boxplot). Boxes indicate the 25 & 75% quartile and lines the median, with smaller points indicating individual samples. Species significantly ($p < 0.05$) differ from each other if they do not share a letter.

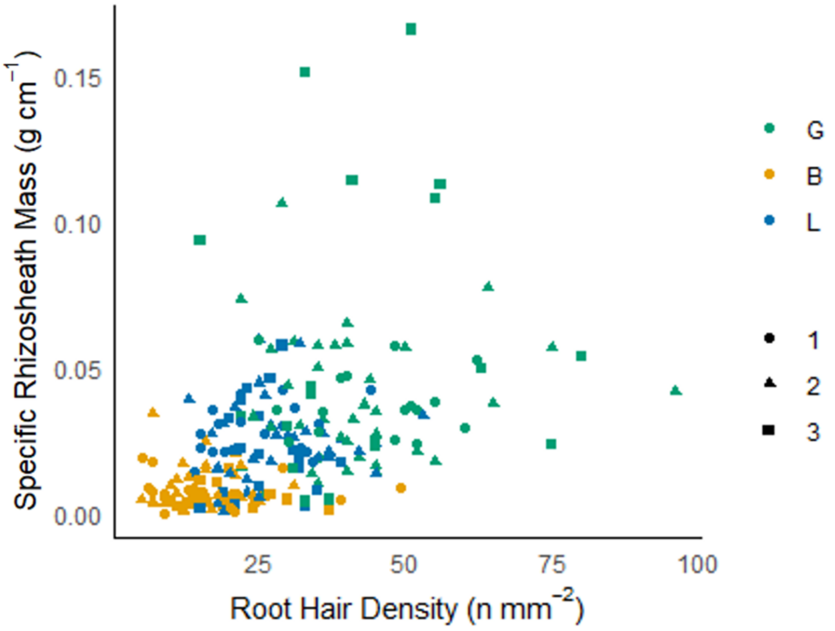
Across plant species, specific rhizosheath mass was significantly positively related to root hair length (F (1, 213) = 155.50, $p < 0.001$; $r^2 = 0.41$) and density (F (1, 213) = 75.56, p

< 0.001; $r^2 = 0.25$), with both independent variables being correlated with each other (F (1, 213) = , $p < 0.001$, $r^2 = 0.57$) and together explaining 42% of specific rhizosheath mass variation (Fig. 4.7a,b). When total root hair length per unit of root length was calculated, specific rhizosheath mass was also significantly positively related with this variable (F (1, 213) = 141.1, $p < 0.001$; $r^2 = 0.39$) (Fig. 4.7c). However, within each plant species, specific rhizosheath mass was not significantly related to root hair length, to root hair density, or total root hair length per unit of root length (Table 4.3). Diversity level did not affect root hair length (F (2, 185) = 0.33, p = 0.71), density (F (2, 185) = 1, p = 0.36), or total root hair length per unit of root length (F (2, 185) = 0.39, p = 0.67), but all differed significantly between species (F (2, 185) = 950.61, $p < 0.001$; F (2, 185) = 120.43, $p < 0.001$; and F (2, 185) = 578.82, $p < 0.001$, respectively). Brown mustard had the shortest and least dense root hairs, field bean had longer and denser root hairs, and rye had the longest and densest root hairs (Table 4.4, Fig. 4.8). Thus, variation in root hair traits affected rhizosheath mass.

a)



b)



c)

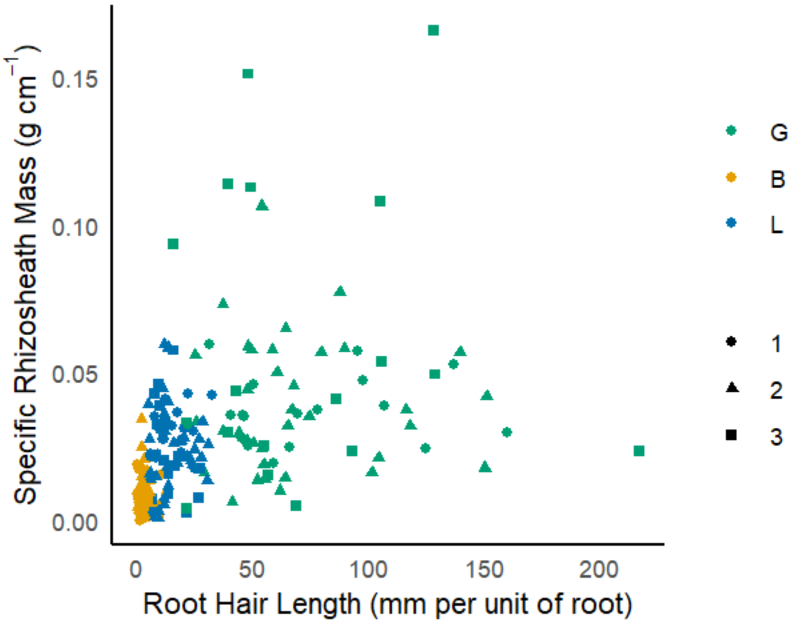


Figure 4.7 Specific rhizosheath mass (rhizosheath dry mass per unit of root length, g cm^{-1}) plotted against root hair length (A), root hair density (B) and total root hair length per unit of root length (C) with species distinguished by colour (*Secale cereale* (G) in green, *Brassica juncea* (B) in yellow and *Vicia faba* (L) in blue), and diversity levels distinguished by shape (n in species B = 72, G = 72 and L = 72; n in diversity level 1 = 54, 2 = 108 and 3 = 54).

Table 4.3 Relationships between specific rhizosheath mass (g cm^{-1}) and root hair length (RHL, µm) and density (RHD, number mm^{-2}), and total root hair length per unit of root length (RHL mm^{-1} root) for each species, overall, and the overall combined effect of root hair length and density on specific rhizosheath mass with slope, F-value and degrees of freedom, significance ($p < 0.05$) and coefficient of determination (r^2) reported.

<table>
<tr><th>Species</th><th>RHL (µm)</th><th>RHD (n mm^{-2})</th><th>RHL mm^{-1} root</th></tr>
<tr><td>Brassica juncea (B)</td><td>$y = 0.08 - 0.01x$
$F(1, 69) = 0.01$
$p = 0.99$
$r^2 = -0.01$</td><td>$y = 0.1 - 0.003x$
$F(1, 70) = 0.71$
$p = 0.40$
$r^2 = -0.01$</td><td>$y = 0.08 - 0.004x$
$F(1,69) = 0.51$
$p = 0.47$
$r^2 = -0.006$</td></tr>
<tr><td>Secale cereale (G)</td><td>$y = 0.21 – 0.01x$
$F(1, 70) = 0.01$
$p = 0.95$
$r^2 = -0.01$</td><td>$y = 0.16 - 0.005x$
$F(1, 70) = 0.71$
$p = 0.48$
$r^2 = -0.01$</td><td>$y = 0.17 + 0.004x$
$F(1,70) = 0.09$
$p = 0.75$
$r^2 = -0.01$</td></tr>
<tr><td>Vicia faba (L)</td><td>$y = 0.46 + 0.05x$
$F(1, 70) = 0.16$
$p = 0.68$
$r^2 = 0.01$</td><td>$y = 0.11 + 0.007x$
$F(1, 70) = 1.27$
$p = 0.26$
$r^2 = 0.01$</td><td>$y = 0.12 + 0.01x$
$F(1,70) = 0.92$
$p = 0.33$
$r^2 = -0.001$</td></tr>
<tr><td>Overall</td><td>$y = 0.04x - 0.16$
$F(1, 213) = 155.50$
$p < 0.001$
$r^2 = 0.41$</td><td>$y = 0.01 + 0.02x$
$F(1, 213) = 75.56$
$p < 0.001$
$r^2 = 0.25$</td><td rowspan="2">$y = 0.06 + 0.03x$
$F(1,213) = 141.1$
$p < 0.001$
$r^2 = 0.39$</td></tr>
<tr><td>Combined effect</td><td colspan="2">$y = −0.16 + 0.04RHL + 0.002RHD$
$F(2, 212) = 77.63$
$p < 0.001$
$r^2 = 0.42$</td></tr>
</table>

Table 4.4 Average root hair length (RHL, µm), density (RHD, number mm^{-2}) and total root hair length per unit of root length (RHL mm^{-1} root) per species with the standard error of the mean in brackets. Species are significantly different from each other ($p < 0.001$) if they do not share a letter.

Species	n	RHL (µm)	RHD (n mm^{-2})	RHL mm^{-1} root
Brassica juncea L. (B)	72	197 (± 7) ^{c}	17 (± 1) ^{c}	3.5 (± 0.3) ^{c}
Secale cereale L. (G)	72	1,636 (± 59) ^{a}	42 (± 2) ^{a}	71.3 (± 4.6) ^{a}
Vicia faba L. (L)	72	545 (± 16) ^{b}	26 (± 1) ^{b}	15.2 (± 0.9) ^{b}

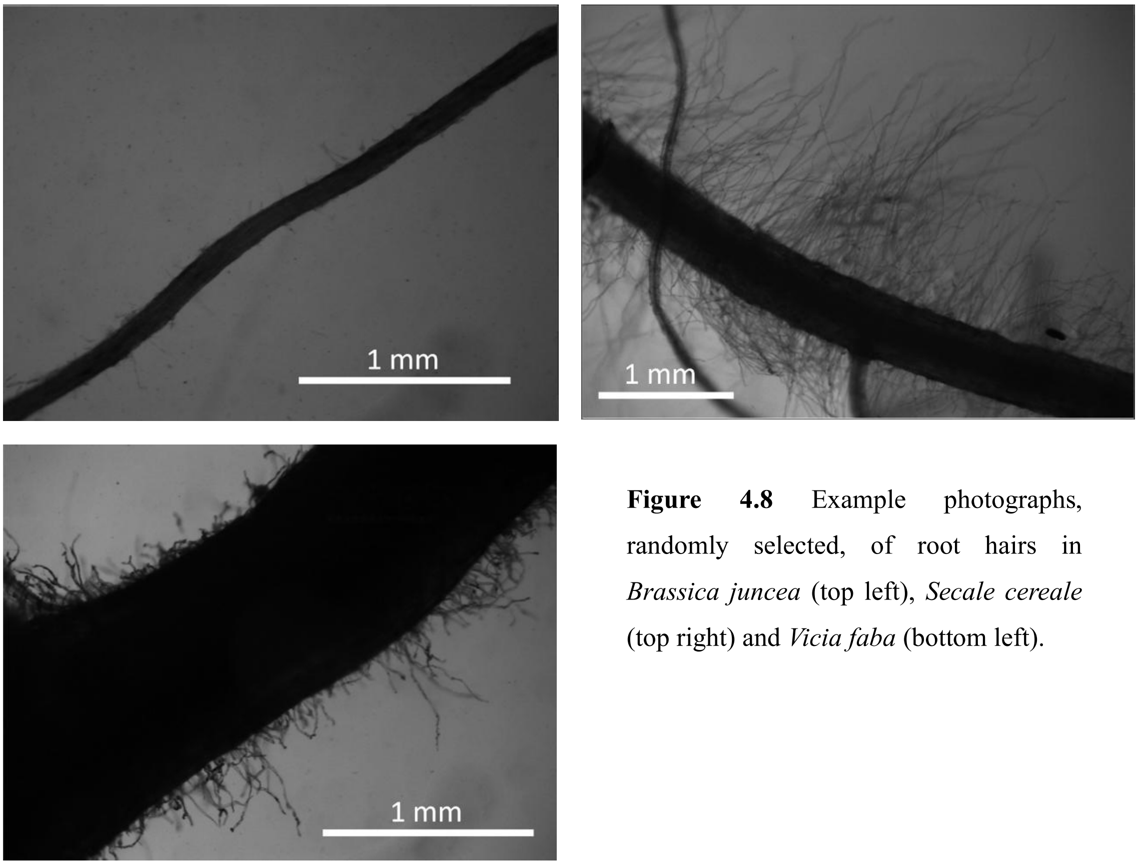


Figure 4.8 Example photographs, randomly selected, of root hairs in *Brassica juncea* (top left), *Secale cereale* (top right) and *Vicia faba* (bottom left).

4.4 Discussion

To our knowledge, this study is the first to assess the persistence of rhizosheath mass in three functionally diverse cover crop species grown in the field. We provide evidence that cover crop species rather than diversity is key for rhizosheath formation, with specific root traits such as those of rye retaining more soil regardless of sowing density. While farmer selection of cover crop species may be determined by their other benefits to cropping systems, species differences in rhizosheath formation suggest opportunities for maximising soil aggregation.

