Exploring synergistic interactions between arbuscular mycorrhizal fungi and Silwet adjuvants to combat drought stress in crops.

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Abstract

Drought is a major jeopardy to global food security. Soil adjuvants such as organosilicone surfactants are a possible tool to increase crop drought tolerance. In this study, the effect of the interaction between organosilicone surfactants and arbuscular mycorrhizal fungi (AMF) on crop drought tolerance was investigated. An *in vitro* ecotoxicology study revealed that exposure to organosilicone surfactants did not affect germination of AMF spores but reduce length of hyphae produced by spores in the asymbiosis growth phase. The organosilicone surfactants significantly improved water retention of hydrophobic soils and impacted nutrient accumulation and distribution in both *Zea mays* L. and *Vicia faba* L.. There was an antagonistic relationship between phosphorus and calcium uptake and silicon uptake in *Z. mays*, while distribution of potassium and silicon was altered in the presence of the surfactant, favouring aboveground accumulation. Nitrogen accumulation in *Z. mays* facilitated by AMF was inhibited by the surfactants. Increased concentration of the osmolyte proline in *V. faba* indicated heightened drought stress in the presence of the surfactant. Both soil water retention benefits and ecotoxicological impacts of the surfactant were greater when the surfactant was present at a low concentration compared to the higher concentrations tested. These findings suggest that while organosilicone surfactants enhance soil water retention, their application alongside AMF biofertilizer may adversely affect arbuscular mycorrhizal symbiosis, plant nutrient uptake, and stress response mechanisms, potentially exacerbating drought stress by compromising the symbiotic relationship between plants and AMF and impeding their ability to efficiently cope with water scarcity and environmental stressors. These results demonstrate the importance of careful consideration when utilizing organosilicone surfactants in agricultural systems, highlighting the intricate balance between soil water management, symbiotic relationships, and nutrient dynamics crucial for sustainable crop resilience in the face of escalating climate challenges.

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Ammonium	NH_4^+
Ammonium molybdate tetrahydrate	$(NH_4)6Mo7O_{24}.4H_2O$
Arbuscular mycorrhizal fungi	AMF
Arbuscular mycorrhizal	AM
Boric acid	H_3BO_3
Calcium nitrate tetrahydrate	$Ca(NO_3)24H_2O$
Carbon dioxide	CO_2
Carbon-to-nitrogen ratio	C:N
Carbon-to-phosphorus ratio	C:P
Centimetres	cm
Circa	ca.
Copper sulfate pentahydrate	$CuSO_4.5H_2O$
Days	d
Days post sowing	DPS
Degrees celcius	^oC
Deionised water	dH_2O
Double deionised water	ddH_2O
Dry weight	DW
Ferric sodium EDTA	NaFeEDTA
Fresh weight	FW
Generalised linear model	GLM
G-force	*g*

Grams	g
Hour	h
Hyphal length density	HLD
Inductively coupled plasma optical emission spectroscopy	ICP-OES
Intergovernmental Panel on Climate Change	IPCC
Iron	Fe
Kilogram	kg
Litre	l
Magnesium sulfate heptahydrate	$MgSO_4.7H_2O$
Magnesium sulfate heptahydrate	$MnSO_4.4H_2O$
Methane	CH_4
Microgram	µg
Micrometre	µm
Micromole	µmol
Millilitres	ml
Millimetre	mm
Modified Strullu and Romand media	MSR media
Molar	M
Monopotassium phosphate	KH_2PO_4
Most probable number	MPN
Nitrate	NO_3^-
Nitrous oxide	N_2O
Number	n
Percent	%
Polyvinyl chloride	PVC

Potassium chloride	KCl
Potassium nitrate	KNO_3
Potassium sulphate	K_2SO_4
Pre-penetration apparatus	PPA
Percentage root length colonisation	% RLC
Second	s
Silwet™ 408 super-spreader	S408
Silwet™ Power superwetter	SP
Sodium molybdate dihydrate	$Na_2MoO_4.2H_2O$
Soil moisture content	SMC
Soil water holding capacity	SWHC
Standard error	SE
Surfactant-based adjuvants	SBA
Volume in volume	v/v
Watts	W
Weight in volume	w/v
Weight in weight	w/w
Zinc Sulphate Heptahydrate	$ZnSO_4.7H_2O$

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Declaration

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

Micronutrient quantification of digested plant samples by ICP-OES was carried out by Blaine Hancock, Environment Department, University of York. Carbon and nitrogen quantification of plant samples by elemental analysis was carried out by Dr Matt Pickering, Environment Department, University of York.

1 General introduction

1.1 The impacts of global climate change on crop cultivation and agricultural practices

Climate change has emerged as one of the most significant global challenges of the present era, posing threats to ecosystems, economies, and human well-being (Friel *et al*., 2014; IPCC, 2022). Climate change is a global phenomenon caused by an abnormal increase in atmospheric greenhouse gases such as carbon dioxide (CO_2), nitrous oxide (N_2O) and methane (CH_4). Greenhouse gases absorb the sun's heat and prevent it from leaving the atmosphere, thus artificially increasing average global temperatures (Manabe & Wetherald, 1967; Davis *et al*., 2010; Meehl & Tebaldi, 2004; Yang *et al*., 2022). These gases are released as a result of anthropogenic activities such as burning fossil fuels and high intensity rearing of ruminant animals (Cassia *et al*., 2018; Lynas *et al*., 2021).

The effects of climate change manifest in varying magnitude as alterations in temperature, precipitation patterns, extreme weather events or a combination of these factors depending on local geography, latitude, and ecosystems (IPCC, 2014; Trenberth *et al.,* 2014; Barker *et al.,* 2015; Rubel & Kottek, 2010). High temperatures and reduced rainfall can lead to drought, extended periods of uncharacteristically low precipitation or moisture levels in a specific region resulting in prolonged dryness (Tate & Gustard, 2000). The manifestations of drought cause severe impacts on agriculture, ecosystems, and various socio-economic aspects of a community or region (Dumenu & Obeng, 2016; Vicente-Serrano *et al*., 2010). Furthermore, certain outcomes of drought, such as biodiversity loss leading to species extinctions and, in some cases, loss of entire ecosystems, are irreversible (Avila-Flores *et* al., 2012; Harrison, 2000; Kéfi *et al*., 2007). Considering the frequency and severity of droughts are predicted to increase as global temperatures rise (Spinoni *et al.,* 2018), finding ways to successfully adapt to drought is vital for the continuation of human life.

A major effect of drought is a reduction in soil moisture content (Otkin *et al*., 2018), with the rate depending on drought severity, regional climate, soil composition and depth, and vegetation density (Tang & Piechota, 2009; Du *et al*., 2024). Reduced soil

moisture content can significantly affect the soil biota including the soil microbiome population. This, in turn, may impact the soil functions that they contribute to, including soil carbon (C) cycling and storage (Evans & Wallenstein, 2014; Munjonji *et al*., 2020). Also, reduced soil organic matter decomposition by soil microbes decreases soil respiration and C and nitrogen (N) mineralisation, reducing nutrient availability (Toberman *et al*., 2008; Plaza-Bonilla *et al*., 2015). A reduction in the soil volumetric water flux also impedes the flow of nutrients through the soil matrix (Butcher *et al*., 2020). Therefore, reduced soil moisture content results in degraded soils which have reduced fertility and structure, diminishing their potential as a growth substrate for plants and their associated microbes.

Low soil moisture content impacts plant growth from the initial germination stage throughout plant development by altering photosynthesis, respiration, and molecular pathways involved in plant stress responses; these alterations to plant growth ultimately decrease crop quality and yield (Hatfield & Prueger, 2015; Barnabás *et al.,* 2008; Verma *et al*., 2016). Many plant-stress responses are species-specific, but common ones include partial or complete stomatal closure to prevent water loss during transpiration, limiting the entry of CO_2 and reducing photosynthesis hence carbon fixation (Flexas & Medrano, 2002; Van Ha *et al*., 2014). Thus, plants affected by drought stress typically have reduced biomass (Chaves, 2002; Lloret *et al*., 2017). Longer and more frequent drought periods are intensifying crop yield reductions (Lobell *et al*., 2011; Prodhan *et al*., 2022). Overall, soil degradation caused by drought reduces food security, particularly when combined with other pressures associated with a growing human population such as rising food demand and increasing competition for land and water (van Dijk *et al*., 2021; Hertel, 2011).

Over time, reduced soil moisture content leads to land degradation, especially when combined with other stresses, such as wind erosion, overgrazing by livestock and tilling practices (Cook *et al.,* 2009; Lal, 2003). Extensive tilling breaks down the soil structure, reducing soil pore space (Panday & Nkongolo, 2021) and organic matter content (Jakab *et al*., 2023). This contributes to soil compaction (Voltr *et al*., 2021) and reduced soil fertility (Lv *et al*., 2023), creating anaerobic conditions that are unfavourable for many soil microbes. Mycorrhizal networks are also damaged through disruption to fungal hyphae (Tatewaki *et al*., 2023). Thus, the microbial community is limited in its capacity to maintain soil quality, so soil degradation is

perpetuated (Sun *et al*., 2018). Degraded soils have reduced soil water holding capacity (SWHC; the maximum amount of water that soil can retain after excess water has drained away), meaning that even after normal precipitation levels are resumed, soil water content remains low relative to its original state (Zhang *et al*., 2020a; Groh *et al*., 2020). Significant periods without tilling after the return of normal precipitation are necessary to enable the revival of soil biota and nutrient cycling that recover degraded soils (Deng *et al*., 2023). However, constant demand for arable land use means that breaks in tilling practices are often not possible (Kopittke *et al*., 2019). As a result, agricultural soils remain degraded following drought periods (Görlach *et al*., 2004).

Current conventional agricultural systems use intensive synthetic inputs (agrochemicals) in large scale monoculture crops and manipulate soil physical properties through rigorous tilling. This maximises yields and economic outputs (both factors of which are vital for meeting food demands and supporting economies) (Sumberg & Giller, 2022). When synthetic agrochemicals enter the environment (through application to plants or via disposal routes), they can have unintended effects on non-target organisms, including humans, either through accumulative or direct toxicity from the chemical or its degradation products (Scholtz & Bidleman, 2007; Kim *et al*., 2017). Nonetheless, conventional agricultural practices have developed over time as the cheapest strategy for coping with biotic and abiotic stresses in crop cultivation. Until recent decades, the use of synthetic pesticides and fertilisers has been successful in producing the highest yields possible (Tilman *et al*., 2011). However, the cumulative effects of agrochemical inputs, tilling practices and instability in environmental conditions caused by climate change has reduced the efficacy of conventional agricultural practices. In fact, global crop yields are decreasing despite growing food demands (IPCC, 2014). Transformation of conventional agricultural practices to be more sustainable is now necessary to adapt to the rapid environmental changes caused by climate change and produce enough food for the global population.

Sustainable agriculture is an alternative approach to farming and food production that seeks to meet current food demands using practices that are environmentally sound, socially responsible, and economically viable (Springmann *et al*., 2018). Practices following these principles maintain equal inputs and outputs (Janker *et al*.,

2018). Adopting sustainable agricultural practices could enhance drought resilience by promoting soil quality and biodiversity through reduced agrochemical usage (Foley *et al*., 2011; Kassie *et al*., 2013; Teklewold *et al*., 2013). However, the transition from conventional to sustainable agriculture may not currently be a viable option for many farmers due to economic or systemic constraints. These constraints may include factors such as institutional policies, market structures, infrastructure limitations, or socio-economic dynamics that impede the adoption of sustainable agriculture methods (Siebrecht, 2020). Therefore, there is a need for the development of technologies that are both economical and can be supplemented into current conventional agricultural practices to enable transition to sustainable agriculture (Rockström *et al*., 2016).