Overall, species grown in monocultures bind more soil per m^2 than when grown in mixtures (Fig. 4.3), in contrast to our hypothesis that cover crop diversity would increase rhizosheath mass. This is partly due to the different soil binding ability of certain species as well as the reduction of plant density with increasing diversity. Sowing rates for each species were halved or reduced to a third in the mixtures, leading to a reduction in rhizosheath mass per m^2 particularly for field bean, probably due to its already low sowing density. However, rye rhizosheath mass per m^2 maintained a large rhizosheath mass irrespective of whether sown at low (mixtures) or high (monoculture) density (Fig. 4.4), suggesting that it is highly competitive for space both with itself (intraspecific) and with the other two species (interspecific). This result suggests that rye could be sown at a lower monocrop density than recommended by cover crop seed suppliers.

Rye suppressed the soil binding capacity of field bean through competition, as field bean bound more soil per m^2 when combined with brown mustard than when combined with rye. The complementary relationship between brown mustard and field bean and competitive effect of rye was also observed in the differences in canopy ground coverage (Table 4.1), aboveground biomass (Table 4.2), root dry mass (Appendix 3 Table 9.1) and root length (Fig. 4.2) and specific rhizosheath mass (Fig. 4.6b) between species and cropping combinations. Rapidly establishing grass species typically dominate in mixed systems, especially over legumes (Hauggaard-Nielsen et al., 2012; Farney et al., 2018). Root interactions determine this competitive effect, as physical barriers in the soil between grass and legume species doubled legume aboveground biomass (Walker and King, 2009). Rye's fibrous rooting system extends throughout the topsoil, while the taproots of brown mustard and field bean grow towards the subsoil (Kemper et al., 2020). These differences in root spatial distribution complement each other, leading to positive effects on root development (root length and mass density, specific root length) and nutrient acquisition (Kemper et al., 2022). Although we expected to see niche differentiation allowing each species to develop relatively larger root and rhizosheath masses when grown in mixtures, the root spatial distribution of rye and its ability to extend and fill the available space suppressed the soil binding capacity of other species.

Over time, the cover crops bound less rhizosheath soil, confirming our second hypothesis and in line with findings in Chapter 2. Although there was no reduction in root length with time, soil binding capacity (rhizosheath mass per m^2) and specific rhizosheath mass overall decreased by 27 % and 22 %, respectively, from the start to the end of the trial. Nevertheless, specific rhizosheath mass of each individual species did not statistically change over time (Fig. 4.6a).

Similar temporal declines in rhizosheath mass have been observed, for example, in barley over a comparable timeframe (George et al., 2014), where reductions were attributed to increased rainfall and higher soil moisture content. Rhizosheath development is known to be plastic in response to soil moisture, with larger rhizosheaths (volume) observed under drier conditions in maize (Watt et al., 1994), while field studies have reported larger rhizosheaths (rhizosheath thickness) in wetter years, likely due to better growing conditions that encouraged root activity (Hartnett et al., 2013).

In this study, although rhizosheath mass declined with time, this was not explained by changes in soil moisture. Soil moisture decreased slightly (from 22.1% to 16.6%) between January and March, and this limited drying appeared insufficient to significantly influence soil aggregation or rhizosheath formation. Soil moisture explained little of the variability in rhizosheath mass ($r^2 = 0.01$), suggesting that other factors were more influential in driving the observed decline.

Plant maturity may have diminished rhizosheath formation over time. Younger, actively growing root systems with greater root exudation may stimulate rhizosheath formation, while root hair death and lignification in older roots likely weakened rhizosheath attachment to the roots. Soil retention capacity of ryegrass and alfalfa, with a fibrous and taproot systems respectively, was greater during early growth stages and then gradually decreased as plants aged due to stunted root growth following unfavourable weather conditions (Liu et al. 2024). In the present field trial, the cover crops were sown in September and sampled from January to March, when the winter months constrained growth and temperatures were gradually increasing but not yet reaching favourable temperatures for greater root development.

Root hair length and density contributed to rhizosheath formation, confirming our third hypothesis. Longer root hairs are associated with larger rhizosheaths (Delhaize et al., 2012; Marin et al., 2021; Opoku et al., 2022) as our results show overall (Fig. 4.7a and Table 4.3). Rongsawat et al. (2021) suggested root hair length is the primary driver of rhizosheath formation when root hairs are < 300 µm, and, beyond 300 µm, other root hair traits, such as density, become more important. Here, no relationships were found for individual species between root hair length or density and specific rhizosheath mass (Table 4.3), although the shorter (< 300 µm) and sparser root hairs in mustard bound less soil than the longer (< 300 µm) and denser root hairs in field bean and rye (Fig. 4.7a). This suggests hair density is of greater importance in < 300 µm long root hairs than proposed. Across all species that varied in root hair length (197-1636 µm), overall root hair density explained 25% of the variation in rhizosheath similar to sorghum (14% - Adu et al., 2023) and cowpea (20% - Opoku et al., 2022). While root hair length explained more (41%) of the variation of specific rhizosheath mass (increasing to 42% when combined with root hair density), both variables were correlated. When root hair length and density were combined into a single variable (total root hair length per unit of root length), the variation in specific rhizosheath mass accounts for slightly lesser (39%) variation than average root hair length alone. This suggests that while both traits contribute to rhizosheath development, average root hair length may have a stronger individual influence compared to average root hair density, or that their combined effect does not enhance explanatory power beyond average root hair length alone. Although average root hair length seems the primary driver for rhizosheath formation, other factors such as mucilage (Akhtar et al., 2018; Galloway et al., 2022) are likely to also be important.

Interestingly, rye had the greatest total root hair length per unit of root length *i.e.* both the longest and most dense root hairs of the three species, a combination that allows it to provide the most suitable root structure for rhizosheath expansion and therefore form the largest rhizosheath mass of the three species. However, not all variation in rhizosheath mass was explained by root hair length and density. Although rye is clearly an important species for binding soil compared to field bean and brown mustard, further work is needed to clarify the mechanisms by which these species bind soil and form a rhizosheath, such as through mucilage production.

The greater soil binding capacity of rye, along with other above-ground factors, may have facilitated its dominance over the other species through better access to resources. Root hairs enhance nutrient and water acquisition (Haling et al., 2013; Marin et al., 2021; Duddek et al., 2022) by increasing root-soil contact. The long and dense root hairs of rye coupled with its fibrous rooting system, which can expand and fill the available space, increases the volume of accessible soil, both for resource exploitation and for soil binding and retention. This may result in rye's further expansion and ability to outcompete the other species, binding more soil in the process. Therefore, selecting rye and other cover crop species with similar root traits will help retain and protect the soil over the winter months.

A key finding here is that the presence of certain species dictates the cover crop mixture's soil binding capacity. Notably, the soil binding capacity of rye was not affected by adding *e.g.* a legume to fix nitrogen, indicating that mixtures of species with complementary functions bring greater potential benefit to agroecosystems than monocultures. This highlights the importance of selecting cover crops for functional diversity rather than species diversity. Mahaut et al. (2020) found that species do not contribute equally to ecosystem functioning suggesting some play a greater role than others in regulating a given function, and in the present study rye could be considered a key species for improving the soil binding capacity of a system. Further, it may be possible to design cover crops mixtures for greater soil binding by selecting for root traits such as root hair length and density and root exudation.

The persistence of cover crop rhizosheaths over time, particularly after cover crop termination, remains largely unknown. Since the rhizosheath is a hotspot for plant-soil-microbe interactions, its potential to influence soil function beyond the lifespan of the cover crop is of significant interest. Rhizosheath-associated microbial communities, including those promoted by nitrogen-fixing legumes, could persist in the soil and shape microbial dynamics that could benefit the subsequent cash crop. These microbial legacies may enhance nutrient cycling, soil structure, and ultimately benefit productivity of the subsequent crop. For example, the extent to which arbuscular mycorrhizal associations, which promote soil aggregation and structure formation (Peng et al., 2013), persist after the cover crop and benefit the following crop is unknown. Understanding this legacy effect could inform better cover crop management strategies to optimise long-term soil health.

4.5 Conclusion

This study contributes novel field data on the mass and persistence of the rhizosheath and the traits associated with its formation in several functionally diverse cover crop species. The presence of certain species rather than diversity *per se* is more important in determining rhizosheath formation. While overall rhizosheath mass decreased with plant age, rye maintained the largest rhizosheath out of the three species regardless of time, diversity level or species combination and outcompeted brown mustard and field bean. Root morphological traits in rye, such as its fibrous rooting system and long, dense root hairs, make this cover crop potentially highly effective at binding soil. Root hair length and density explained only moderate to weak variation in rhizosheath mass, therefore other factors like mucilage production or composition are likely important. The ability of rye to extend and fill the space equally regardless of sowing density coupled with its extensive soil binding capacity, makes this species an excellent choice to include in a cover cropping system for soil retention. Importantly, the soil binding capacity of rye was not affected when grown with other species, supporting the use of mixtures that combine rye with other species providing additional ecosystem benefits. Further research on rhizosheath formation mechanisms could enable the design of cover crop mixtures with better soil binding by selecting complementary root traits such as root hair length, density, and exudation, while also considering the competitive nature of the species. Whether the persistence of cover crop rhizosheaths and their associated bacterial and fungal communities influence soil function for the subsequent crop is unknown and may be an important consideration for cover crop selection.

4.6 Acknowledgements

This work was funded by the Perry Foundation and the James Hutton Institute (JHI) as part of a 2021 – 2025 Joint PhD Scholarship. The JHI's proportion of the funding was provided by the Scottish Government's Rural and Environment Science and Analytical Services (RESAS) as part of the 2022-2027 Strategic Research Programme. We gratefully thank Katharine Preedy from BioSS at JHI and Rachel Baxter from Lancaster Environment Centre (Lancaster University) for statistical advice, as well as Jack Fletcher and Jacqui Brandt from the JHI's field team for their assistance with fieldwork and Molly Brown from the Plant Ecophysiology group (JHI) for her expertise in measuring root hairs.

Chapter 5. General Discussion

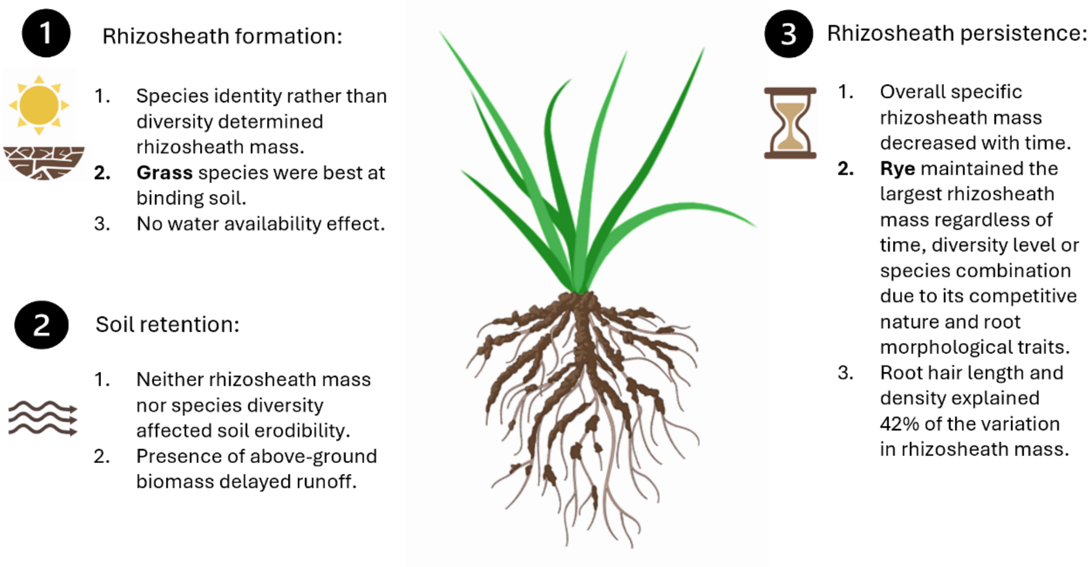
Cover crops have attracted significant interest for their potential to improve agricultural resilience to climate change by benefiting several ecosystem services, such as pest management, soil fertility, and weed suppression. There is, however, limited understanding about the direct effects of cover crop roots on soil stabilisation. How they bind soil (through rhizosheath development) and whether multi-species cover crops offer additional benefits has attracted little attention.

To address this knowledge gap, the research presented in this thesis tests the hypothesis that increased cover crop diversity improves soil binding capacity, thereby enhancing soil erosion control and resilience to water stress. To achieve this, a series of mesocosm and field scale experiments investigated how different levels of cover crop diversity affect rhizosheath formation, and whether there are additive or synergistic effects under drought stress (Chapter 2), in controlling soil water erosion (Chapter 3), and in rhizosheath persistence over time (Chapter 4).

The novel contributions of this thesis are:

- Quantitative evidence of the effect of different cover crop species and their mixtures on rhizosheath mass under varying water availability (Chapter 2).
- Field evidence of the relationship between root diversity, rhizosheath mass, and soil erosion from overland flow (Chapter 3).
- Novel insights into the persistence of rhizosheath mass over time in field-grown winter cover crop mixtures (Chapter 4).
- Evidence of the relative contribution of root hair length and density on rhizosheath formation for three cover crop species with divergent root morphologies (Chapter 4).

The research presented in this thesis addresses knowledge gaps regarding the role the rhizosheath plays in cover crop species grown in monocultures and mixtures and informs cover crop species selection in the field to help build resilience to abiotic stress. The main findings of this thesis are synthesised in the diagram below (Fig. 5.1) and, in the following sections, they are discussed along with their implications in practice and future research needs.



1 Rhizosheath formation:

1. Species identity rather than diversity determined rhizosheath mass.
2. **Grass** species were best at binding soil.
3. No water availability effect.

2 Soil retention:

1. Neither rhizosheath mass nor species diversity affected soil erodibility.
2. Presence of above-ground biomass delayed runoff.

3 Rhizosheath persistence:

1. Overall specific rhizosheath mass decreased with time.
2. **Rye** maintained the largest rhizosheath mass regardless of time, diversity level or species combination due to its competitive nature and root morphological traits.
3. Root hair length and density explained 42% of the variation in rhizosheath mass.

Figure 5.1. Diagram synthesising the main findings of the thesis.

5.1 Species identity is more important than diversity

Consistently throughout the studies, individual species identity, rather than species diversity, determined root soil binding capacity at both the mesocosm and field scales. Although this may appear to contradict the key ecological principle that increasing plant diversity can improve ecosystem function, these results agree with research that highlights the importance of functional diversity over species richness (Tilman et al., 1997; Cardinale et al., 2012; Hernandez and Picon-Cochard, 2016; Bakker et al., 2016). Some species contribute more than others to regulating a specific ecosystem function (Mahaut et al., 2020), and in this case, rye can be considered a key species for soil binding. Rye specific rhizosheath mass was circa 2-fold greater than brown mustard in Chapter 2 (Fig. 2.12b) and 50% greater when treatments contained rye in Chapter 3 (Fig. 3.5), and rye specific rhizosheath mass was 1.75 times greater than field bean and 6.60 times greater than brown mustard in Chapter 4 (Fig. 4.4). Root morphology and high relative growth rate are key reasons behind the excellent soil binding capacity of rye.

Vertical niche differentiation was expected to allow each species to develop relatively larger root and rhizosheath masses in mixtures, as it would reduce resource competition and allow further exploitation (Bakker et al., 2016). However, the extensive root spatial distribution of rye dominated the available space, suppressing the soil-binding capacity of other species (brown mustard in Fig. 2.12b and field bean in Fig. 4.6b). Grasses like rye often dominate in mixed systems as they establish quicker (higher relative growth rate) than other species, especially legumes (Hauggaard-Nielsen et al., 2012; Farney et al., 2018). The competitive nature of rye allowed it to maintain its specific rhizosheath mass and length whether grown in a monoculture or a mixture.

The greater soil binding capacity of rye regardless of species combination was likely due to its dominant nature over other species and its root traits. Rye had the longest and densest root hairs of the three species (Table 4.4). Together with its expanding fibrous rooting system, this may have facilitated its dominance over other species through greater resource acquisition, as root hairs enhance nutrient and water uptake through greater root-soil contact (Haling et al., 2013; Marin et al., 2021; Duddek et al., 2022). In addition, larger rhizosheaths are associated with longer (Delhaize et al., 2012; Marin et al., 2021; Opoku et al., 2022) and denser root hairs (Adu et al, 2023; Opoku et al., 2022) as indicated in Figure 4.7 and Table 4.3, therefore making rye an excellent cover crop choice for binding soil in the field.

However, root hair length and density explained only 41% and 25% of variation in specific rhizosheath mass respectively (42% when combined). The positive relationship between these variables has been found in a variety of species such as *Solanum lycopersicum* (Karanja et al., 2021), *Hordeum vulgare* (Marin et al., 2021), *Triticum aestivum* (Delhaize et al., 2012) and *Vigna unguiculata* (Opoku et al., 2022). However, no relationship was found between specific rhizosheath mass and root hair length or density within each species in this work, just as Zhang et al. (2020) reported across four *Oryza sativa* varieties or Pang et al. (2017) across 100 *Cicer arietinum* genotypes. Since root-hairless mutants of *Hordeum vulgare* and *Zea mays* still form a limited rhizosheath (Burak et al., 2021b), other factors such as mucilage production (Akhtar et al., 2018; Galloway et al., 2022) and interactions with soil microorganisms are likely important in rhizosheath formation and these may dominate or complement each other depending on species and genotype. Therefore, further work involving high-throughput root exudate analysis is crucial to understand the mechanisms and contributions of different factors to rhizosheath formation to assess the suitability of cover crop species for optimum soil binding.

5.2 The role of the rhizosheath under abiotic stress

As climate change intensifies abiotic stresses like drought and erosion, plants will primarily experience these challenges at the root-soil interface, *i.e.* the rhizosheath. This critical interface can mitigate the effects of these stresses (George et al., 2024b), but the potential of cover crop rhizosheath development to mitigate drought and erosion has been largely overlooked in the literature. The research presented in this thesis contributes to the understanding of its functional role in enhancing plant resilience to abiotic stresses.

The presence and mass of the rhizosheath is considered a key trait for drought tolerance (Steiner et al., 2024; Rabbi et al., 2021; Basirat et al., 2019). On the one hand, drought tolerant species such as chickpea (Rabbi et al., 2021) form a greater specific rhizosheath mass that retains more water than the surrounding bulk soil, sustaining transpiration (Basirat et al., 2019) and maintaining nutrient and water uptake during soil drying (North and Nobel, 1997; Carminati et al., 2010; George et al., 2014; Pang et al., 2017; Cheraghi et al., 2023). Furthermore, drought stress enhances rhizosheath formation (e.g. in maize, Watt et al., 1993, 1994; in rice, Zhang et al., 2020), linked to greater root hair development (length and density) under drought (Watt et al., 1994, Haling et al. 2010) and the viscosity and adhesiveness of mucilage encouraged by dry-wet cycles (Pang et al., 2017; Naveed et al., 2018; Aslam et al., 2022). However, other research (savannah grasses in Hartnett et al, 2013; barley in Marin et al. 2021), including here (rye and mustard in Chapter 2), has not found evidence of drought enhancing specific rhizosheath mass, instead finding greater rhizosheath mass under well-watered conditions. This can be attributed to better growing conditions (*i.e.* larger root mass) under well-watered that droughted treatments. Further, Rahim et al. (2023), who found that wet soil conditions increased rhizosheath mass to a greater extent than dry-wet cycles, using chia seed mucilage and artificial roots, suggesting that maintaining wet conditions and avoiding drying cycles allows uninterrupted rhizosheath accumulation with mucilage diffusing into the bulk soil and binding further soil particles, as opposed to the interruptions to rhizosheath accumulation occurring in dry-wet cycles due to soil cracking and shrinkage during soil drying.

Rhizosheath development differs across species and varieties in response to drought. For example, some maize varieties explore the soil through root elongation, maintaining rhizosheath structure (*i.e.* preserving stable macroaggregates), whilst others focus on maintaining plant-microbial interactions by enhancing rhizodeposit accumulation in microaggregates inhibiting macroaggregate formation (Steiner et al., 2024). The range of responses to drought between varieties, attributed to differences in functional plant traits and enzymes produced by the microbial community in the rhizosheath, can also be expected between plant species. For instance, barley produces exudates that may disperse the soil, while maize exudes polysaccharides that may bind soil (Naveed et al. 2017). Therefore, rhizosheath formation under drought may be expected to be less in grasses like barley or rye. Greater rhizosheath masses under drought occur in maize (Morel et al., 1991; Watt et al., 1993; 1994), especially in experiments conducted under glasshouse conditions on young plants (harvested approximately 1 week after sowing) (Watt et al., 1994; Haling et al., 2014). This suggests that greater rhizosheath masses in response to drought may form only in early root development stages and depends on plant species and their root traits and exudates, providing the opportunity for further research into the mechanisms by which different rhizosheath formation capacities across species can improve tolerance to this abiotic stress.

Rhizosheaths also play a crucial role in improving soil structure and stabilisation and therefore have great potential for mitigating soil erosion. Rhizosheaths allow grasses in arid and semi-arid environments to anchor in sandy soils, stabilising loose soils and mitigating soil erosion (Tian et al., 2019; T. Y. Liu et al., 2019; Wang et al., 2022). Compounds in rhizodeposits stabilise (Naveed et al., 2018) and strengthen the aggregates bound around the roots (Czarnes et al., 2000) and Burak et al. (2021b) found the presence of root hairs, which enhanced rhizosheath development in barley, significantly decreased soil detachment in a mesocosm-scale rainfall simulation. While the rhizosheath has clear potential to mitigate soil erosion (Burak et al., 2021a), the greater soil binding capacity of rye did not lead to a reduction in sediment loss during the overland flow experiments in the field (Fig. 3.6). This might have been explained by soil particles detaching from the soil surface rather than the root zone, with divergent root systems having no effect on surface soil detachment. Fibrous rooting systems (*e.g.* rye) decreased sediment loss compared to taproots (*e.g.* brown mustard) in overland flow experiments (Liu et al., 2024). However, soil structural and hydrological properties were likely more important for sediment loss than the plant treatments and their differing soil binding capacities in this field trial. This unexpected result suggests further work is needed to unmask rhizosheath effects in the field (see section 5.5).

Although rhizosheath mass did not respond to drought or mitigate soil erosion, differences in soil binding capacities between species highlight the need to further investigate the mechanisms underlying rhizosheath formation. Exploring these mechanisms could reveal their potential role in mitigating abiotic stresses across a broader range of cover crop species and species combinations. While this work did not demonstrate the soil stabilisation potential of the rhizosheath, it indicates species differences in soil binding capacity. Further research should clarify the rhizosheath impacts on mitigating soil erosion, but soil variability in field trials may make it difficult to identify robust effects. Rhizosheaths provide numerous functional benefits, being hotspots for plant-soil-microbe interactions, that have the potential to improve the delivery of ecosystem services and therefore they remain a crucial trait for further investigation and consideration for land managers.

5.3 Rhizosheath persistence

Since the rhizosheath can bring many benefits to soil function and ecosystem services (Pang et al., 2017; Aslam et al., 2022), investigating its persistence is essential to optimise and inform crop selection and decision-making in the field. Overall rhizosheath mass consistently decreased over time across two mesocosm trials (over 5 and 4 weeks, respectively, Table 7.1 and 7.2) and a 3 month field trial (Fig. 4.5). Rhizosheath mass can fluctuate with soil moisture content, with soil drying-induced increases under short-term controlled experiments (Watt et al., 1994; Haling et al., 2014; T. Y. Liu et al., 2019) and with soil wetting in the field (Marin et al., 2021, Hartnett et al., 2013), yet rhizosheath mass was not related to soil moisture here. Root-bound soil decreased with plant age, as George et al. (2014) also found in a barley field trial of similar duration to Chapter 4, attributed to diminished root activity. Younger, actively growing root systems with greater root exudation may promote rhizosheath formation, whereas in older roots, root hair death and lignification likely weakened rhizosheath attachment. When unfavourable weather conditions stunted ryegrass and alfalfa growth, soil retention capacity decreased compared to early growth stages (Liu et al. 2024). As the winter months constrained cover crop growth, root length and rhizosheath mass remained constant over time in Chapter 4 while drought stress exacerbated the reduction in bound soil over time in Chapter 2. Favourable growing conditions are therefore essential for rhizosheath formation and persistence as suggested by Hartnett et al. (2013) and as seen in well-watered grass treatments (Chapter 2). Hartnett et al. (2013) suggested greater rhizosheath masses form under favourable growing conditions due to root exudation and growth of root hairs, dependent on plant carbon gain during its growth. However, in Chapter 2, favourable growing conditions allowed stability of rhizosheath mass over time regardless of root growth, further suggesting that rhizosheath formation occurs in the early stages of plant development, i.e. actively growing root tips, and does not keep expanding with time. As Section 5.1 mentions, although root hairs provide a physical network for rhizosheath formation, their length and density only explained a proportion of variation in bound soil. Understanding the fluctuation in mucilage production with plant age across a wide variety to cover crop species would be beneficial for understanding the factors regulating optimum soil binding in the field.