1.2 Arbuscular mycorrhizal fungi and their symbiotic associations

Arbuscular mycorrhizal fungi (AMF) form mutualistic symbiotic associations with the roots of approximately 80% of land plants, in which photosynthetic carbohydrates and lipids are exchanged for enhanced water and nutrient uptake (Smith & Read, 2010a; Salmeron-Santiago *et al*., 2023; Keymer *et al*., 2017). These beneficial fungi belong to the phylum Glomeromycota and have a unique anatomical structure that develops within the cortical cells of roots (Corradi & Bonfante, 2012). There are various stages to an established AMF- plant association, which begins prior to physical contact between fungi and root (Tian *et al*., 2019). When a host plant becomes nutrient deficient, it releases root secretions, chemical compounds such as strigolactones, into the soil (Besserer *et al*., 2006). Strigolactones function as signalling molecules in the rhizosphere, triggering genes involved in AMF spore germination and hyphal branching to become activated (Akiyama *et al*., 2005). In response, AMF release various chemical signals known as mycorrhizal factors, including lipochitooligosaccarides, which trigger activation of genes in the common symbiosis signalling pathway – a signalling pathway conserved in all plants capable of forming mycorrhizal relationships (Cope *et al*., 2019). This causes calcium (Ca) ion channels to open, triggering Ca spikes in the cytosol of root epidermal cells (Navazio *et al*., 2007). Downstream effects include the activation of genes that are

involved in structurally preparing the plant for entry of hyphae into the cell (Kosuta *et al*., 2003). Specifically, the nucleus of the root cell anticipating fungal entry migrates towards the anticipated entry point (Parniske, 2008). A pre-penetration apparatus (PPA), a bridge across the cell vacuole formed by clustering of endoplasmic reticulum and cytoskeleton, is then formed in the plant cell, which enables the plant to pre-determine the path in which fungal hyphae enter and colonise the root cell (Genre *et al*., 2008). Upon contact with root epidermal cells, the fungus produces a hyphopodium, a specific hyphal branch comprising of 1-2 lobed cells (Bruijn, 2020). This specialised structure allows the fungus to attach and move through the root epidermis and enter the root cortex via the PPA (Genre *et al*., 2005). Within the cortex, intraradical hyphae are produced and colonise along the root, terminating in cortical cells with the formation of highly branched, tree-like arbuscules (Floss *et al*., 2013). The establishment of arbuscules in the root cortex provides a large surface area for nutrient and water exchange between plant and fungus. Following establishment in the root cells, fine filamentous extraradical hyphae develop and extend into the soil, increasing the size of the depletion zone that the plant has access to, thus improving water and nutrient access for the host plant (Gutjahr & Paszkowski, 2013). When the AMF receive energy resources from the host plant, some species can produce lipid storage organs called vesicles (Smith & Smith, 1997). An overview of the AMF structures which form within root cells and the benefits that the mycorrhizal relationship can provide for host plants are shown in Figure 1.1.

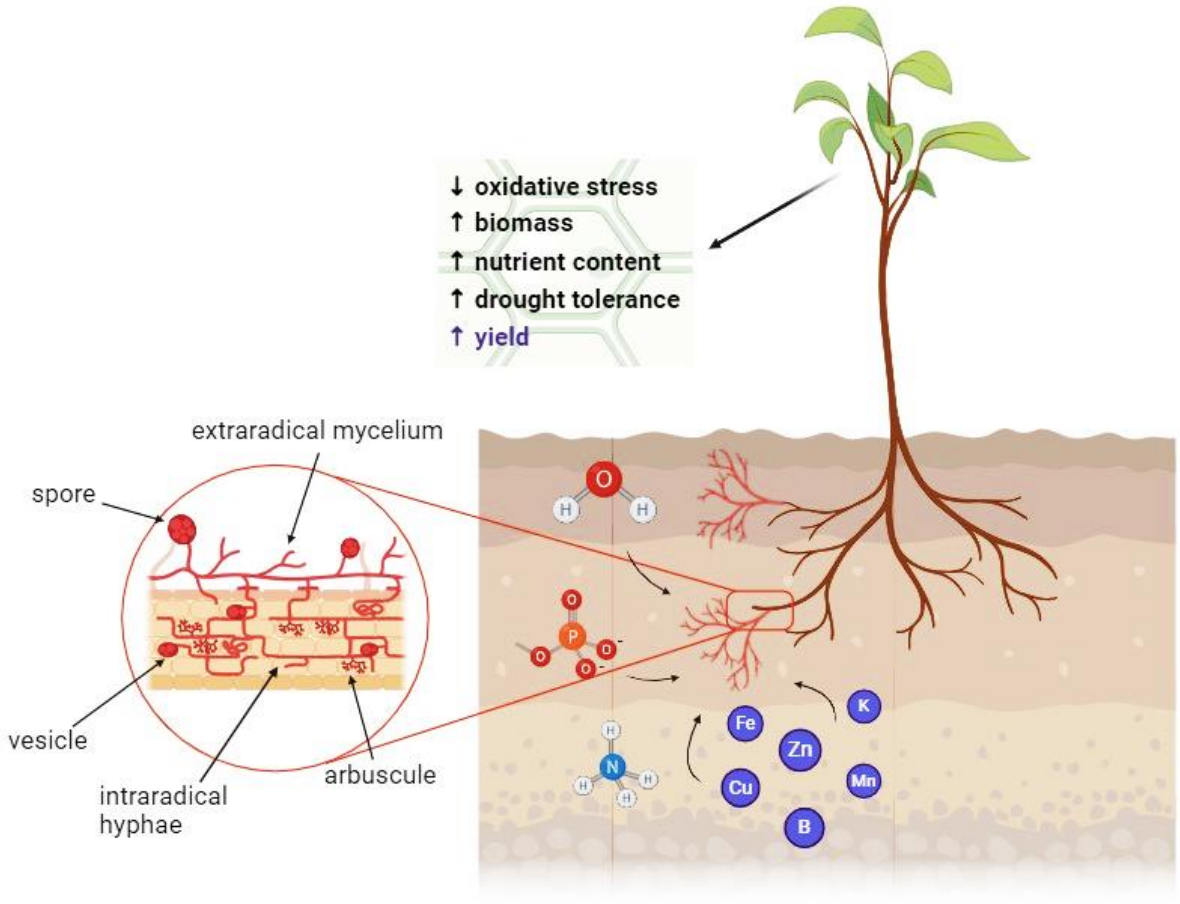

Figure 1.1. Arbuscular mycorrhizal fungi (AMF) have specialised anatomical structures which enable them to initiate and support a mutualistic relationship with a host plant. Through specific morphological changes in both the fungus and plant, AMF enter root cortical cells and produce arbuscules to enable water and nutrient exchange. Macro- and micro- nutrients acquired through the soil phase hyphal or mycelial network are passed between AMF and the host plant via the arbuscules. Some AMF species store C sources acquired from the host plant in vesicles, while plants use nutrients accessed by the AMF to improve photosynthetic rate and immune responses, reduce oxidative stress, increase biomass, ultimately increasing crop yields. Through this interaction, both organisms are able to survive symbiotically in stressful environments. Up arrows indicate an increase in biomass, nutrient content, drought tolerance and yield, and down arrows show a decrease in oxidative stress, as a result of AM symbiosis. Diagram created with BioRender.com.

An established mycorrhizal relationship can provide the host plant with a range of benefits. By virtue of their size, extraradical mycelia are able to access water in soil pores which are too small for host plant roots to access (Kakouridis *et al*., 2022). Moreover, the glycoprotein glomalin, present on the surface of AMF extraradical

hyphae, binds soil particles together to increase soil aggregation, thus frequency of soil pores (Syamsiyah *et al*., 2018). These soil structural changes improve soil water retention and hydraulic conductivity, thus water (Pauwels *et al*., 2023) and water-soluble nutrient availability (Clark & Zeto, 2000) for the host plant. Organic acids released by AMF and roots into the soil interact with insoluble forms of phosphorus (P) such as such as calcium phosphate or iron phosphate (Andrino *et al*., 2021). There is some evidence that these interactions result in the solubilization of bound P, converting it into forms that are more readily available for plant uptake (Tawaraya *et al*., 2006). Improved uptake of essential nutrients can improve photosynthetic rate, leading to significant increases in host plant height and biomass (Shen *et al*., 2022). Mobilisation of metal ions such as zinc (Zn), boron (B) and iron (Fe) are also facilitated by the release of organic acids and phenols (Haselwandter *et al*., 2020). Secondary nutrients such as these are used in various structural, signalling and synthesis pathways throughout the plant system, hence improving uptake of these nutrients has significant positive impacts on plant growth and productivity (Marschner, 1995; Ishfaq *et al*., 2022; Narayan *et al*., 2022). Increased micronutrient uptake aids overall host plant resistance to biotic stresses, namely, presence of pathogenic bacteria, fungi, viruses, and nematodes, and increases tolerance of abiotic stresses such as high heavy metal and salt content in soil, or low temperatures (Zhu *et al*., 2022). Stress responses are also regulated by AMF through modulation of relevant molecular pathways (Kaur & Suseela, 2020), such as those controlling biostimulants such as polyamine (Zhang *et al*., 2020b), and channel proteins such as aquaporins that enable water to pass through cell membranes (Krajinski *et al*., 2000). Furthermore, AMF regulate plant responses to different levels of precipitation, such as reducing stomatal conductance so that water loss is reduced and upregulating osmolyte production to reduce cell damage during drought periods (Doubková *et al*., 2013; Hashem *et al*., 2016). Therefore, colonisation with AMF can be greatly beneficial for the growth and productivity of host plants.

However, AM plants do not usually experience the benefits of the symbiotic relationship immediately after colonisation has occurred. In fact, AM plants can have decreased growth compared to non-AM plants during early establishment of AMF in host roots, as the host plant delivers resources to the AMF, but the external hyphal network has not yet been established (Ven *et al*., 2019). Moreover, if environmental

conditions limit photosynthetic capacity or plants are under stress, providing C to the fungus may become costly for the plant. This competition for C can lead to reduced plant growth and fitness (Wang *et al*., 2023). Nonetheless, fully established AM plants often out-perform non-AM plants, especially in low-moisture and nutrient-deficient soils (Marro *et al*., 2022). AMF can entirely take over the P acquisition pathway in a host plant, such that although there may be no observable difference in plant P between AM- and non-AM plants, AM plants are able to acquire P from sources inaccessible to non-AM plants. This increases survival of AM plants in soils with much lower available P concentrations compared to non-AM plants (Smith *et al*., 2003). Therefore, the relationship between AMF and their host plant can be an expensive, but ultimately beneficial, trade-off for plant resilience to fluctuations in soil conditions.