Whether cover crop rhizosheaths persist over time (after cover crop termination) is unknown. As the rhizosheath is a hotspot for plant-soil-microbe interactions, rhizosheath-associated bacterial communities of the preceding cover crop may contribute to soil function under the subsequent crop. For example, the benefits of arbuscular mycorrhizal associations, recognised promoters of soil aggregation (Peng et al., 2013), may still be evident in the soil for the following crop. In addition to plant-root interactions with beneficial microbes, the role of rhizosheaths in interactions with other organisms such as pathogenic microbes, nematodes, and other invertebrate pests both for the cover crops and the following crop is also unknown. This is an interesting pathway for future research, which could identify differences and synergies across a range of cover crop species combinations and inform species selection in the field not only for the winter months but also for the subsequent crop.

5.4 Practical recommendations

Up to March 2025, UK government policies incentivised arable farmers to use cover crop mixtures over the winter months, offering £129 per ha per year under the Sustainable Farming Incentive (SFI) scheme (Defra, 2024). However, agricultural policy is evolving, and the SFI scheme in England has since been discontinued, highlighting the dynamic nature of policy support for cover cropping, with a new framework set to be announced under the upcoming spending review. Despite these policy changes, cover crop mixtures remain a key strategy for improving soil health and resilience. A mixture is defined as at least two species from two or more families (e.g. brassicas, legumes or grasses), advocating that a mixture offers combined benefits and also mitigates potentially poor performance of some cover crop species. While grass and brassica mixes are favourable for erosion control, brassica and legume mixes are good for improving soil structure (AHDB, 2015). However, field trials quantifying the additive or interactive effects of different cover crop species combinations are severely lacking as most guidance focuses on individual species benefits and considerations.

Field trials in this thesis found cover crop diversity had no soil binding benefit over monocultures, however this may not be true for all species combinations, soil types, and environmental conditions, and, importantly, maintaining a diverse cover cropping system is still desirable as other species can improve other ecosystem functions. For example, despite the limited soil binding capacity of brown mustard, their large water demand could prove them to be a good companion to rye (that is good at binding soil) under high soil moisture conditions. Since dominance and species competition is also crucial, and rye outcompeted mustard (Fig. 2.10, Fig. 4.2), maintaining a large soil binding capacity regardless of being grown in a monoculture or a mixture is beneficial. Understanding the links between synergies and dominance among species like rye is essential for land managers to optimise cover cropping strategies for achieving desired environmental outcomes.

All farmers have the pressure of improving the resilience of agricultural systems to abiotic stress if livelihoods and agricultural productivity are to be sustained for our society. Abiotic stresses associated with climate change such as erosion, drought and flooding will be mainly perceived at the root-soil interface (George et al., 2024b), therefore selecting cover crop species with appropriate functional root traits can help mitigate these effects. Cover crop choice should be determined by coupling species functional root traits to the objective farmers have set to achieve in the field *e.g.* erosion control.

Although this thesis did not detect the potentially beneficial role of the rhizosheath in erosion control, differences in rhizosheath formation across species suggest opportunities to identify soil binding capacities of a wider range of cover crop species and their combinations to maximise soil binding and water availability management in the field. While cover crop aboveground biomass delayed runoff (Fig. 3.8a) as previously reported (Wang et al., 2015; Ludwig et al., 2005), this occurred in a field trial where field variability masked other plant treatment effects. This highlights the importance of using cover crops and making sure as much of the bare soil is covered over the winter months to delay soil loss during heavy rainfall.

Cover crop establishment is also key to protect soil over the winter months and can be a challenge, especially in the north of the UK, where conditions can be too wet or cold to allow optimum establishment. Defra suggest cover crop selection should consider local land and weather conditions, *e.g.* selecting species that can germinate in cooler weather if growing seasons are short and the seed supplier should provide guidance. In the field trials of this thesis, although cover crop plant biomass remained small (compared to cover crops grown in a warmer polytunnel) due to the cold overwinter Scottish weather conditions, they were well-established. Field trials were drilled rather than broadcast for a more reliable germination success, however the lines of bare soil between crops that this method produces may have allowed preferential water runoff pathways to form during the overland flow simulations of Chapter 3 and therefore masked diversity or rhizosheath effects on sediment loss. Although farmers commonly drill crops, broadcast sowing is cheaper and, despite the potential for poorer establishment, would improve soil erosion control and allow a better assessment of the effects of crop diversity. Further, rye demonstrates a strong ability to extend its canopy and rooting systems in the available space regardless of sowing rate, which coupled with its great soil binding capacity, suggests growers could sow rye at a lower density than recommended and still make this species an excellent choice to include in a cover cropping system for soil retention.

In the EU, where farmers can also access subsidies for implementing cover cropping practices (CAP Strategic Plan Regulation, 2021), policy is the strongest determinant for EU farmers to adopt cover cropping, while the environment and climate change do not play a significant role in farmer's decisions (Kathage et al., 2022). Therefore, greater policy obligations and subsidies would lead to increased adoption of these kinds of practices that protect the environment and livelihoods. However, with the increasing frequency of extreme weather events and variable rainfall patterns (Kendon et al., 2023), farmers should implement a sustainable plan tailored to the location and environmental conditions of their system if agricultural resilience to the detrimental effects of climate change is to be achieved. It is worth acknowledging that pest and disease issues, which are expected to worsen with climate change (Chakraborty et al., 2011), should be taken into account when selecting cover crop species as these can act as reservoirs for pests and pathogens for the following crop (Rand et al., 2022). In the UK, cover crop adoption is on the rise, with increasing farmer and government awareness of the importance of sustainably managing soil (Storr et al., 2019). While this may be due to policy, UK farmers are already experiencing the effects of extreme weather events and variable rainfall patterns through the loss of soil (Rickson et al., 2020) and reduction of crop yields (Defra, 2024), and therefore optimum cover cropping solutions along with greater farmer environmental awareness and stronger government support are necessary.

This thesis highlights the importance of selecting cover crop species for functional diversity rather than species diversity. Continued efforts identifying rhizosheath formation capacity and associated root traits of different species may allow complementary effects under different field conditions and help us understand the links between functional trait composition and biodiversity. Selecting cover crops for root traits such as root hair length and density may optimise cover crop mixtures for better soil binding. This work provides quantifiable evidence on the effect of cover crops and their mixtures on soil binding, helping inform on-farm decision-making and policy to achieve agricultural system resilience to climate change.

5.5 Future Research

This work provided mesocosm and field scale evidence of the effects of increasing cover crop diversity on the soil binding capacity of the system, a soil-associated ecosystem service largely ignored in the literature. This research has contributed to understanding the determinants of rhizosheath formation and its potential role in improving resilience to abiotic stress in cover crop monocultures and mixtures commonly grown in temperate climates like the UK. Further research opportunities identified throughout this thesis are summarised below:

1. **Drought effects on rhizosheath formation across species.** Chapter 2 found watering regime had no effect on rhizosheath formation in cover crop species of up to 11 weeks old. Most studies demonstrating larger rhizosheaths under drought use young maize plants in glasshouse conditions. This thesis applied different watering regimes to older plants in polytunnel conditions. The larger mesocosm pots and the varying temperature and humidity in polytunnel conditions made maintaining consistent watering regimes a challenge despite the help of the soil moisture retention curve in the second experiment. Although data are needed at larger scales, greater control over watering regimes in glasshouse trials would help clarify whether water availability affects rhizosheath formation in cover crops of

varying diversity and identify any synergistic or additive effects in soil binding capacity. Including a wide range of species with different root traits and exudates provides opportunities to identify mechanisms by species variation in rhizosheath formation might affect drought tolerance. Further research on water availability effects on rhizosheath formation of species at different growth stages would help clarify whether water availability only affects rhizosheath formation in early stages of plant growth. As the need to translate evidence from small scale to large scale remains, this knowledge gap could be addressed by conducting multiple field trials in areas with different rainfall patterns and over several years.

2. **Root diversity and soil binding effect on sediment loss.** Chapter 3 found increasing cover crop diversity or rhizosheath mass did not affect sediment loss. Future work should investigate the soil binding capacity of a greater range of cover crop species as well as a greater range of diversity levels (3+ species combinations) that can complementarily use resources in time and space with particular focus in the field. After identifying suitable species combinations in overland flow simulations in the lab, the effect of root functional diversity and the associated soil binding capacity on mitigating overland flow erosion should be field-tested. Field variability in Chapter 3 masked any potential diversity and rhizosheath effects, therefore studies including multiple fields to account for between-field variability would help clarify the effects of increasing cover crop root diversity on soil retention. Although difficult to achieve in practice, using a higher flow rate than the 'low' rate applied in Chapter 3 may have a greater impact on sediment loss and therefore help discern plant diversity effects, especially with the differences in turbulence caused by species with varying stem diameters. While challenging in field conditions (primarily due to wind affecting rainfall uniformity and distribution), conducting rainfall simulations instead of overland flow simulations may allow a clearer understanding of the soil retention capacity of root systems as the vertical impact of raindrops may interact more with the roots and therefore the rhizosheath than the horizontal movement of overland flow.
3. **Role of exudates in rhizosheath formation and persistence across species.** Chapter 2 identified decreased soil binding capacity over time in two mesocosm experiments of up to 11-week-old plants, especially under drought conditions.

Chapter 4 also identified this decrease during a period of three months in the field. As root hair length and density explained moderate to weak variation in rhizosheath formation, the change was attributed to plant maturity and the associated reduction in root activity *i.e.* potentially less mucilage production. Further research should identify underlying mechanisms that limit rhizosheath persistence, as it could affect crop choices for different environment conditions. Understanding **mucilage production** (composition, concentration and exudation timing) of a wide range of cover crop species provides opportunities to identify optimised cover crop combinations to build resilience to abiotic stress.

4. **Effects of cover crop rhizosheaths on the subsequent crop.** There is potential to determine whether rhizosheath-associated bacterial communities and arbuscular mycorrhizal associations that aggregate soil contribute to soil function during the subsequent crop (legacy effects). In addition to plant-root interactions with beneficial microbes, there is a knowledge gap regarding the role of rhizosheaths in interactions with other organisms such as pathogenic microbes, nematodes, and other invertebrate pests both for the cover crops and the following crop. Identifying the contribution of rhizosheaths to soil functioning over time offers opportunities to select for improved cover crop species and combinations not only for soil binding but for other ecosystem functions and for the subsequent crop.

5.6 General conclusion

This thesis quantifies the effect of increasing cover crop diversity on soil binding capacity (rhizosheath formation). At the mesocosm and field scales, it investigates whether there are additive or synergistic effects on soil binding under drought, soil water erosion control and rhizosheath persistence over time. This contributes to a better understanding of the role of rhizosheaths and their formation across functionally diverse cover crop species and informs land managers on cover crop species selection to improve agricultural resilience to abiotic stress.

For the ecosystem functions measured in this thesis, greater cover crop diversity provided no benefit over monocultures, with the presence of rye maximising soil binding capacity of any cover crop treatment. Rye bound the largest amount of soil regardless of species combination, sowing density, water availability and time. Although species with fibrous rooting systems and long and dense root hairs such as rye are a strong cover crop choice for binding soil, maintaining a diverse cover cropping system is still desirable as other species can improve functions that have not been investigated here, such as soil fertility or pest management. While rhizosheath formation did not respond to drought or directly contribute to erosion control in these experiments, species-specific differences in rhizosheath formation highlight the opportunity to design optimum cover crop combinations for binding soil. Although rye is clearly more effective at binding soil than field bean and brown mustard, further research is needed to understand the specific mechanisms of soil binding and rhizosheath formation in these species.

Despite the valuable insights gained, some limitations remain, particularly regarding field variability. Incorporating a broader range of species and diversity levels, plant ages, and controlled rainfall simulations, could better understand increased cover crop diversity effects on soil binding. Since rhizosheaths are critical to plant and soil function, continued research into this trait is important. Expanding our knowledge in this area will help optimise cover crop selection for improving soil health and mitigating the impacts of climate change.

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7. Appendix 1: Chapter 2 – Supplementary material

Single species experiment

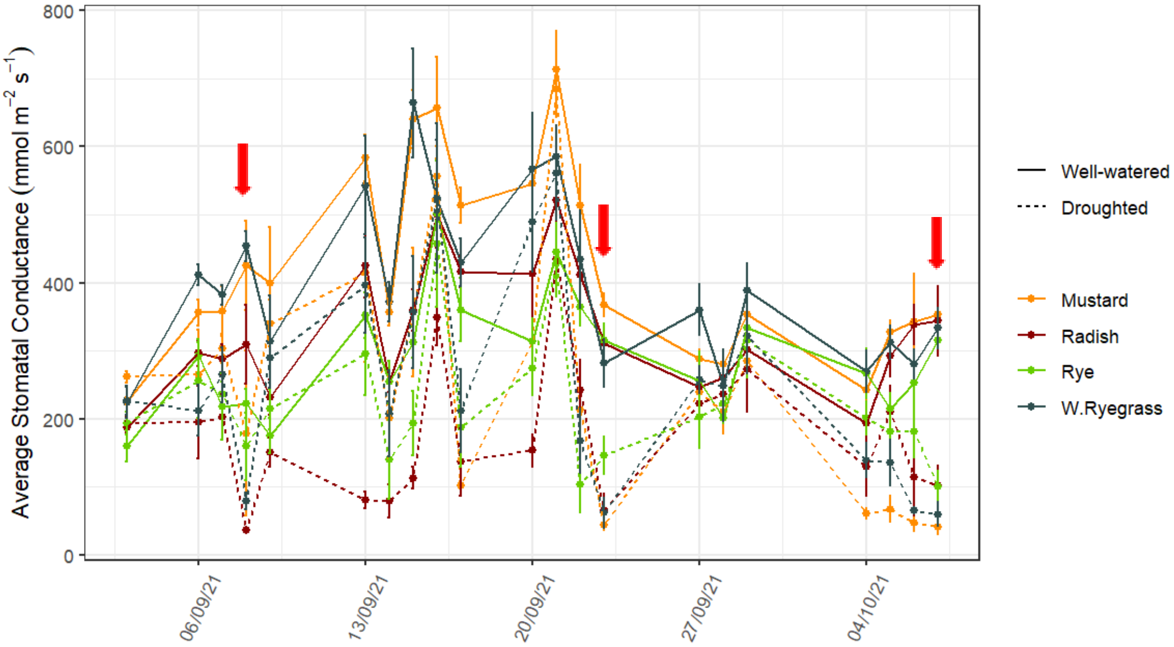

Figure 7.1 Average stomatal conductance (mmol m^{-2} s^{-1}) from the start of differing watering regimes to harvest, measured every other day (n = 5). Red arrows mark sampling times. Treatments include four species belonging to two functionally diverse families (brassicas and grasses) under two different watering regimes. Species include mustard in orange, radish in red, rye in green and W. ryegrass in blue. Watering regimes include well-watered, represented by a continuous line, and droughted, represented by a dotted line. Lines only aid visualization of stomatal conductance fluctuation over time and do not imply a relationship between points.

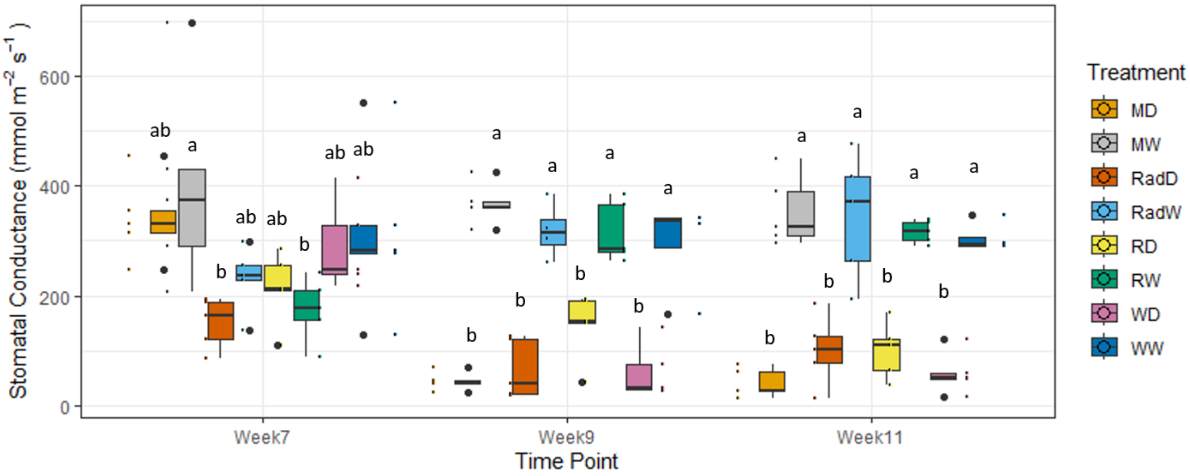

Figure 7.2 Stomatal conductance (mmol m^{-2} s^{-1}) of all treatments at each time sampling point (n = 5). Treatments include droughted mustard (MD), rye (RD), radish (RadD) and W, ryegrass (WD), and well-watered mustard (MW), rye (RW), radish (RadW) and Westerwold ryegrass (WW). Boxplots that share a letter are not significantly different from each other. Letters of significance only address the significant differences within each sampling time point, not across all.

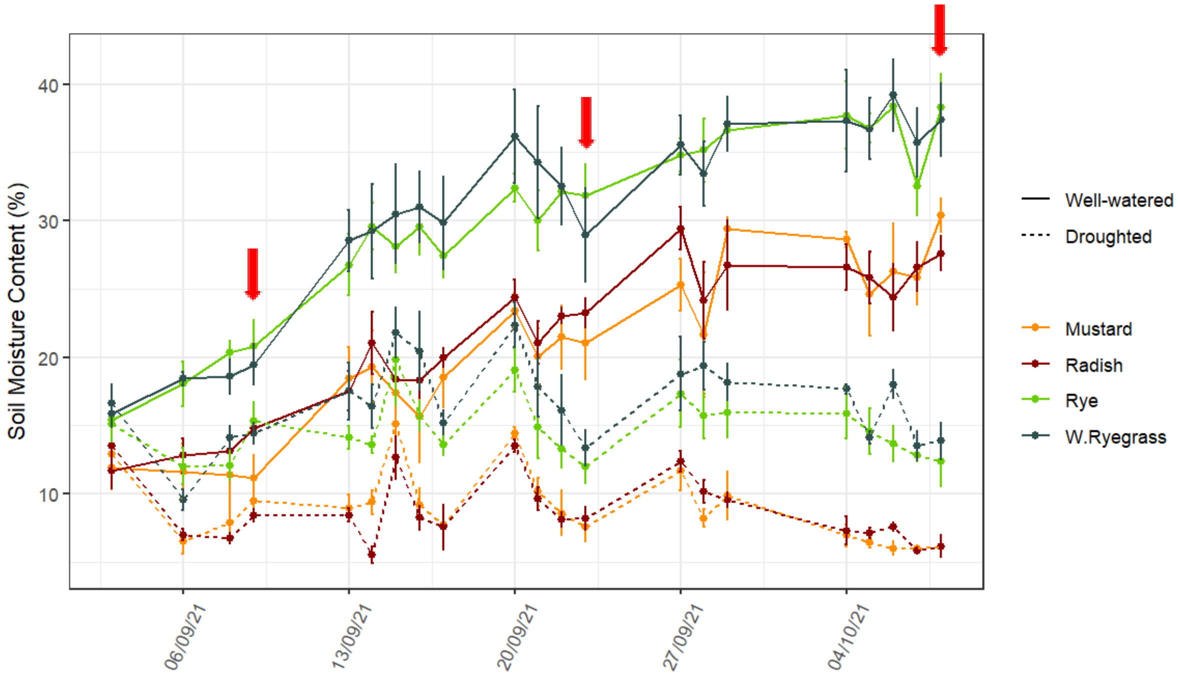

Figure 7.3 Volumetric soil moisture content (%) from the start of differing watering regimes to harvest (n = 5). Red arrows mark the sampling times. Treatments include four species belonging to two functionally diverse families (brassicas and grasses) under two different watering regimes. Species include mustard in orange, radish in red, rye in green and W. ryegrass in blue. Watering regimes include well-watered, represented by a continuous line, and droughted, represented by a dotted line. Lines only aid visualization of volumetric soil moisture content fluctuation over time and do not imply a relationship between points.

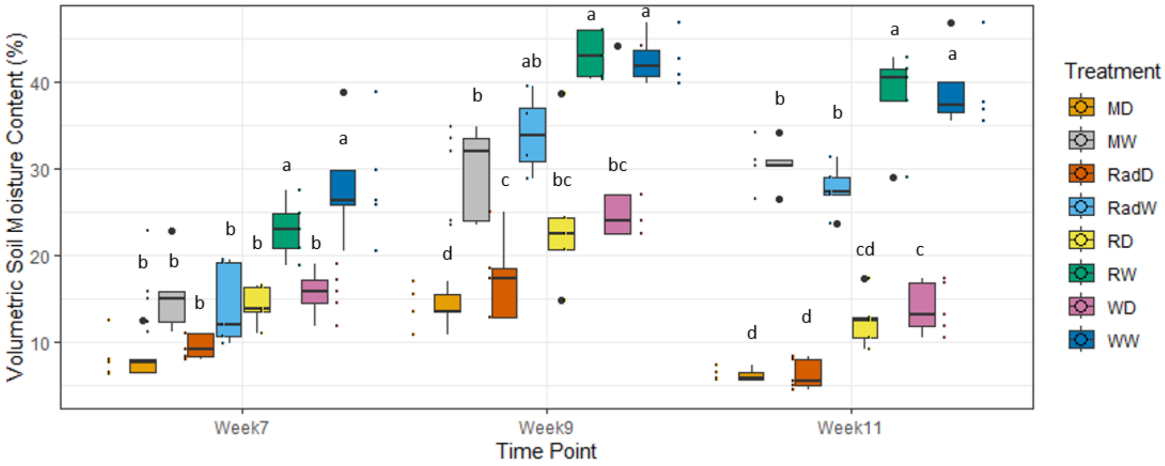

Figure 7.4 Volumetric soil moisture content (%) of all treatments at each time sampling point (n = 5). Boxplots sharing a letter are not significantly different from each other. Treatments include droughted mustard (MD), rye (RD), radish (RadD) and W. ryegrass (WD), and well-watered mustard (MW), rye (RW), radish (RadW) and W. ryegrass (WW). Boxplots that share a letter are not significantly different from each other. Letters of significance only address the significant differences within each sampling time point, not across all.

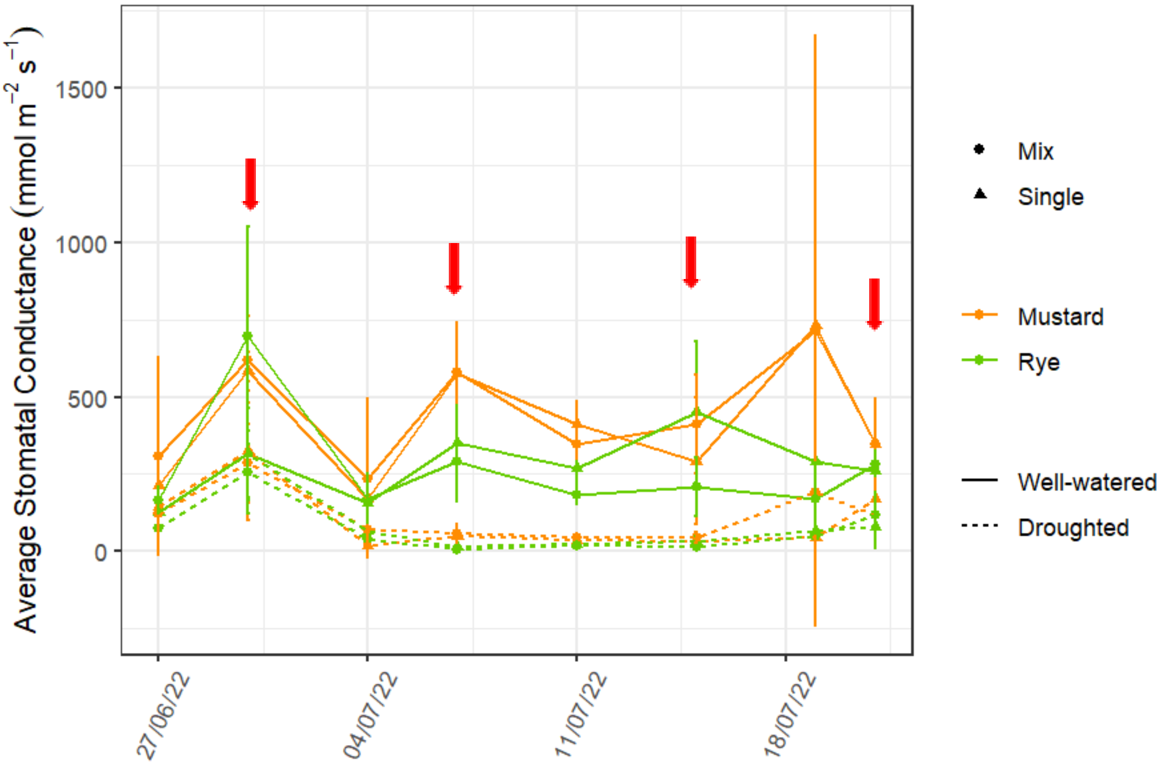

Figure 7.5 Average stomatal conductance (mmol m^{-2} s^{-1}) during the second mesocosm experiment, measured every second day (n = 5). Red arrows mark sampling times. Treatments include two species belonging to two functionally diverse families (brassicas and grasses) under two different watering regimes. Species include mustard in orange, and rye in green, with shapes representing whether they were grown in monocultures (triangles) or mixtures (circles). Watering regimes include well-watered, represented by a continuous line, and droughted, represented by a dotted line. Lines only aid visualization of stomatal conductance fluctuation over time and do not imply a relationship between points.