AMF are important ecosystem engineers and key beneficial plant symbionts. As such, they can be used as biofertilizers by improving soil fertility and plant growth by enhancing the availability of nutrients to plants (Daniel *et al*., 2022). Evolution of biofertilizers such as AMF with plants makes them ideal for targeted crop enhancement (Redecker *et al*., 2000; Bahadur *et al*., 2019). However, by nature biofertilizers such as AMF are variable in delivering their intended benefits consistently, with different observed outcomes on crop productivity. This can be due to plant and AMF species combination, the quality and species of AMF inoculum (Deja-Sikora *et al*., 2023), the environmental context of the host plant (Zaller *et al*., 2011), or the presence of synthetic fertilisers (Kim *et al*., 2017). The need for an undisturbed soil environment to gain the maximum benefits of AMF for host plants means that using AMF in current conventional agricultural systems is not necessarily possible or effective (Lu *et al*., 2018). Also, significant periods of edaphic drought can reduce P transfer from AMF to host plants (Bitterlich *et al*., 2024). Therefore, there is a need to find ways of adapting conventional agriculture so biofertilizers such as AMF can be employed under the variable conditions caused by climate change.

1.3 Defining soil adjuvants: their properties, biochemistry, and applications

Adjuvants cover a spectrum of substances added to agrochemical formulations for foliar or soil spray applications, enhancing their efficacy. They may also be applied alone directly to the soil to optimise soil water dynamics. In this way, they are similar to soil conditioners that target soil structure and fertility. Adjuvants range from naturally derived products such as plant oils and extracts, to synthetically produced compounds such as surfactants and acidifiers (Lin *et al*., 2023; Baratella *et al*., 2016). Adjuvants can be defined by the following categories: wetter-spreaders, which reduce the contact angle of water on organic matter to reduce water surface tension and increase water distribution and infiltration; stickers, nonevaporating materials that bind with agrochemicals and maintain them on a target surface (usually plant leaves); humectants, similar to stickers, but vary in that they maintain the active chemical in a liquid form, making it more bioavailable to target tissues; and penetration agents or activators, which aid the active chemical in infiltrating target tissues (if this is via leaves, this can involve softening or dissolving cuticular waxes to allow entry or stomatal infiltration; Hazen, 2000). Via these mechanisms, adjuvants are utilised to improve efficiency of agricultural processes both above- and belowground.

Soil adjuvants are a group of compounds that can increase wetting, water infiltration and distribution in soils with a low SWHC by reducing water surface tension. They can also be applied to soilless growth substrates such as those composed of coir or rock-wool (Urrestarazu *et al*., 2008). This enables water and other liquid agrochemical formulations to absorb and spread evenly through the growth substrate (Karagunduz *et al.*, 2001). These properties enable soil adjuvants to improve soil moisture and nutrient availability (Lehrsch *et al*., 2011; Starr *et al*., 2005). Moreover, they reduce rainfall run-off, by binding water molecules to organic matter and other soil colloids. Hard-to-wet soils often contain organic compounds or mineral coatings on soil particles that repel water, making it difficult for water to penetrate and be absorbed into the soil (Bauters *et al*., 2000). Thus, application of soil adjuvants to hard-to-wet soils helps to overcome issues related to water repellency or hydrophobicity (Moore *et al*., 2010), reducing pressure on water resources

(Baumhardt & Blanco-Canqui, 2014). The ability of soil adjuvants to reduce drought effects and improve agrochemical efficacy (Palma-Bautista *et al*., 2020) means that lower concentrations of agrochemicals can be used to aid crop cultivation (Magor *et al*., 2023), reducing the environmental impacts of conventional agricultural practices while maintaining crop yields (Singh *et al*., 2020). Thus, soil adjuvants are a vital tool for improving sustainability of conventional agricultural practices. However, excessive application of some surfactant-based adjuvants (SBA) can cause phytotoxicity in crop plants (Knoche *et al*., 1992; Räsch *et al*., 2018) and impacts on the wider ecosystem, particularly when applied with pesticides (Wernecke *et al*., 2021). Considering diverse and abundant agroecosystems are vital for crop productivity and successful food production, the nature in which soil adjuvants are applied should be considered for long-term food security goals (Ruemmele & Amador, 1999; Moffett & Morton, 1975; Willett *et al*., 2019). Soil adjuvants that are more compatible with the surrounding ecosystem also provide opportunities to develop practices which can utilise biofertilizers such as AMF while maintaining more intensive growing methods to maintain high yields.

SBA, more commonly utilised as spreader-wetters, are composed of amphipathic molecules (Daneshnia *et al*., 2016; Figure 1.2A). The hydrophilic side chains form cohesive bonds with water molecules and reduce their surface tension, improving the spreading and wetting properties of water or agricultural formulations. Meanwhile, the hydrophobic backbone binds to soil colloids such as organic matter and clay (Raeisi *et al*., 2021; Figure 1.2B). This unique structure enables SBA to decrease the contact angle between organic matter and water molecules, therefore reducing the water surface tension and helping it to spread more evenly in soil and other growth substrates (Figure 1.2C; Arriaga *et al.,* 2009).

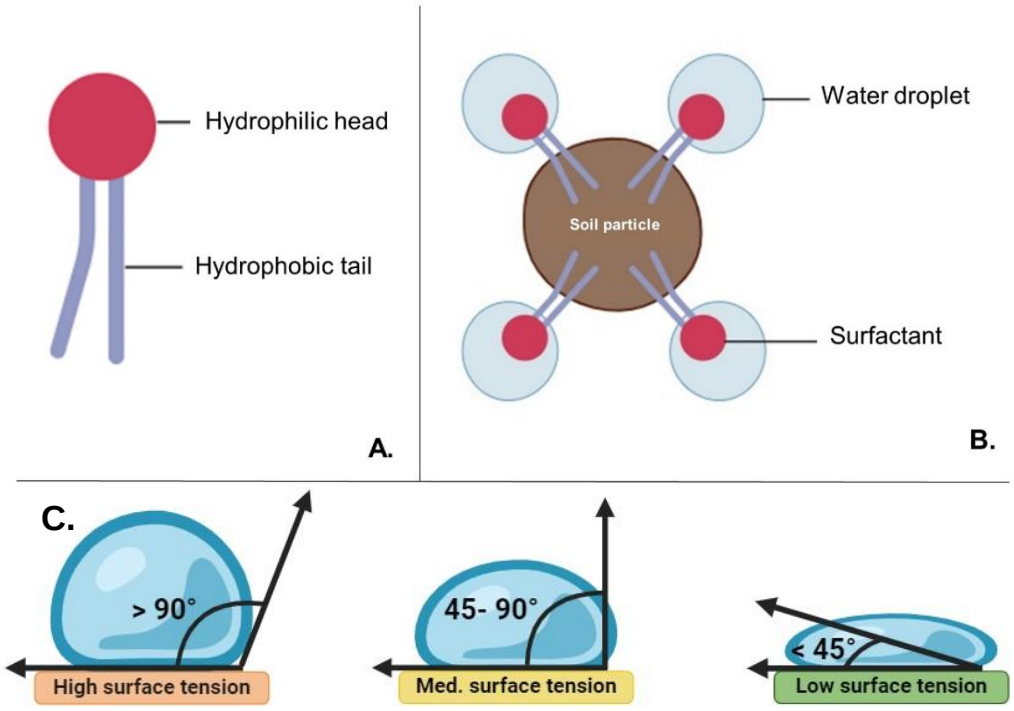

Figure 1.2. The unique chemical structure of surfactants enables them to improve water distribution and retention in soil. Figure 1.2A. Surfactants are amphipathic molecules composed of a hydrophobic head and hydrophilic tail. Figure 1.2B. This amphipathic structure allows the surfactant molecule to simultaneously bind soil particles and water molecules. Figure 1.2C. Thus, surfactants reduce the contact angle between water molecules and the soil particles, reducing surface tension. As a result, water can both spread and penetrate the soil surface more effectively. Adapted from Raeisi *et al*. (2021) and Akbari *et al*. (2018) and made using Biorender.com.

SBA can also help plants to maintain ionic balance, thus increasing yield output for crops grown in water deficit and high salinity conditions (Chaichi *et* al., 2017; Mohammad *et al*., 2016). This is due to differences in ionic charge between SBA types. Some SBA are non-ionic (uncharged), while others can be anionic (negatively charged) or cationic (positively charged) (Hazen, 2000). Compatibility with agrochemical formulations often depends on the overall charge of the surfactant molecule (Ishiguro & Koopal, 2016). The lack of charge of non-ionic surfactant molecules makes them less chemically active than their charged counterparts, thus less toxic for plants, soil microorganisms and biological products such as biofertilizers (Reinikainen & Herranen, 1997). SBA come in powder and liquid form

for specific purposes and agrichemical combinations – this can be as specific as the soil or crop type to which they are designed to be applied. Table S1 provides a summary of some SBA currently commercially available that are effective as soil adjuvants and conditioners, their proposed properties in soil and benefits for plant growth. While SBA encompass a range of common benefits for soil water retention and distribution, there are unique selling points for many of them that make them more suitable for different application contexts. It is important to consider, however, that these unique selling points are often based on tests carried out by the associated company and therefore may be an under- or overrepresentation of the product's capabilities.

Organosilicone surfactant-based adjuvants, such as Silwet™ Power superwetter (SP; see Figure S1 for TDS), are non-ionic, making them better suited for sustainable agriculture than more reactive ionic products. They are commonly used either as standalone soil conditioners or as adjuvants alongside fertilizers and/or pesticides. Their role is to enhance water absorption in soils by improving infiltration, drainage, retention, and hydrophilicity. By optimizing these soil properties, organosilicone surfactants can also facilitate the efficient delivery of active ingredients, like fertilizers or pesticides, when incorporated together into the soil. Organosilicone surfactants typically consist of a hydrophobic silicon (Si)-based backbone and hydrophilic side chains (Baratella & Trinchera, 2018). Some organosilicone surfactants have the potential to degrade in the environment dependent on chemical structure, but it is recommended to test specific structures of interest for degradability (Ying, 2006). This is an important consideration for the longevity of the effects of organosilicone surfactants, as rewetting events may reduce their efficacy over time (Song *et al*., 2014). Chemically, organosilicone surfactants such as SP contain a backbone of dimethyl silyl, and hydrophobic methyl silyl groups in a blocked or random order. Pendant polyalkylene oxide, polyethylene oxide, or a combination of polyethylene oxide and polypropylene oxide, form the hydrophilic part of the molecule and enable the formation of a comb-like structure (Mojsiewicz-Pieńkowska *et al*., 2016; Figure 1.3).

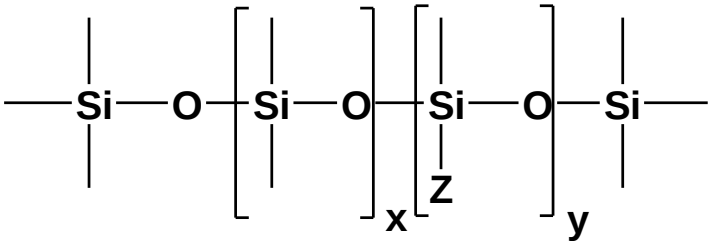

Figure 1.3. Organosilicone surfactants are polymers composed primarily of repeating units of silicon (Si), oxygen (O) and carbon (C), where Z is $CH_2CH_2CH_2O$-($CH_2CH(R)O$)-R^1 and R^1 is hydrogen (H), an alkyl group, or acetyl. The number of repeating units (represented by x and y above) and side chain composition significantly impacts organosilicone surfactant properties, making them highly adaptable tools for a range of different agricultural applications. Diagram provided by Dr George Policello (Momentive Performance, Tarrytown, NY, USA).