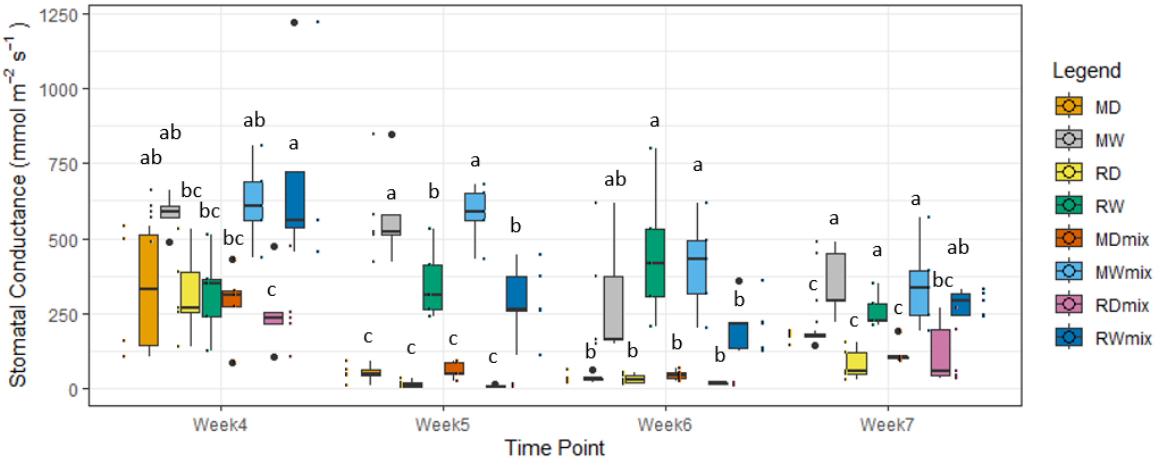

Figure 7.6 Stomatal conductance of all treatments at each sampling time (n = 5). Treatments include: droughted mustard in monoculture (MD) and in mixture (MDmix), rye in monoculture (RD) and in mixture (RDmix), and well-watered mustard in monoculture (MW) and mixture (MWmix), and rye in monoculture (RW) and mixture (RWmix). Boxplots that share a letter are not significantly different from each other. Letters of significance only address the significant differences within each sampling time point, not across all boxplots.

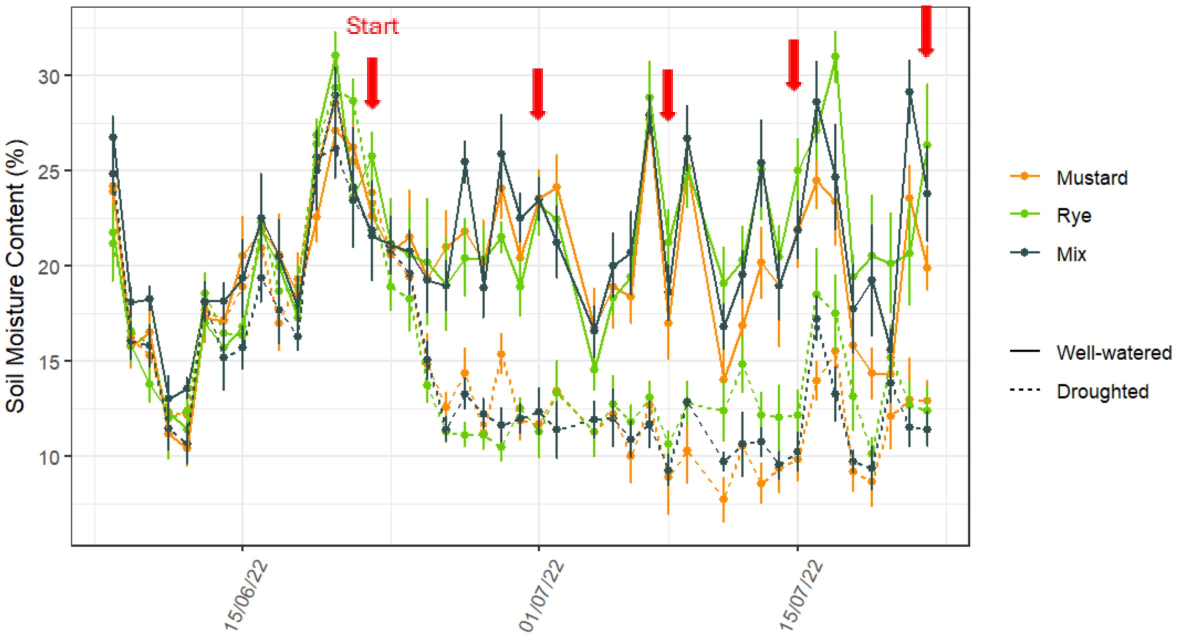

Figure 7.7 Volumetric soil moisture content (%) over the duration of the second mesocosm (n = 5). First ´start´ arrow marks the beginning of the differing watering regimes and the subsequent arrows mark the sampling days. Treatments include two species belonging to two functionally diverse families (brassicas and grasses) under two different watering regimes and grown in monocultures or mixtures. Species include mustard and rye, represented in orange and green respectively when grown in monocultures, and blue when grown together. Watering regimes include well-watered, represented by a continuous line, and droughted, represented by a dotted line. Lines only aid visualization of volumetric soil moisture content fluctuation over time and do not imply a relationship between points.

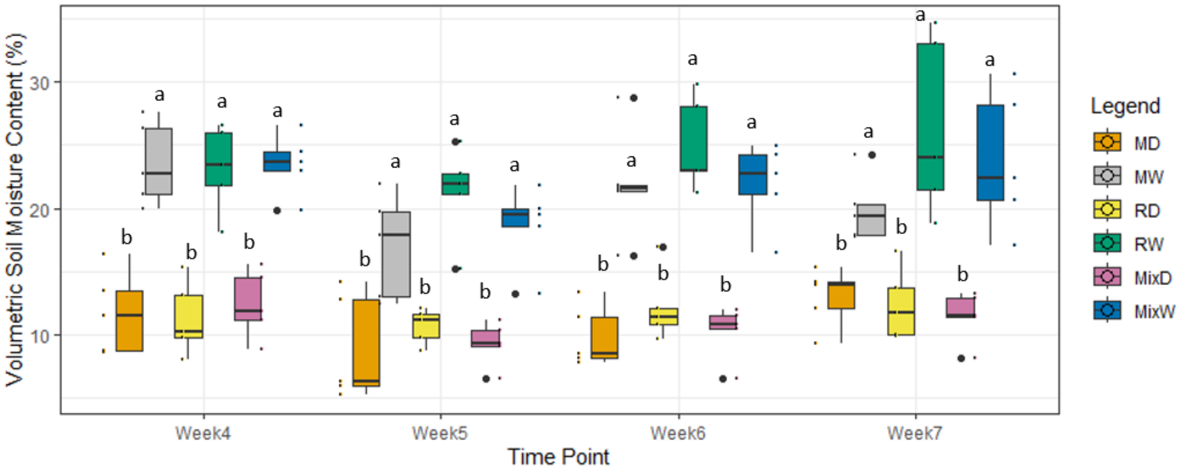

Figure 7.8 Volumetric soil moisture content of all treatments at each time sampling point (n = 5). Boxplots sharing a letter are not significantly different from each other. Letters of significance only address the significant differences within each sampling time point, not across all boxplots.

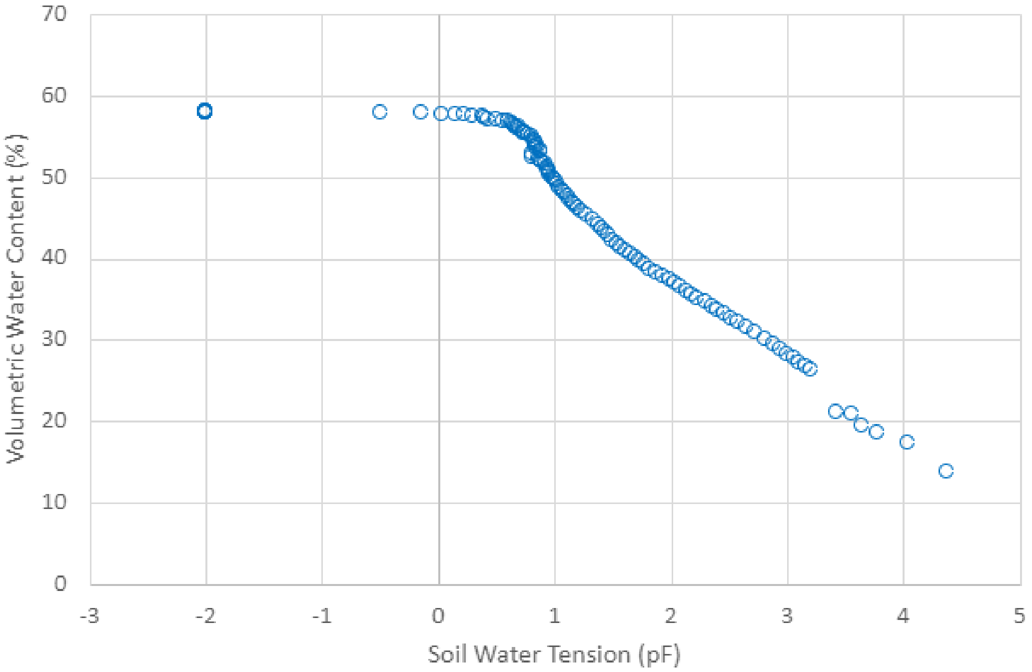

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Figure 7.9 Soil moisture retention curve of Upton topsoil at the bulk density of mesocosm 1 (0.91 g cm^{-3}) measured by HYPROP and WP4C (METER Group AG, München, Germany). This curve shows the decrease of volumetric soil moisture content as the soil water tension decreases from saturation to dry soil passing through the conventionally accepted permanent wilting point of pF 4.2.

Table 7.1 Single species mesocosm means and standard error of the mean in brackets of soil moisture, stomatal conductance, aboveground dry mass, root dry mass, rhizosheath dry mas and specific rhizosheath mass across all species and watering regime treatments and time points. Factorial repeated measures ANOVA F and p values are stated below with significant ($p < 0.05$) main and interaction effects marked in bold.