This combination of hydrophobic and hydrophilic groups makes silicon polyether copolymers surface active, enabling it to reduce the surface tension of aqueous solutions. Differences in side chain composition determine chemical properties of organosilicone surfactants, such as variation in the ability of organosilicone surfactants to affect water surface tension, and thus increase spreading ability (Svitova *et al*., 1996; Lee *et al*., 2009). Also, the length of non-polar carbon chain regions determines the physical property of the surfactant, i.e., whether it is liquid or solid (Czajka *et al*., 2015). Silwet™ S408 super-spreader (S408; Figure S2) is a trisiloxane alkoxylate based organosilicone surfactant (Figure 1.4). Trisiloxane alkoxylates are highly effective in reducing surface tension, which allows liquids to spread more uniformly across surfaces. Therefore, they are more often used as adjuvants in pesticide formulations to enhance the coverage and penetration of pesticides, herbicides, and fertilizers. The ability to alter and fine-tune the properties of organosilicone surfactants makes them useful tools for a range of different settings, such as water management, soil structure improvement, and enhancement of delivery of biological products to ultimately protect crops against drought stress (Baratella & Trinchera, 2018).

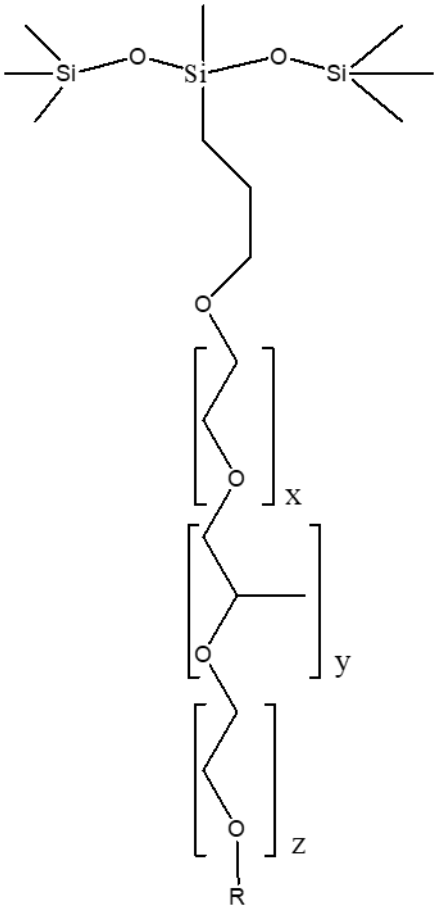

Figure 1.4. The general chemical structure of trisiloxane alkoxylates, a sub-group of organosilicone surfactants which are typically utilised as “super-spreaders” due to their capabilities in reducing surface tension, which allows liquids to spread more uniformly across surfaces, enabling the formation of thin, even films of liquids. Si is silicon, O is oxygen and R is hydrogen, an alkyl group, or acetyl. Diagram provided by Dr George Policello (Momentive Performance, Tarrytown, NY, USA).

The toxicity of organosilicone surfactants has been investigated, both for crop plants and their surrounding environment (Nobels *et al*., 2011; Falk *et al*., 1994). The Si−O−Si linkage in organosilicone surffactant molecules is hydrolysed in the presence of moisture, under acidic or basic conditions (i.e. outside the range of pH 6.5-7.5). Above or below this pH range trisiloxane alkoxylates are subject to rapid hydrolysis (Policello *et al*., 1995). SP is a degradable molecule, while S408 is readily biodegraded in the environment. Another benefit suggested among organosilicone surfactants is that they may enhance lateral root development, leading to improved establishment of plants (Baratella *et al*., 2016). Exploring the direct effects of organosilicone surfactants on the growth and development of specific crop species

could elucidate the mechanisms at play that lead to these phenotypes. Further, it could inform how these compounds might interact with root-associated microbes such as AMF and their host plants. There are currently no known studies investigating synergistic effects between organosilicone surfactants and soil microbes such as AMF. If there were evidence that certain organosilicone surfactant products are compatible with root-associated microbes, development of agricultural practices that includes the precision capabilities of organosilicone surfactants and the ecosystem synergy of AMF could be possible. The cumulative benefits of these soil additives could be valuable for protecting crops from droughts and other climate change-induced stresses. As products which have already accumulated field trial and laboratory-based evidence of their ameliorating effects on hydrophobic soils, SP and S408 are suitable candidates for further evaluation in this area. Their degradability and potential for crop-specific benefits are promising indicators that they could improve drought resilience and sustainability of agricultural practices.

1.4 Project Aims

Limited research has been conducted to investigate the combined use of AMF and soil adjuvants as tools for crop health (Chaichi *et al*., 2017). Furthermore, their combined application to improve crop drought tolerance and direct interactions between AMF and organosilicone surfactants have not yet been investigated. Addressing these gaps would provide valuable insights into the potential benefits or limitations of using adjuvants in conjunction with AMF for enhancing the growth and drought stress tolerance of important crop species. Therefore, this project aims to investigate the effects of different Silwet soil adjuvants on soil moisture content and explore the compatibility of organosilicone surfactants with AMF. Furthermore, it aims to explore whether Silwet Power can aid seed germination under drought conditions. Finally, crops cultivated with SP and AMF will be assessed to determine whether the two soil treatments produce synergistic effects for crop drought tolerance.

2 The effect of Silwet Power on germination of *Vicia faba and Zea mays* seeds and *Rhizophagus irregularis* spores

2.1 Introduction

Organosilicone surfactants are chemical tools that can alleviate drought stress in crops by retaining moisture in the root zone of the soil (Kostka *et al*., 2007; Martin *et al*., 2022). Enhancement of water retention at the root zone can increase seed germination and seedling emergence rate (McMillan *et al*., 2023). Organosilicone surfactants can be considered a sustainable solution to agricultural issues relating to water scarcity as they are inert chemicals which can break down in soil (Baratella & Trinchera, 2018). The compatibility of organosilicone surfactants with AMF biofertilizers has not been tested. If organosilicone surfactants prove to be compatible with AMF germination and symbiosis with host plants, then more sustainable agricultural systems could be developed which utilise both AMF and organosilicone surfactants to reduce reliance on synthetic agrochemical inputs while reducing crop drought stress. The following aims to give an overview of germination initiation in plant and AMF species, elucidate existing knowledge on the effects of organosilicone surfactants on germination of plants and AMF, and identify where further research is needed to delimit potential uses for organosilicone surfactants in germination processes.

2.1.1 Germination in plants and arbuscular mycorrhizal fungi

Germination is the process by which the first growth stages of a previously dormant seed or spore begin, triggered by favourable environmental conditions (Kozlowski & Pallardy, 1997; Giovannini *et al*., 2020). In plants, this is followed by postgerminative development of the enclosed embryo (the germ developing from a zygote [Ingensiep, 2004]) and endosperm (the storage organ which feeds the germ [Li, 2017]) tissues, leading to the emergence of cotyledons (one in monocots and two in dicots) from the seed case and subsequent growth of a seedling (Rajjou *et al*., 2012). Leaf cell

division takes place at the apical meristem (base and site of cell division), of which cells eventually form shoots and leaf structures (Undersander, 2019). Development of these structures can dictate drought tolerance strategies. For example, differences in the site of leaf cell expansion gives rise to a typically broader, rounded leaf shape in dicots as opposed to the long, straight leaves of monocots (Nelissen *et al*., 2016; Fournier *et al*., 2005; Perico *et al*., 2022). Also, under drought conditions, monocot leaves have a lower stomatal density, preventing moisture loss through evaporation (Robertson *et al*., 2023), while stomatal density increases in some dicot species in order to maintain photosynthetic processes (Lei *et al*., 2018).

For AMF, germination is more complex: environmental factors are thought to be responsible for the initiation of the asymbiotic phase of growth, in which spores can produce limited hyphae in the absence of a host plant (Giovannetti, 2000). However, a lack of host plant signalling molecules prevents asymbiotic spores from moving to the next phase of germination, namely presymbiosis, and as such the lifespan of asymbiotic spores is limited to a few weeks (Giovannetti *et al*., 2003). Development to the presymbiotic growth stage starts when signalling molecules known as strigolactones are secreted from host plant roots and detected by asymbiotic AMF, as described in section 1.2. *Rhizophagus irregularis* is a model AMF species whose spores are typically pale in colour and round or oval-shaped. They have an inner and outer wall layer (Rosas-Moreno *et al*., 2023; Kokkoris *et al*., 2023) but can also present dimorphically, in which spore colour and thickness of the second wall layer varies. *R. irregularis* spores produce aseptate hyphae that branch dichotomously (Lee, 2011). *R. irregularis* is commonly used as a biological product in agricultural practices due to its symbiosis with many crop species and enhancement of plant nutrient uptake, particularly P (Kokkoris *et al*., 2023; Manteghi *et al*., 2022; Wang *et al*., 2023).

Although resilient by nature, AMF spore germination can be reduced or halted altogether in the presence of various chemical soil additives such as mineral fertilizers and synthetic pesticides (Karpouzas *et al*., 2014). Heavy fertiliser inputs reduce AMF colonisation of crop plants as the higher P availability in soil reduces plant reliance on AMF colonisation for P acquisition (Ma *et al*., 2021). Also, artificially increased N availability in soil reduces the soil N-to-P ratio, altering AMF species composition in soil microbial communities (Johnson *et al*., 2003). The active

ingredients of some pesticides have been shown to delay germination in *R. irregularis* (Buysens *et al*., 2015), or reduce the elongation of hyphae during the pre-symbiotic phase (Zocco *et al*., 2008). AMF are also affected by tilling practices in conventional agriculture which damage AMF hyphal networks (Alguacil *et al*., 2008). As such, the use of AMF as biofertilizers in conventional agriculture is slow, with the most common use currently in organic growing contexts (Gosling *et al.* 2006). Considering this, agricultural practices which enable widespread use of AMF would require minimal artificial inputs or physical manipulation of the soil. Soil adjuvants permit agricultural practices which utilise less water and chemicals with active properties (such as pesticides) whilst achieving high outputs. Thus, they could introduce an economic and sustainable security against drought stress in key crop species.

2.1.2 Organosilicone surfactants: effects on plants and arbuscular mycorrhizal fungi

SP is a powder organosilicone surfactant (see Figure S3) with a copolymer structure, comprised of trisiloxane and polyalkyleneoxide polymers (pers. comms. Dr. Benjamin Langendorf, Momentive [London, UK]). The trisiloxane component is hydrophobic with a binding affinity for organic matter (Yılgör *et al*., 1993), while polyalkyleneoxide is water soluble (Bailey & Koleske, 1990). The powder formulation of SP makes it suitable for both liquid and dry broadcast applications. Other benefits of SP reported by Momentive include improving soil-air exchange capacity of treated soil and promoting feeder root development by reducing soil compaction.

Improvement of soil-air exchange capacity is reported to support a healthy soil microbiome (Howe & Smith, 2021) These soil enhancements can improve both crop yield and quality (Szatanik-Kloc *et al*., 2018). S408 is an organosilicone surfactant, also produced by Momentive. It is described as having similar benefits for water distribution and retention as SP, but is a liquid adjuvant, making it useful for different user applications such as in foliar sprays.