Time	***Species***	***Watering Regime***	***Soil Moisture (%)***	***Stomatal Conductance (mmol m^{-2} s^{-1})***	***Aboveground Dry Mass (g $plant^{-1}$)***	***Root Dry Mass (g $plant^{-1}$)***	***Rhizosheath Dry Mass (g $plant^{-1}$)***	***Specific Rhizosheath Mass***
Week 7	Mustard	Well-Watered	15.30 (±2.03)	399.00 (±83.00)	0.96 (±0.09)	0.35 (±0.06)	13.40 (±3.77)	38.20 (±8.62)
		Droughted	8.10 (±1.12)	340.00 (±33.80)	1.10 (±0.21)	0.36 (±0.05)	16.70 (±4.38)	47.20 (±9.84)
	Radish	Well-Watered	14.20 (±2.11)	231.00 (±26.20)	17.50 (±5.89)	5.71 (±3.35)	126.00 (±58.60)	27.30 (±3.88)
		Droughted	9.46 (±0.65)	151.00 (±20.90)	9.20 (±1.70)	3.02 (±0.66)	46.50 (±17.10)	16.00 (±5.58)
	Rye	Well-Watered	22.90 (±1.52)	175.00 (±26.10)	1.97 (±0.23)	2.98 (±0.86)	60.80 (±20.40)	22.30 (±5.27)
		Droughted	14.20 (±1.02)	214.00 (±29.50)	1.23 (±0.10)	1.23 (±0.16)	38.90 (±11.50)	35.20 (±12.50)
	W. Ryegrass	Well-Watered	28.20 (±3.03)	313.00 (±67.90)	0.81 (±0.09)	0.62 (±0.06)	26.20 (±3.89)	44.10 (±5.31)
		Droughted	15.60 (±1.21)	290.00 (±36.40)	1.04 (±0.49)	0.66 (±0.33)	25.90 (±11.30)	49.70 (±16.30)
Week 9	Mustard	Well-Watered	29.50 (±2.41)	367.00 (±16.90)	2.01 (±0.35)	0.53 (±0.11)	8.41 (±1.24)	18.10 (±4.18)
		Droughted	14.00 (±1.05)	44.30 (±7.64)	1.76 (±0.31)	0.49 (±0.08)	9.61 (±2.70)	19.50 (±2.49)
	Radish	Well-Watered	33.90 (±2.39)	319.00 (±25.50)	16.90 (±4.81)	4.61 (±1.98)	18.80 (±6.16)	5.44 (±1.10)
		Droughted	17.20 (±2.24)	65.60 (±24.10)	15.00 (±4.07)	5.19 (±2.84)	28.50 (±12.70)	7.52 (±1.44)
	Rye	Well-Watered	43.10 (±1.23)	316.00 (±24.80)	2.94 (±0.44)	2.03 (±0.46)	22.60 (±2.70)	20.00 (±7.48)
		Droughted	24.20 (±3.95)	146.00 (±27.50)	2.12 (±0.46)	2.56 (±0.35)	26.00 (±4.14)	11.70 (±1.79)
	W. Ryegrass	Well-Watered	42.50 (±1.52)	295.00 (±42.90)	1.53 (±0.44)	1.05 (±0.32)	13.00 (±4.14)	14.10 (±3.16)
		Droughted	28.00 (±4.11)	61.40 (±21.90)	1.64 (±0.44)	0.89 (±0.29)	17.20 (±5.34)	21.60 (±1.07)
Week 11	Mustard	Well-Watered	30.40 (±1.21)	354.00 (±28.90)	2.06 (±0.56)	0.77 (±0.23)	5.95 (±0.81)	9.76 (±1.94)
		Droughted	6.12 (±0.30)	41.20 (±11.70)	2.58 (±0.96)	0.56 (±0.12)	7.21 (±1.57)	13.90 (±0.63)
	Radish	Well-Watered	27.60 (±1.26)	344.00 (±51.30)	20.30 (±3.08)	3.30 (±0.62)	21.70 (±4.27)	6.98 (±1.27)
		Droughted	6.16 (±0.78)	102.00 (±28.50)	15.90 (±3.55)	5.66 (±2.76)	23.10 (±5.53)	6.97 (±2.11)
	Rye	Well-Watered	38.30 (±2.48)	316.00 (±9.37)	4.02 (±1.28)	1.65 (±0.48)	32.90 (±8.15)	21.20 (±1.79)
		Droughted	12.40 (±1.37)	101.00 (±23.00)	2.62 (±0.49)	2.69 (±0.34)	31.70 (±2.69)	13.60 (±1.23)
	W. Ryegrass	Well-Watered	39.20 (±2.55)	306.00 (±13.50)	2.03 (±0.61)	1.02 (±0.13)	26.80 (±3.88)	26.80 (±2.98)
		Droughted	13.90 (±1.33)	59.70 (±17.20)	2.15 (±0.91)	2.20 (±0.62)	23.50 (±4.66)	12.00 (±1.89)

Species	F(3, 91) = 32.13 **p < 0.001**	F(3, 91) = 3.54 **p = 0.01**	F(3, 91) = 104.04 **p < 0.001**	F(3, 91) = 45.47 **p < 0.001**	F(3, 91) = 21.59 **p < 0.001**	F(3, 91) = 22.23 **p < 0.001**
Watering	F(1, 91) = 296.46 **p < 0.001**	F(1, 91) = 157.85 **p < 0.001**	F(1, 91) = 1.88 p = 0.17	F(1, 91) = 0.19 p = 0.89	F(1, 91) = 0.09 p = 0.75	F(1, 91) = 0.22 p = 0.63
Time	F(2, 91) = 62.57 **p < 0.001**	F(2, 91) =10.09 **p < 0.001**	F(2, 91) = 11.91 **p < 0.001**	F(2, 91) = 3.64 **p = 0.03**	F(2, 91) = 9.23 **p < 0.001**	F(2, 91) = 42.61 **p < 0.001**
Species : Watering	F(3, 91) = 0.55 p = 0.64	F(3, 91) = 2.91 **p = 0.03**	F(3, 91) = 0.68 p = 0.56	F(3, 91) = 0.09 p = 0.96	F(3, 91) = 0.35 p = 0.78	F(3, 91) = 1.36 p = 0.25
Species : Time	F(6, 91) =0.72 p = 0.63	F(6, 91) = 5.58 **p < 0.001**	F(6, 91) = 0.3 p = 0.93	F(6, 91) = 0.98 p = 0.43	F(6, 91) = 1.67 p = 0.13	F(6, 91) = 1.92 p = 0.08
Watering : Time	F(2, 91) = 25.33 **p < 0.001**	F(2, 91) = 27.25 **p < 0.001**	F(2, 91) = 0.09 p = 0.9	F(2, 91) = 1.73 p = 0.18	F(2, 91) = 1.93 p = 0.15	F(2, 91) = 1.68 p = 0.19
Species : Watering : Time	F(6, 91) = 0.49 p = 0.81	F(6, 91) = 0.33 p = 0.91	F(6, 91) = 0.14 p = 0.98	F(6, 91) = 1.03 p = 0.4	F(6, 91) = 0.59 p = 0.73	F(6, 91) = 2.71 **p = 0.01**

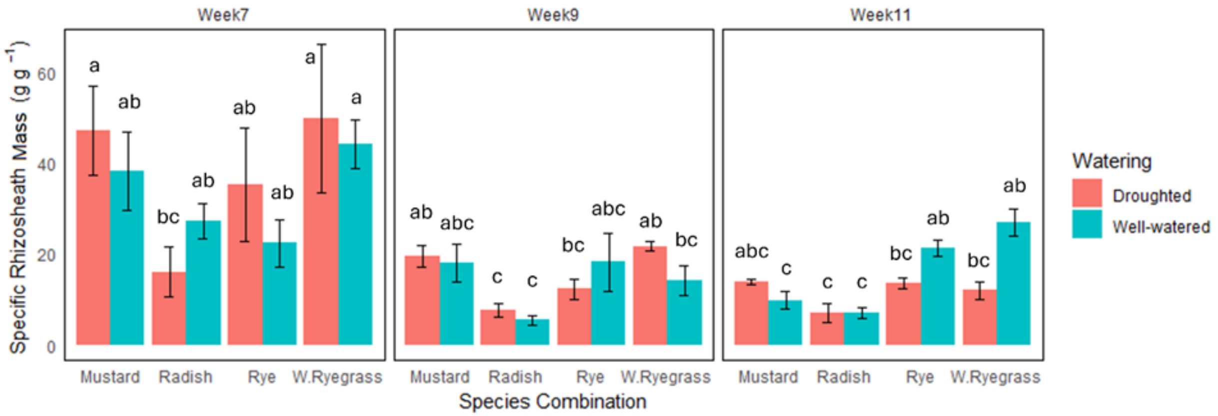

Figure 7.10 Specific rhizosheath mass (rhizosheath dry mass per unit of root dry mass) across species, watering regimes and time points (n = 5 per boxplot except n = 4 for well-watered radish in week 9, and well-watered W. ryegrass in week 9 and 11). Treatments are significantly different ($p < 0.05$) from each other if they do not share a letter.

Table 7.2 Mixed species mesocosm means and standard error of the mean in brackets of soil moisture, stomatal conductance, aboveground dry mass, root dry mass, rhizosheath dry mas and specific rhizosheath mass across all species, diversity and watering regime treatments and time points. Factorial repeated measures ANOVA F and p values are stated below with significant ($p < 0.05$) main and interaction effects marked in bold.

Time	*Species*	*Watering Regime*	*Species Combination*	*Soil Moisture (%)*	*Stomatal Conductance (mmol m^{-2} s^{-1})*	*Aboveground Dry Mass (g plant^{-1})*	*Root Dry Mass (g plant^{-1})*	*Rhizosheath Dry Mass (g plant^{-1})*	*Specific Rhizosheath Mass*
Week 4	Mustard	Well-watered	Single	23.50 (±1.48)	584.00 (±27.85)	0.53 (±0.12)	0.07 (±0.01)	1.00 (±0.36)	19.30 (±7.41)
			Mix	23.50 (±1.09)	681.00 (±137.09)	0.43 (±0.25)	0.05 (±0.02)	0.49 (±0.20)	16.80 (±6.46)
		Droughted	Single	11.70 (±1.48)	325.50 (±101.18)	0.47 (±0.12)	0.10 (±0.04)	2.79 (±1.33)	23.80 (±5.02)
			Mix	12.40 (±1.22)	257.00 (±60.44)	0.38 (±0.06)	0.05 (±0.01)	0.71 (±0.17)	15.00 (±4.55)
	Rye	Well-watered	Single	23.20 (±1.54)	317.40 (±65.03)	1.06 (±0.39)	0.30 (±0.07)	7.77 (±1.61)	28.90 (±3.60)
			Mix	23.50 (±1.09)	623.75 (±72.46)	1.75 (±0.56)	0.24 (±0.03)	8.73 (±1.91)	36.10 (±6.34)
		Droughted	Single	11.30 (±1.30)	315.00 (±66.30)	0.67 (±0.07)	0.24 (±0.03)	11.20 (±1.68)	47.10 (±7.01)
			Mix	12.40 (±1.22)	284.60 (±56.46)	0.74 (±0.14)	0.18 (±0.01)	8.10 (±2.32)	44.30 (±12.40)
Week 5	Mustard	Well-watered	Single	17.00 (±1.86)	576.00 (±73.11)	1.48 (±0.25)	0.19 (±0.03)	3.40 (±1.18)	16.40 (±4.63)
			Mix	18.60 (±1.45)	290.85 (±7.10)	0.72 (±0.22)	0.10 (±0.03)	1.31 (±0.67)	10.30 (±1.88)
		Droughted	Single	8.88 (±1.91)	49.56 (±13.76)	0.42 (±0.08)	0.10 (±0.02)	1.75 (±0.69)	13.90 (±4.10)
			Mix	9.26 (±0.77)	5.96 (±2.42)	0.35 (±0.08)	0.06 (±0.01)	0.62 (±0.29)	12.30 (±5.59)
	Rye	Well-watered	Single	21.20 (±1.67)	350.40 (±53.63)	1.38 (±0.19)	1.06 (±0.18)	17.90 (±3.58)	17.60 (±2.84)
			Mix	18.60 (±1.45)	582.00 (±43.52)	0.65 (±0.06)	0.68 (±0.27)	11.90 (±2.36)	23.70 (±7.06)
		Droughted	Single	10.70 (±0.62)	12.25 (±5.33)	0.69 (±0.14)	0.25 (±0.09)	6.61 (±2.36)	25.80 (±2.91)
			Mix	9.26 (±0.77)	57.94 (±13.02)	0.73 (±0.13)	0.24 (±0.06)	7.21 (±2.20)	30.60 (±8.12)
Week 6	Mustard	Well-watered	Single	21.90 (±2.01)	291.80 (±91.90)	1.94 (±0.37)	0.26 (±0.03)	2.07 (±0.56)	7.96 (±1.58)
			Mix	21.90 (±1.50)	208.90 (±41.89)	1.41 (±0.14)	0.22 (±0.04)	0.97 (±0.25)	4.40 (±1.14)
		Droughted	Single	9.82 (±1.08)	32.77 (±7.10)	0.69 (±0.15)	0.13 (±0.03)	0.87 (±0.19)	7.72 (±2.35)
			Mix	10.20 (±0.96)	15.39 (±2.07)	0.42 (±0.15)	0.08 (±0.03)	0.60 (±0.42)	4.36 (±2.02)
	Rye	Well-watered	Single	25.00 (±1.67)	452.30 (±102.49)	1.49 (±0.20)	0.59 (±0.11)	5.97 (±1.81)	9.88 (±1.69)
			Mix	21.90 (±1.50)	412.50 (±72.01)	2.22 (±0.23)	1.40 (±0.22)	14.60 (±1.92)	11.40 (±2.06)