Considering their beneficial effect on water distribution and retention, both SP and S408 could be valuable for improving seed germination under drought conditions (Martinez-Ghersa *et al*., 1997; Lu *et al*., 2022). Preliminary tests carried out by Momentive showed that SP improves germination rate in *T. aestivum, Arachis hypogaea* and *Z. mays* (pers. comms. Dr. Benjamin Langendorf, Momentive [London, UK]). However, as with any chemical application to plants, organosilicone surfactants may also have detrimental impacts on plants during the vulnerable germination stage, such as increasing susceptibility to bacterial infections (Zidack *et al*., 1992), phytotoxicity (Volgas & Lopez, 2003) and preventing germination at high concentrations (Gálvez *et al*., 2018). Achieving the right balance is crucial for leveraging the benefits of organosilicone surfactants such as SP and S408 for seed germination under drought conditions, while also addressing their limitations. Therefore, studies which aim to define the appropriate contexts in which organosilicone surfactants can be used to benefit plant germination and cultivation are vital for the progression of sustainable agricultural practices.

Organosilicone surfactants can be used as an adjuvant in combination with biological products such as biofertilizers. However, the combined use of these adjuvants with AMF has not yet become common practice, so the effects of organosilicone surfactants on AMF spore germination have not yet been reported. The inert nature of organosilicone surfactants suggests they may have no effect on the germination potential of AMF. Furthermore, in the field, organosilicone surfactants can reduce the need for heavy pesticide and fertiliser use, which would benefit AMF colonisation in most crop plants. Yet certain surfactants can be toxic to microbes as they disrupt microbial cell membranes or interfere with cellular processes (Nobels *et al*., 2011). This can lead to reduced microbial activity or cell death (Farkas *et al*., 2017). The extent of toxicity varies significantly depending on factors such as microbial species, environmental conditions, and surfactant properties (Chen *et al*., 2018). Therefore, it is unclear how organosilicone surfactants could impact AMF spore germination and host plant colonisation. Should organosilicone surfactants and AMF be compatible, more sustainable conventional agricultural practices could be developed which utilise improved soil structure to aid seed germination and subsequent establishment of crop plants under drought conditions.

Considering the above possibilities, the following studies aim to evaluate the effect of SP on seed germination under drought conditions. *V. faba* and *Z. mays* are commonly cultivated crop species across the globe and, as such, are vulnerable to drought stress due to global climate change (Kibbou *et al*., 2022; Kim & Lee, 2023). They also represent the dicot and monocot genetic groups (respectively), thus, the effect of SP on germination of *Z. mays* and *V. faba* seeds will be tested. It is hypothesised that under low watering and relative humidity conditions, seeds cultivated in growth substrate treated with SP will have an increased germination and emergence count due to the improved water retention of the growth substrate. The improved water retention of the growth substrate will also lead to larger treated plants that have a higher water content than untreated plants. The direct effects of SP and S408 on the germination of *R. irregularis* spores will also be explored. Due to their inert nature, it is hypothesised that the organosilicone surfactants will not negatively impact AMF spore germination or growth of hyphae, irrespective of concentration or exposure duration.

2.2 Materials and methods

2.2.1 *In vitro* compatibility assessment of two Silwet adjuvants with arbuscular mycorrhizal fungi

The effect of exposure to two Momentive (New York, USA) Silwet™ adjuvants, SP (a powder adjuvant) and S408 (a liquid adjuvant) on germination of *Rhizophagus irregularis* (Błaszk., Wubet, Renker & Buscot) C. Walker & A. Schüßler spores was investigated in an *in vitro,* fully factorial assay. There were 18 treatment groups and 16 replicates for each treatment (Table 2.1).

Table 2.1. A summary of the 18 treatment groups that *Rhizophagus irregularis* spores were exposed to prior to incubation on Modified Strullu and Romand (MSR) medium for 27 d. The length of hyphae (cm) produced by the spores was measured every 3 d throughout the incubation period. Low, medium, and high concentrations of aqueous SP and S408 were as follows: SP – 0.1, 1, 10% w/v and S408 – 0.05, 0.5, 5% v/v. For each treatment, *n* = 16.

Treatment	Exposure time (h)	Adjuvant concentration
Silwet Power	0.25	Low
		Medium
		High
	3	Low
		Medium
		High
	24	Low
		Medium
		High
Silwet 408	0.25	Low
		Medium
		High
	3	Low
		Medium
		High
	24	Low
		Medium
		High
Control (ddH2O)	0.25	N/A
	3	
	24	

Sterility of the adjuvant solutions was tested prior to experimental use. Three bottles of Modified Strullu and Romand (MSR) medium, lacking vitamins and solidified with Bacto Agar (Le Pioufle & Declerck, 2018; Table S2), were prepared and autoclaved (one cycle, 121°C for 15 min). In a laminar flow hood, 10 g SP was added to one of the bottles containing MSR media, and 5 ml S408 to the other. The bottles were swirled until the adjuvants were completely combined in the medium. No adjuvant was added to the final bottle, which served as the control. Each bottle of media was decanted into five 10 cm^2 plastic Petri dishes (10 cm^2), respectively, and the dishes were transferred to a 27ºC incubator (Labnet, Edison, NJ, USA) and monitored daily for 27 d for signs of bacterial or fungal contamination. No contamination was present on any of the plates following 27 d incubation, thus there was no need to autoclave adjuvant solutions before use in the main bioassay.

Low, medium, and high concentrations of aqueous SP and S408 (Table 2.1) were prepared with ddH_2O by serial dilution. These concentrations were chosen based on the trisiloxane content of each adjuvant, where SP has half the concentration of trisiloxane compared to S408, such that double the concentration of SP was used compared to S408. Therefore, the concentrations for low, medium, and high adjuvant preparations are equivalent for each respective adjuvant. Adjuvant solutions were buffered to pH 7 with 1 M sodium phosphate dibasic to prevent adjuvant hydrolysis in solution.

Sterile cell culture multi-well plates (Costar™ 24-well TC-treated, Corning™, New York, USA) were prepared to contain 2 ml of the autoclaved (one cycle, 121°C for 15 min) MSR medium per well. In a laminar flow cabinet, intact *R. irregularis* spores (Premier Tech, Quebec, Canada) were selected using a dissecting microscope (Wild M3Z, Heerbrugg, Switzerland) and P1000 micropipette that were cleaned with 70 % ethanol before and between uses. Approximately 100 spores were transferred to 15 ml tubes containing 5 ml of each adjuvant treatment or the untreated control and stored at 4°C for either 0.25, 3 or 24 h to prevent germination from initiating prior to transfer to the MSR media. Spores were then rinsed in autoclaved (one cycle, 121°C for 15 min) ddH_2O and one spore was transferred to each well of the prepared cell culture multi-well plates in a randomised block design. Plates were incubated at 27°C in the dark for 27 d (method adapted from Buysens *et al*., 2015). Spore germination was monitored using a light microscope (Nikon Eclipse, Tokyo, Japan)

at 100x magnification every 3 d, where hyphal length was measured by gridline intersect method (Newman, 1966). Measurement of hyphae encompassed both primary and secondary hyphal structures. Total germ tube length (cm) was determined via the following equation adapted from Tennant (1975):

$$\text{Length} = \frac{11}{14} \text{ x N x g}$$

Where:

$\frac{11}{14}$ is a constant that represents the relationship between hyphal dispersal in a finite area and the repetitive use of a short line for intercept counts.

g is the area of the grid unit

N is the number of intersections where hyphae crossed the gridlines.

2.2.2 Impact of Silwet Power on seed germination in low humidity conditions

2.2.2.1 Growth substrate

A loamy sandy soil (Rolawn®, Yorkshire, UK) was sieved to 6 mm and dried at room temperature for a minimum of 7 days with fan assistance. Dried sharp horticultural sand (0-3 mm particle size; Keith Singletons, Cumbria, UK) was added to the soil in a 3:1 ratio to reduce SWHC. The resulting growth substrate was analysed by NRM Cawood (Berkshire, UK) for composition and nutrient content. The growth substrate had the following composition: sand (0.063-2.00 mm) 87 % w/w, silt (0.002-0.063 mm) 5 % w/w, clay (< 0.002 mm) 8 % w/w, organic matter 17.3% w/w with a pH of 8.3 as determined through analysis carried out by NRM (Berkshire, UK). Available P was determined by extraction with Olsen's reagent to be 49 µg g^{-1}, and available potassium (K) and magnesium (Mg) were determined by extraction with ammonium nitrate to be 942 µg g^{-1} and 172 µg g^{-1}, respectively. Available nitrate (NO_{3}^{-}) content was determined by extraction with potassium sulphate (K_2SO_4) to be 0.9 µg g^{-1}, while available ammonium (NH_{4}^{+}) content was determined by extraction with potassium

chloride (KCl) to be 3.6 µg g^{-1}. The SWHC, calculated from the saturation and desiccation weight of the substrate (dried at 105 °C for 7 d), was 0.414 ml g^{-1} dry weight (DW) growth substrate. The growth substrate was not sterilised to ensure soil structure and micronutrient composition remained representative of that found in field conditions. As such, background AMF inocula in the media were measured and reported in section 3.3.3.1.

2.2.2.2 Plant materials

Vicia faba L. cultivar Sutton Dwarf (Moles Seeds, Essex, UK) and *Zea mays* L. Rising Sun seeds (Moles Seeds, Essex, UK) were used in the following assay due to their common cultivation as food crops and their genetic differences which represent both monocotyledons (*Z. mays*) and dicotyledons (*V. faba*). The seeds were assessed for germination quality prior to experimental use. Plastic Petri dishes (2 x 9 cm diameter) were lined with filter paper soaked in ddH_2O. For each species, 10 seeds were transferred into a Petri dish sealed with standard electrical tape. Plates were incubated at room temperature in the dark and emergence of radicles was monitored daily for 7 d. After 7 d, percentage of germinated seeds was determined, and found to be 80% and 100% for *Z. mays* and *V. faba*, respectively.

2.2.2.3 Experimental design

The efficacy of SP in enhancing the germination of *V. faba* L and *Z. mays* in low relative humidity and low soil moisture content (SMC) conditions was evaluated through a controlled environment pot trial. It was a randomised block design with 5 seeds per pot and 20 replicate pots in total for each treatment group.

On the same day as sowing, three batches of growth substrate, as described in section 2.2.2.1, were prepared as follows: one batch was made up to 40% SMC (substrate A), a second was made up to 65% SMC (substrate B) and a third was made up to 65% SMC and then thoroughly combined with SP at a rate of 5 g kg^{-1},

ensuring even distribution of the adjuvant throughout the growth substrate (substrate C; Figure 2.1). Square, plastic 10 cm^3 pots were filled with 525 g of substrate A and the SMC of the growth substrate layer was confirmed using a capacitance probe (Delta-T ML3 ThetaKit, Cambridgeshire, UK). For each crop species, 5 seeds were sown in each pot ca. 1 cm below the surface of growth substrate A. Then, 125 g of substrate B or C was added on top, depending on the treatment, keeping the seeds ca. 4 cm below the surface (see Figure 2.1). Additionally, three pots for each crop and growth substrate treatment were prepared so that SMC could be monitored throughout the experiment without disrupting germinating seeds in the main experimental pots.

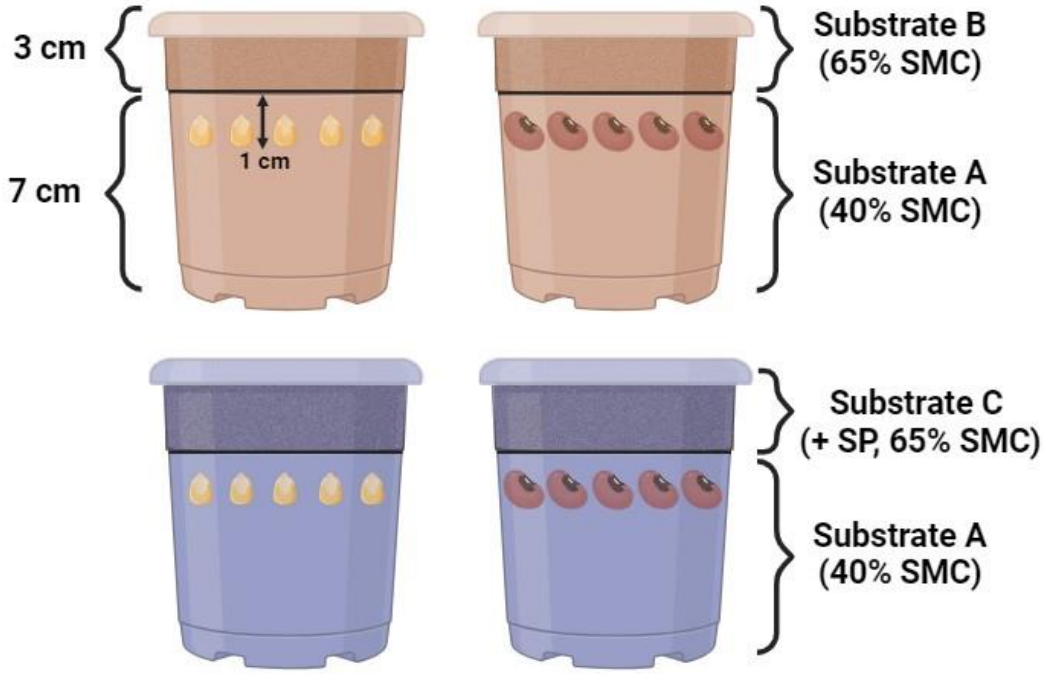

Figure 2.1. Pot setup to test the effect of Silwet Power (SP) on seed germination and emergence under drought conditions. *Zea mays* L. or *Vicia faba* L. seeds were planted ca. 1 cm below the surface of growth substrate A (40 % of the substrate moisture content [SMC]) and covered with 125 g of either growth substrate B (65 % SMC) or C (65% SMC and treated with SP at 5 g kg^{-1}). The pots were maintained in a low relative humidity growth chamber (ca. 40%) for 2 weeks to assess the effect of SP on seed germination and emergence from the soil surface under drought conditions (n = 20 per treatment). Figure created with Biorender.com.

Pots were transferred to a growth chamber (MC1000, Snijders, Tilberg, the Netherlands) set at the following conditions: 23:20 °C, 40% relative humidity and a light:dark cycle of 16:8 h. Illumination in the cabinet was provided by 4 fluorescent tubes (36 W, 4000 Kelvin) and light intensity in the cabinet was ca. 225 $\mu mol^{-2}\ s^{-1}$ at shelf level. Temperature and relative humidity were recorded every 10 min using a data logger (View 2 TV-4500, TinyTag, Sussex, UK) and plants were monitored for 14 d or until full seed emergence. No additional water was applied to pots throughout the trial.

At 4-, 5-, 7-, 11-, and 14-d post sowing (DPS), the number of emerged plantlets and plant height was recorded. Emerged plantlets were monitored for signs of phytotoxicity, and the SMC of the additional pots measured. At 14 DPS, the total number of geminated seeds (whether emerged or not) was recorded and root length of any germinated seeds measured using WinRHIZO™ software (Regent Instruments Inc, Quebec, Canada). Specific leaf area of emerged seedlings was measured using a portable area meter and belt conveyer (LI-3000A and LI-3050A, respectively; LI-COR inc., Nebraska, USA). The fresh weight (FW) and dry weight (DW; dried at 105°C for 7 d) of any ungerminated seeds was also recorded and the seed moisture content calculated.

2.2.3 Data analysis

Analyses were performed in SPSS version 28.0.1.1 (Illinois, USA). For all data, a Shapiro-Wilk normality test was carried out to determine fit to a normal distribution and a Levene's test was used to assess homogeneity of variance. Significance was set at $P \leq 0.05$ for all analyses.

The number of spores which had germinated after 27 d were counted to determine germination rate for each treatment group, and the proportion data were arcsine transformed. The effect of treatment on germination rate was tested in a 3-way ANOVA with Bonferroni correction. Block was initially included in the model, but as there was no block effect, it was subsequently removed, and the ANOVA was repeated. Adjuvant exposure did not appear to affect spore germination, so the effect

of adjuvant exposure on the length of hyphae produced by germinated spores was then tested. The main and interactive effects of adjuvant type, adjuvant concentration and exposure time on germ tube elongation of *R. irregularis* spores over time were tested in an unbalanced repeated measures ANOVA. Hyphal length data were not normally distributed, so a Box-Cox transformation was performed to remove skew and meet the assumptions of the ANOVA. Block was also tested but as this was found to be not significant, it was removed from the model to help improve model strength and simplify interpretation. Pairwise comparisons with Bonferroni correction were used to examine significant interactions and Tukey post-hoc tests were used to interpret significant main effects. To test the interaction between adjuvant concentration and adjuvant exposure time, the analysis was repeated excluding control data. This did not affect the *P* value of the interaction, so results from the original analysis were portrayed in the results section.

An unbalanced one-way ANOVA was used to analyse the effect of cultivating seeds in growth substrate treated with SP on seed moisture content of ungerminated seeds, which was calculated using FW and DW.

2.3 Results

2.3.1 Effect of two Silwet adjuvants on *Rhizophagus irregularis* spore germination rate

After 27 d of incubation on MSR media, 85% ± 3% of the total *R. irregularis* spores in the bioassay had germinated (Table 2.2). Differences in germination (%) among treatments were not significant (Table S3).

Table 2.2. The percentage of *Rhizophagus irregularis* spores that germinated after 27 d following exposure to Silwet™ Power (SP) or Silwet™ 408 (S408) for 0.25, 3 or 24 h. Spores were exposed to varying concentrations of SP or S408, which were categorized as low, medium, or high (SP: 10, 1, 0.1 % w/v; S408: 5, 0.5, 0.05 % v/v). These concentrations were chosen based on the trisiloxane content of each adjuvant, where SP has half the concentration of trisiloxane compared to S408. Therefore, the concentrations for low, medium, and high adjuvant preparations are equivalent for each respective adjuvant. Control spores were exposed to sterile ddH_2O for 0.25, 3, or 24 h. There were no significant differences in the mean percentage of germinated spores among treatments which was tested by an unbalanced 3-way ANOVA with Bonferroni correction ($P \leq 0.05$). For all groups, the SE was <1 so was not included in the table. n is the number of replicates for each treatment that successfully germinated.

Treatment	Exposure time (h)	Adjuvant concentration	Spore germination (%)	*n*
Silwet Power	0.25	Low	81	13
		Medium	81	13
		High	100	16
	3	Low	94	15
		Medium	88	14
		High	100	16
	24	Low	81	13
		Medium	75	12
		High	88	14
Silwet 408	0.25	Low	88	14
		Medium	94	15
		High	100	16
	3	Low	94	15
		Medium	63	10
		High	75	12
	24	Low	100	16
		Medium	75	12
		High	56	9
Control (ddH2O)	0.25	N/A	88	14
	3		75	12
	24		94	15

2.3.2 Effect of two Silwet adjuvants on *Rhizophagus irregularis* spore hyphal length

The outcomes of exposing *R. irregularis* spores to SP and S408 at low, medium and high concentrations (see section 2.2.1) for 0.25, 3 or 24 h are presented below. Two examples of treated spores that have germinated on MSR medium are shown in Figure 2.2.

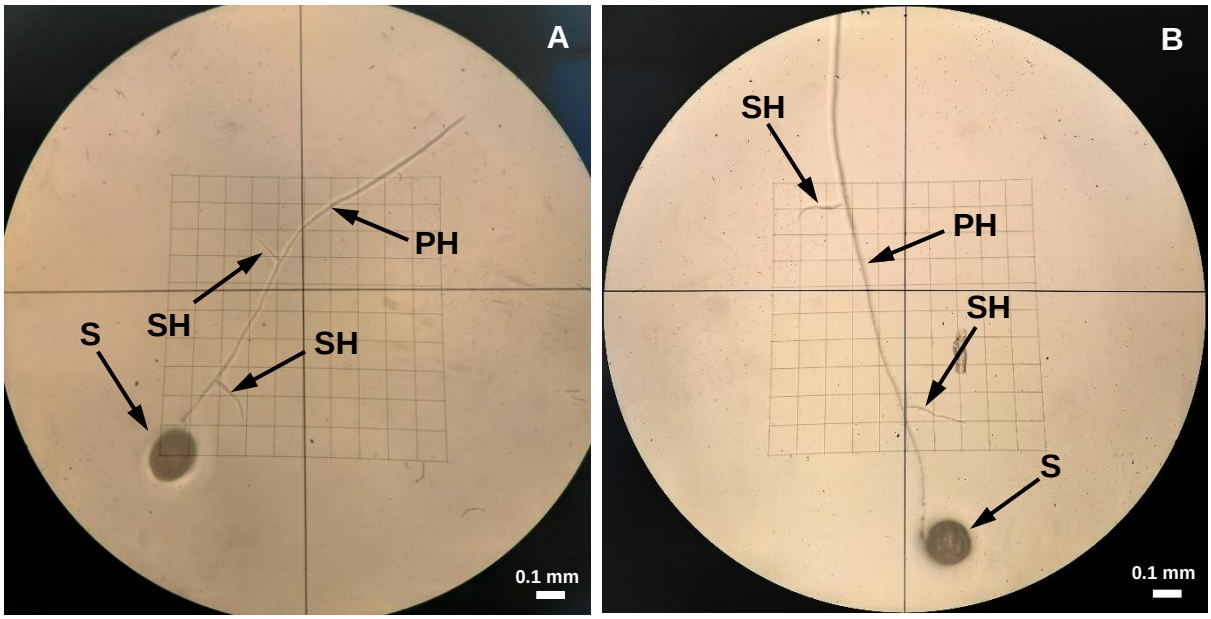

Figure 2.2. An example of germinated *Rhizophagus irregularis* spores (S) with primary hyphae (PH) and secondary hyphae (SH) after 9 d on Modified Strullu Romand (MSR) medium. The 1 x 1 mm grid allowed hyphal quantification as intersections where grid lines and hyphae crossed were counted to determine total hyphal length (see section 2.2.1). White scale bar is equal to 0.1 mm. Figure 2.2A shows a spore that was initially exposed to Silwet™ Power at 0.1% w/v for 3 h before incubation on MSR medium, while Figure 2.2B shows a spore that was exposed to Silwet™ 408 at 0.05% v/v for 0.25 h before incubation on MSR medium.

Hyphae length produced by *R. irregularis* was not significantly affected by exposure to either the type or concentration of adjuvant (adjuvant type: $F_{1, 344} = 0.001$, $P = 0.979$, adjuvant concentration: $F_{2, 344} = 0.263$, $P = 0.769$), but it was affected by adjuvant exposure time ($F_{2,344} = 20.9$, $P < 0.001$). Regardless of adjuvant type,

spores exposed to an adjuvant for 3 h or more produced significantly shorter hyphal lengths than those exposed to an adjuvant for 0.25 h only. There was no significant difference in the hyphal length produced by spores exposed for either 3 or 24 h (Figure 2.3).