		Droughted	Single	12.20 (±1.25)	30.40 (±7.61)	0.69 (±0.11)	0.20 (±0.03)	2.55 (±0.45)	13.10 (±2.35)
			Mix	10.20 (±0.96)	42.88 (±7.89)	1.16 (±0.31)	0.38 (±0.04)	7.05 (±2.94)	17.00 (±5.57)
Week 7	Mustard	Well-watered	Single	19.90 (±1.18)	348.40 (±51.69)	1.77 (±0.31)	0.26 (±0.04)	1.45 (±0.22)	5.80 (±0.75)
			Mix	23.80 (±2.48)	284.20 (±18.27)	2.34 (±1.07)	0.35 (±0.15)	0.73 (±0.21)	2.64 (±0.75)
		Droughted	Single	12.90 (±1.04)	171.00 (±8.20)	0.49 (±0.04)	0.09 (±0.01)	0.65 (±0.08)	7.03 (±0.63)
			Mix	11.40 (±0.90)	118.30 (±48.09)	0.35 (±0.09)	0.06 (±0.01)	0.45 (±0.11)	7.02 (±1.78)
	Rye	Well-watered	Single	26.40 (±3.16)	258.80 (±26.09)	1.72 (±0.11)	0.83 (±0.17)	7.36 (±1.36)	9.79 (±1.54)
			Mix	23.80 (±2.48)	346.20 (±65.70)	2.87 (±0.81)	1.09 (±0.28)	10.40 (±2.45)	10.10 (±0.99)
		Droughted	Single	12.40 (±1.26)	78.80 (±23.89)	0.81 (±0.14)	0.27 (±0.03)	2.43 (±0.50)	9.16 (±1.77)
			Mix	11.40 (±0.90)	116.60 (±18.27)	1.47 (±0.57)	0.32 (±0.03)	5.73 (±1.47)	16.80 (±2.51)
			Species *	F(2, 94) = 117.6 **p = 0.01**	F(1, 126) = 0.34 p = 0.55	F(1, 126) = 31.25 **p < 0.001**	F(1, 126) = 197.27 **p < 0.001**	F(1, 126) = 308.36 **p < 0.001**	F(1, 126) = 66.73 **p < 0.001**
			Diversity	NA	F(1, 126) = 0.07 p = 0.78	F(1, 126) = 1.34 p = 0.24	F(1, 126) = 3.28 p = 0.07	F(1, 126) = 0.38 p = 0.53	F(1, 126) = 3.26 p = 0.07
			Watering	F(1, 94) = 347.76 **p < 0.001**	F(1, 126) = 372.45 **p < 0.001**	F(1, 126) = 60.64 **p < 0.001**	F(1, 126) = 63.49 **p < 0.001**	F(1, 126) = 24.02 **p < 0.001**	F(1, 126) = 2.53 p = 0.11
			Time	F(3, 94) = 7.49 **p < 0.001**	F(3, 126) = 22.91 **p < 0.001**	F(3, 126) = 11.15 **p < 0.001**	F(3, 126) = 18.74 **p < 0.001**	F(3, 126) = 4.89 **p = 0.002**	F(3, 126) = 27.76 **p < 0.001**
			Species: Diversity	NA	F(1, 126) = 22.91 **p < 0.001**	F(1, 126) = 13.31 **p < 0.001**	F(1, 126) =9.47 **p = 0.002**	F(1, 126) =14.04 **p < 0.001**	F(1, 126) = 10.49 **p = 0.001**
			Species: Watering	F(2, 94) = 1.71 p = 0.18	F(1, 126) = 0.02 p = 0.86	F(1, 126) = 2.77 p = 0.09	F(1, 126) = 2.08 p = 0.15	F(1, 126) = 8.66 **p = 0.003**	F(1, 126) = 1.15 p = 0.28
			Diversity: Watering	NA	F(1, 126) = 1.25 p = 0.26	F(1, 126) = 0.02 p = 0.87	F(1, 126) = 0.22 p = 0.63	F(1, 126) = 0.002 p = 0.96	F(1, 126) = 0.27 p = 0.84
			Species:Time	F(6, 94) = 0.79 p = 0.57	F(3, 126) = 4.13 **p = 0.007**	F(3, 126) = 2.98 **p = 0.03**	F(3, 126) = 0.35 p = 0.78	F(3, 126) = 1.31 p = 0.27	F(3, 126) = 0.14 p = 0.93
			Diversity: Time	NA	F(3, 126) = 0.98 p = 0.4	F(3, 126) = 1.33 p = 0.26	F(3, 126) = 2.33 p = 0.07	F(3, 126) = 4.27 **p = 0.006**	F(3, 126) = 0.27 p = 0.84

Watering: Time	F(3, 94) = 1.08 p = 0.35	F(3, 126) = 18.94 **p < 0.001**	F(3, 126) = 4.31 **p = 0.006**	F(3, 126) = 8.93 **p < 0.001**	F(3, 126) = 6.86 **p < 0.001**	F(3, 126) = 0.94 p = 0.42
Species: Diversity: Watering	NA	F(1, 126) = 0.64 p = 0.42	F(1, 126) = 0.08 p = 0.76	F(1, 126) = 0.58 p = 0.44	F(1, 126) = 0.01 p = 0.9	F(1, 126) = 0 p = 0.99
Species: Diversity: Time	NA	F(3, 126) = 3.34 **p = 0.02**	F(3, 126) = 1.08 p = 0.35	F(3, 126) = 1.34 p = 0.26	F(3, 126) = 2.25 p = 0.08	F(3, 126) = 0.27 p = 0.84
Species: Watering: Time	F(6, 94) = 0.7 p = 0.64	F(3, 126) = 2.72 **p = 0.04**	F(3, 126) = 2.71 **p = 0.04**	F(3, 126) = 0.91 p = 0.43	F(3, 126) = 0.61 p = 0.6	F(3, 126) = 1.4 p = 0.24
Diversity: Watering: Time	NA	F(3, 126) = 2.08 p = 0.1	F(3, 126) = 1.32 p = 0.26	F(3, 126) = 0.95 p = 0.41	F(3, 126) = 1.55 p = 0.2	F(3, 126) = 1.27 p = 0.28
Species: Diversity: Watering: Time	NA	F(3, 126) = 0.71 p = 0.54	F(3, 126) = 1.03 p = 0.38	F(3, 126) = 0.17 p = 0.91	F(3, 126) = 0.53 p = 0.66	F(3, 126) = 0.06 p = 0.97

***** ‘Species’ refers to mustard and rye for all variables except for soil moisture, where it refers to mixed, single mustard and single rye treatments.

8. Appendix 2: Chapter 3 – Supplementary material

Table 8.1 Average stem density (per m^2) and standard error of the mean of all treatments (n = 6). Values not sharing a letter are significantly different from each other ($p < 0.05$).

	Average stem density (per m^2)
Grass (G)	313 (± 25) ^{a}
Brassica (B)	278 (± 8) ^{ab}
Legume (L)	36 (± 2) ^{f}
GB-Grass	202 (± 12) ^{cd}
GB-Brassica	144 (± 13) ^{de}
GL-Grass	223 (± 19) ^{bc}
GL-Legume	15 (± 2) ^{f}
BL-Brassica	181 (± 21) ^{cde}
BL-Legume	24 (± 4) ^{f}
GBL-Grass	157 (±15) ^{cde}
GBL-Brassica	130 (± 7) ^{e}
GBL-Legume	12 (± 2) ^{f}

Table 8.2 Linear regression F-values, p-values and r^2 of the relation between rhizosheath dry mass and root dry mass of each treatment (all treatments n = 6 except G and BL n = 5) with significant ($p < 0.05$) effects marked in bold.

Treatment	***F(df1, df2)***	***p-value***	***R²***
Grass (G)	F(1, 3) = 5.88	0.094	0.55
Brassica (B)	F(1, 4) = 492.9	**< 0.001**	0.99
Legume (L)	F(1, 4) = 1.53	0.284	0.10
GB	F(1, 4) = 73.43	**0.001**	0.94
GL	F(1, 4) = 1.87	0.243	0.15
BL	F(1, 3) = 2.98	0.183	0.33
GBL	F(1, 4) = 6.99	0.057	0.55

Table 8.32 Linear regression F-values, p-values and r^2 of the relation between soil moisture (%) and all other continuous variables and, below the thicker black line, factorial repeated measures ANOVA F and p

values of the significant ($p < 0.05$) main and interactive effects in soil moisture between all categorical variables.

Soil moisture (%) vs	***F(df1, df2)***	***p-value***	***R²***
Time until runoff generation (s)	F (1, 38) = 3.43	0.07	0.06
Total sediment (g)	F (1, 38) = 0.25	0.61	0.02
Mean sediment concentration (g L^{-1})	F (1, 38) = 0.02	0.87	0.02
Total runoff (mm)	F (1, 38) = 0.09	0.75	0.02
Rhizosheath dry mass (g)	F (1, 38) = 0.43	0.51	0.01
Root dry mass (g)	F (1, 38) = 0.77	0.38	0.01
Mown	F (1, 38) = 0.09	0.91	-
Diversity	F (2, 38) = 0.40	0.69	-
Species	F (4, 38) = 0.15	0.94	-
Block	F (2, 38) = 1.75	0.31	-
Mown:Diversity	F (2, 38) = 1.21	0.41	-
Mown:Species	F (4, 38) = 1.16	0.46	-
Mown:Block	F (2, 38) = 1.49	0.38	-
Diversity:Block	F (4, 38) = 0.23	0.9	-
Species:Block	F (8, 38) = 1.11	0.51	-
Mown:Diversity:Block	F (4, 38) = 0.53	0.72	-
Mown:Species:Block	F (5, 38) = 0.44	0.81	-

9. Appendix 3: Chapter 4 – Supplementary material

Table 9.1. Average root dry mass (g m^{-2}) and standard error of the mean per species across treatments and the three time points (n = 6). Within time point (columns), values not sharing a letter are significantly different from each other ($p < 0.05$). No significant differences were found within treatment across time (rows).

	T1	***T2***	***T3***
Grass (G)	10.7 (± 1.76) ^{a}	8.32 (± 1.58) ^{ab}	11.6 (± 0.8) ^{a}
Brassica (B)	4.38 (± 1.10) ^{ab}	4.6 (± 1.39) ^{bc}	7.9 (± 3.42) ^{ab}
Legume (L)	9.99 (± 2.21) ^{a}	8.38 (± 0.92) ^{ab}	9.45 (± 2.12) ^{ab}
BL - B	2.27 (± 0.53) ^{b}	2.79 (± 0.74) ^{bc}	11 (± 4.84) ^{bc}
BL - L	5.79 (± 0.91) ^{ab}	5.27 (± 0.77) ^{bc}	5.55 (± 0.86) ^{bc}
GB - G	11.3 (± 4.76) ^{a}	18.2 (± 5.83) ^{a}	13.1 (± 2.79) ^{a}
GB - B	1.58 (± 0.22) ^{b}	1.97 (± 0.42) ^{c}	1.8 (± 0.43) ^{c}
GL - G	7.7 (± 1.36) ^{ab}	8.25 (± 0.63) ^{ab}	19.4 (± 4.54) ^{a}
GL - L	4.13 (± 0.68) ^{ab}	4.59 (± 0.91) ^{bc}	4.89 (± 0.92) ^{bc}
GBL – G	11 (± 1.76) ^{a}	16 (± 2.95) ^{a}	19.4 (± 4.42) ^{a}
GBL - B	1.61 (± 0.56) ^{b}	2.12 (± 0.68) ^{c}	1.7 (± 0.41) ^{c}
GBL - L	2.76 (± 0.53) ^{b}	3.2 (± 0.89) ^{bc}	2.92 (± 0.73) ^{bc}