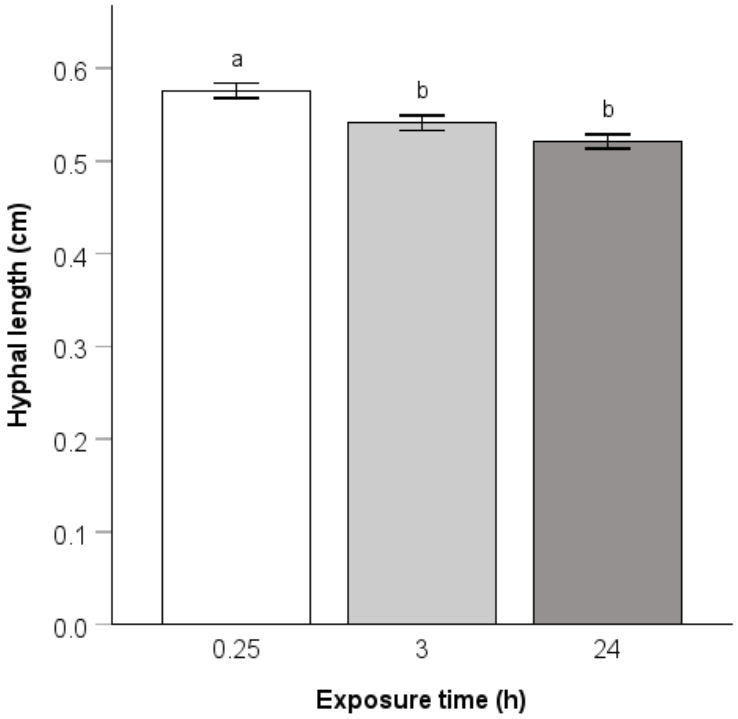

Figure 2.3. The effect of exposure to Silwet™ adjuvants (data combined from both adjuvants, Silwet™ Power and Silwet™ 408) for 0.25, 3 and 24 h on the length of hyphae (cm) produced by *Rhizophagus irregularis* spores. Hyphal length was significantly ($F_{2,344}$ = 20.9, P < 0.001) affected by exposure time to Silwet™ adjuvants, regardless of adjuvant type, and this was tested by a repeated measures 3-way ANOVA with Bonferroni correction. Hyphal length of spores exposed to an adjuvant for 3 h or more was significantly less than of spores only exposed to an adjuvant for 0.25 h. Error bars are ± 1 standard error (n = 87 for 0.25 h; n = 94 for 3 h and n = 91 for 24 h) while different letters indicate significant differences among exposure times ($P \leq 0.05$).

There was also a significant interaction between adjuvant concentration and adjuvant exposure time ($F_{4,344}$ = 7.12, P < 0.001); exposure time significantly reduced hyphal length at both low (0.45 ± 0.1 cm) and high (0.50 ± 0.1 cm) adjuvant concentrations compared to control spores (0.56 ± 0.1 cm). In contrast, the hyphal length of spores exposed to an adjuvant applied at a medium concentration did not differ from those produced by the untreated controls (0.53 ± 0.1 cm). The statistical

analysis was repeated but excluding data of the control spores to determine whether this interaction was in fact being driven by the inclusion of the control data in the model. In the repeated analysis, the interaction was still significant ($F_{4,223}$ = 6.40, P < 0.001) because exposure time significantly reduced hyphae length produced by spores exposed to the low adjuvant concentration, but not the medium or high adjuvant concentrations (Figure 2.4).

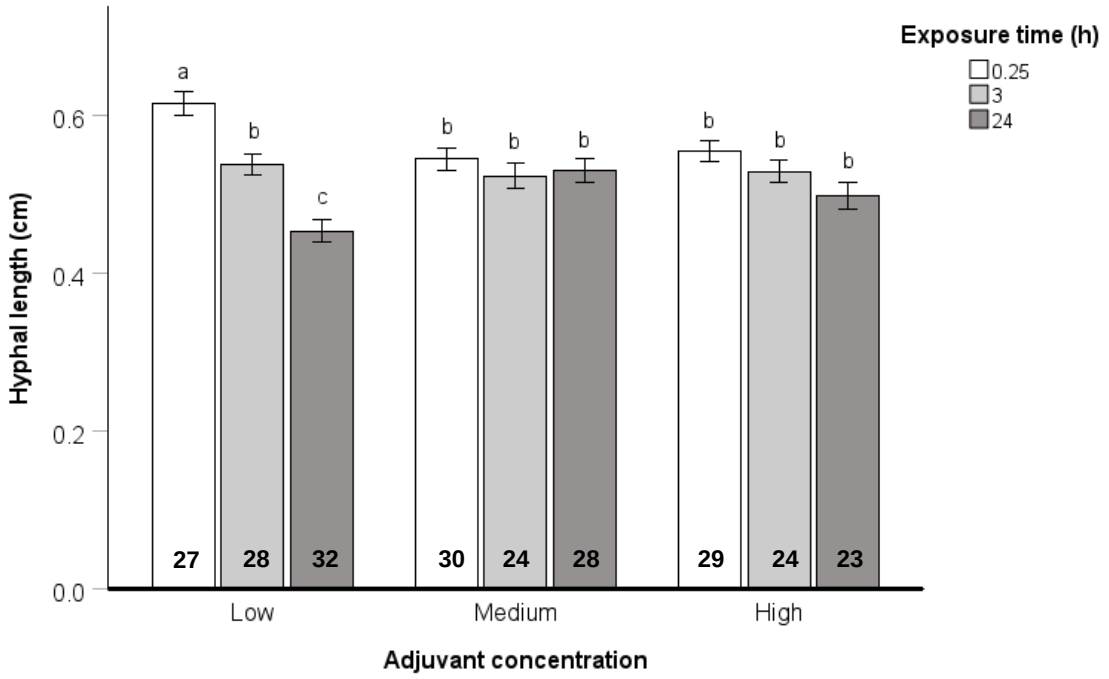

Figure 2.4. Length of hyphae (cm) produced by germinated *Rhizophagus irregularis* spores exposed to three concentrations of Silwet™ adjuvant (SP: 0.1, 1, 10% w/v; S408: 0.05, 0.5, 5% v/v; see Table 2.1 for justification) for 0.25, 3 or 24 h. Control spores were exposed to ddH_2O for 0.25, 3 or 24 h. The figure shows a significant ($F_{4,344}$ = 0.38, P < 0.001) interaction between adjuvant exposure time and concentration, caused by a reduction in hyphae length as exposure time increases, but only when spores were exposed to the low adjuvant concentration. The interaction effect was tested by 3-way ANOVA with pairwise comparison with Bonferroni correction. Error bars are ± 1 standard error, where *n* varied among adjuvant treatments (*n* for each treatment group is shown at the base of each bar) due to germination count for each treatment. Different letters indicate significant differences among treatments ($P \leq 0.05$).

Exposure to an adjuvant at medium or high concentrations decreased hyphal length at 0.25 h exposure, compared to the hyphal length produced under low concentration. However, the low concentration of adjuvant reduced hyphal length as exposure time increased, with 0.25 h exposure leading to a mean hyphal length of 0.6 ± 0.1 cm and 24 h exposure leading to a mean hyphal length of 0.5 ± 0.1 cm.

2.3.3 Effect of Silwet Power on seed moisture content and germination

After 14 d of cultivation, seed germination rates were extremely low, with none of the *V. faba* seeds having germinated and only 10 of the *Z. mays* seeds (most of which were pseudo replicates from a total of 5 pots) out of the 100 seeds sown for each species. Of the 10 germinated *Z. mays* seeds, 7 produced shoots. The plantlets from the growth substrate treated with SP showed no signs of phytotoxicity (Figure 2.5). Among the germinated seeds, germination rate and shoot production were unaffected by treatment with SP. On 14 DPS, the % SMC was 10.4 ± 0.2% regardless of growth substrate treatment.

Figure 2.5. Germinated *Zea mays* L. seeds which were cultivated in a growth cabinet in growth substrate treated with Silwet™ Power (+SP; 5 g kg^{-1}) or in an untreated growth substrate (no treatment). At the start of the trial the growth substrate had an average moisture content of 45%. The average relative humidity of the growth cabinet was 40%. The germinated seeds were pseudo replicates from 5 pots out of the total 40, two of which received no treatment and 3 which received treatment with SP. The 4 plantlets that emerged from the soil surface originated from 1 pot which was treated with SP (indicated by white arrows). The ruler is 30 cm for scale.

There was also no difference in seed moisture content for either *Z. mays* or *V. faba*, when seeds were cultivated in growth substrate treated with SP or left untreated (see Table 2.3).

Table 2.3. Mean (± 1 SE) moisture content (%) of *Zea mays* L. and *Vicia faba* L. seeds that were cultivated at 40% relative humidity in a growth substrate (SMC was 45%) that had been treated with Silwet™ Power (SP) or left untreated (control). Treatment of the growth substrate with SP had no significant impact on seed moisture content for *Z. mays* or *V. faba*, as determined by unbalanced 1-way ANOVA ($P \leq 0.05$). *n* is number of replicates for each species.

	Seed moisture content (%)				
	Mean ± SE	***df***	**Mean square**	***F***	***P***
Zea mays L. (*n* = 189)	33.1 ± 1.0	1, 198	0.01	0.37	0.544
Vicia faba L. (*n* = 200)	89.4 ± 0.8	1, 198	0.15	0.99	0.322

2.4 Discussion

There were a number of spores from each treatment group which did not germinate, but this was not attributed to any individual treatment as spore germination count did not differ from the control spores for any treatment. Hence, the Silwet adjuvants had no observable impact on germination. Therefore, the hypothesis that Silwet organosilicone surfactants would not negatively impact *R. irregularis* spore germination irrespective of organosilicone surfactant concentration or exposure duration was supported. The failure of some spores to germinate is likely due to genetic factors (Hijri & Sanders, 2005) or inoculum quality rather than environmental stress, evident by the high overall germination count from the study (85% ± 3%). This suggests that the presence of an organosilicone surfactant does not interfere with C respiration in *R. irregularis*, which serves to produce the primary energy source for AMF spores during the asymbiosis phase of AMF growth (Bago *et al*., 1999).

It was also hypothesized that the mean length of hyphae produced by germinated spores would not be affected by exposure to an adjuvant, irrespective of adjuvant concentration or exposure duration. However, hyphal length was lower when exposed to an adjuvant for 3 or 24 h (average hyphal length: 0.5 ± 0.1 cm) compared to those exposed to an adjuvant for 0.25 h (0.6 ± 0.1 cm). This effect was the same for both SP and S408 when spores were exposed to the lowest concentration of adjuvant. Moreover, exposure to medium and high adjuvant concentrations did not have an increasing impact on hyphal length over time, where the low adjuvant concentration did. However, after 0.25 h exposure, spores subjected to the medium and high adjuvant concentrations had shorter hyphae than those exposed to the low adjuvant concentration. This suggests that at initial exposure, the low adjuvant concentration was less detrimental to hyphal length than the medium and high concentrations, which both had a negative impact on hyphal length regardless of exposure time. The shorter hyphal length produced by spores that were exposed to the low adjuvant concentration for 3 or 24 h indicates that, under these conditions, there could be a targeted effect of the organosilicone surfactant on particular regulatory pathways in *R. irregularis* that control hyphal growth rate (Besserer *et al*.,

2008; Bahn *et al*., 2007). Crucially, exposure to either of the Silwet organosilicone surfactants did not prevent the *R. irregularis* spores from producing hyphae. Moreover, the effect size of adjuvant exposure on hyphal length was modest compared to control spores, suggesting that *R. irregularis* can tolerate exposure to an organosilicone surfactant. However, this tolerance may be limited by the duration of exposure to low adjuvant concentrations and there may be a threshold for the concentration of organosilicone surfactants that *R. irregularis* spores can tolerate and still successfully produce hyphae at the asymbiotic growth stage.

Due to the lack of studies examining any relationship between AMF and soil adjuvants, it remains unclear how the presence of the Silwet organosilicone surfactants led to a 0.04 ± 0.01 cm reduction in the length of hyphae produced by adjuvant-treated spores compared to the untreated control spores. There have been studies, however, which examine the effect of adjuvants on soil microbial communities as a whole (Banks *et al*., 2014) and the direct impact of adjuvants on other soil organisms such as other fungal species (Poirier *et al*., 2017), various bacteria (Nobels *et al*., 2011, Jibrin *et al*., 2021) and protozoa (Tsui & Chu, 2003). These studies demonstrated that certain non-ionic surfactants can be toxic to both prokaryotic and eukaryotic microorganisms, but the mechanisms responsible are not yet clear. Nobels *et al.* (2011) investigated the acute toxicity of a range of adjuvants, including organosilicone surfactants, on *Escherichia coli* at the gene expression level to deduce their main mechanism of impact. They found that while organosilicone surfactants triggered expression of fewer genes associated with acute toxicity compared to other types of non-ionic surfactants, exposure to the organosilicone surfactant tested did induce oxidative damage to the Superoxide Response pathway (involved in superoxide stress responses in *E. coli*). This differs from ionic adjuvants, which commonly damage cells through alterations in membrane permeability (Chapman *et al*., 1993). There have been no developments on the findings by Nobel *et al*. (2011) and the results of the present study are only representative of very specific conditions. As such, further investigation into this mechanism, particularly *in planta* is necessary before confident conclusions can be drawn about the toxic mode of action of organosilicone surfactants on either prokaryotic or eukaryotic organisms. This could also be investigated utilising transformed roots *in vitro*, a method (Bi *et al*., 2004) that has been successfully employed in previous studies to discern the impact

of various petroleum products and biocides on culturable AMF species (Kirk *et al*., 2005; Campagnac *et al*., 2008; Zocco *et al*., 2008). This would enable observation of how organosilicone surfactants may impact the AMF-plant association in a much more controlled environment than in a soil matrix. Nevertheless, the reduction in the length of hyphae produced by *R. irregularis* spores exposed to the organosilicone surfactant in this study may be due to damage to oxidative stress response pathways in AMF, reducing their activity (Wu *et al*., 2014).

Spores exposed to the lower surfactant concentration for 24 h produced hyphae that had a mean length of 0.06 ± 0.02 cm less than those exposed to the medium or high concentration of surfactant for 24 h. This enhanced impact of the low concentration of organosilicone surfactant exposure on the length of hyphae produced by *R. irregularis* spores is an unexpected result and counter to the findings of other studies that have found adjuvant toxicity to be dose dependent (Donovan & Elliott, 2001; Chen *et al*., 2022). Nevertheless, this could be the result of a non-linear dose-response, leading to an increase in organosilicone surfactant uptake in *R. irregularis* spores at low organosilicone surfactant concentrations (Ma *et al*., 2020). If, as this study suggests, low levels of Silwet adjuvants have a specific mode of action on *R. irregularis* spores which hinders their ability to produce hyphae, then there is a need for further research which explores this mechanism. Repeating the study with longer exposure times and a wider range of concentrations would delimit the threshold tolerance of *R. irregularis* spores to organosilicone surfactants. Such studies could be followed up by molecular indications of oxidative damage to elucidate the mode of action responsible for the effect of organosilicone surfactants on *R. irregularis* spores. These results are from an *in vitro* study under axenic conditions, so the effects in soil could differ substantially. The reduction in hyphal length at the asymbiotic stage may not necessarily have a detrimental effect on the root colonization of a host plant under field conditions. Additionally, it is important to note that trisiloxane-based organosilicone surfactants such as SP and S408 hydrolyse rapidly in soil. Therefore, any potential detrimental effects observed in this study may not be observed *in situ*.

There were no significant differences among the effects of SP and S408 on either AMF spore germination or production of hyphae, despite slight differences in chemical structure between these two trisiloxane-based adjuvants. This implies a

general effect of organosilicone surfactants on *R. irregularis* germination and hyphal production. Furthermore, the impact of the surfactants on *R. irregularis* was limited by concentration. Therefore, other adjuvants from the Silwet catalogue could also be compatible with *R. irregularis* and should be tested accordingly. Furthermore, testing AMF from other phylogenetic families by this method would illuminate whether these effects are species-specific or a general reaction of AMF to organosilicone surfactants. This would inform which AMF consortia are compatible with organosilicone surfactants, such that their combined use could reap the maximum benefits for crop drought tolerance and water management.

Regarding the seed germination assay, only 10 of the *Z. mays* seeds and none of the *V. faba* seeds germinated, so the hypothesis that seeds cultivated in growth substrate treated with SP would have an increased germination and emergence count after 14 d could not be tested. The very low seed germination counts of both species, regardless of soil treatment and despite successful germination in seed quality tests, indicates that these results are due to limitations with the study design and do not reflect the true impact of SP on seed germination. Namely, the environmental conditions for the study were chosen based on a previous study carried out by Momentive which utilised *Z. mays* and *T. aestivum* cultivated in growth substrate composed of an agricultural topsoil and vermiculite in a 50/50 ratio. The composition and structure of a growth substrate determines how it interacts with water molecules due to the availability of polar regions where water and organic matter can associate (Zhang & Lu, 2020a). The high sand content of the growth substrate used in the present study meant its ability to retain moisture was relatively low. Therefore, it is clear from the above results that the environmental conditions chosen were not appropriate for the plant species or growth substrate utilised in the present study. Development of the present study should then consider how the impact of growth substrate composition changes its soil water holding capacity, thus its suitability for cultivation of different crop species.

Overall, the seed germination study has made clear the need to investigate the effect of SP on the water retention of a range of growth substrates varying in composition before plant trials are conducted. This would enable more appropriate environmental conditions to be selected (i.e., drought severity chosen based on the growth substrate SWHC), allowing deduction of the benefits of SP for germination of

different crop species under drought conditions. It is evident from the results of this study that the ability of SP to improve the water retention of a growth substrate is specific to the growth substrate composition. Therefore, these results could also have indications for other studies which make conclusions about the activity of organosilicone surfactants in soil, if they have not observed these properties in a range of different soil types and growth substrates. As such, further investigation is needed to deduce the agricultural contexts in which SP could be of optimal benefit for crop germination under drought conditions.

In conclusion, the ecotoxicity study demonstrated a novel interaction between a model AMF species and commercial adjuvants from a group of compounds commonly used with agricultural formulations. Principally, the two Silwet adjuvants were compatible with *R. irregularis*, causing no impact to spore germination and only slightly reducing the ability of the AMF to produce hyphae in the asymbiosis phase. These findings should be explored further to elucidate the mechanism responsible for the reduction in the length of hyphae produced by *R. irregularis* spores treated with an organosilicone surfactant. The study is a first step in understanding the relationship between a model AMF species and two commercially available organosilicone surfactants.

3 Utilising Silwet soil adjuvants and arbuscular mycorrhizal fungi to improve soil water relations for crop productivity

3.1 Introduction

Climate change poses significant challenges to agriculture by exacerbating drought (Humphrey *et al*., 2018) and flooding conditions (Sefton *et al*., 2021), leading to shifts in growing seasons and disrupting crop yields, ultimately threatening food security and livelihoods worldwide (van Dijk *et al*., 2021). One method of measuring drought is through quantification of the soil moisture content: the percentage of water present per unit of soil (Civeira, 2019). Reduced soil moisture content impacts soil quality. As soil dries out, it becomes more prone to compaction (Mbarki *et al*., 2023). Compaction reduces soil porosity and pore space, restricting root growth and limiting water infiltration and drainage (Mbarki *et al*., 2023; Szatanik-Kloc *et al*., 2018). Furthermore, the loss of soil moisture causes soil particles to shrink and pull away from each other, leading to the disintegration of macroaggregates, thus the loss of soil structure (Quintana *et al*., 2023). Without proper soil structure, water infiltration and air exchange are impaired, decreasing the soil water holding capacity (SWHC) and resulting in increased erosion risk (Masroor *et al*., 2022).

Low soil moisture content can also suppress soil microbial activity by increasing osmotic stress and limiting metabolic activities (Stark & Firestone, 1995). Microorganisms play a crucial role in organic matter decomposition and nutrient mineralization processes that release nutrients such as N, P, and K into plant-available forms (Kallenbach *et al*., 2016; Jacoby *et al*., 2017). Reduced soil nutrient availability leads to deficiencies in plants which impact various plant functions (Waraich *et al*., 2011). Examples include stunting growth thus reducing biomass (Muller *et al*., 2011), reducing leaf expansion and increasing leaf necrosis (Sakuraba, 2022), decreasing stress resilience (Zhang *et al*., 2020c) and limiting root development (Gruber *et al*., 2013). Micronutrient deficiencies increase susceptibility to biotic and abiotic stresses. For example, Zn deficiency reduces the ability of some

plants to respond to pathogens and herbivorous pests (Cabot *et al*., 2019), while B and Si deficiencies impact cell wall strength and repair (Osuna-Canizalez *et al*., 1991), and cell membrane permeability (Brown *et al*., 2002). Furthermore, deficiency of Ca, which is involved in root signalling cascades among other things, reduces the root system size of affected plants (Duan *et al*., 2022). This limits the plant's ability to access water and nutrients from the soil, thus perpetuating stress symptoms caused by drought and inducing premature senescence in affected plants (Wehner *et al*., 2015). Overall, the effects of drought on soil can be detrimental to soil quality and fertility, impacting plant growth, thus reducing crop yields and food security.

Proline accumulation is a well-documented response to drought stress in plants (Arteaga *et al*., 2020; Kijowska-Oberc *et al*., 2023; Zhang *et al*., 2017). Proline is a common plant osmolyte that is produced in the cytoplasm and chloroplast stroma to decrease cellular osmotic potential in response to low soil water potential (Meena *et al*., 2019). It can accumulate in above- or belowground parts of the plant depending on the drought response elicited by the affected plant species (Polavarapu & Nese, 2014). The regulation of cellular osmotic potential then maintains cell turgor and water content (Rivero *et al*., 2007). Higher proline accumulation in leaf cells typically indicates more severe drought stress (Verslues & Sharma, 2010). Therefore, elevated proline levels serve as a biochemical marker indicating that plants are experiencing water deficit conditions. Both monocots and dicots accumulate proline as a common response to drought stress, but there are reported differences in proline accumulation between the two evolutionary lineages. In general, monocots tend to accumulate higher levels of proline in response to drought stress compared to dicots, suggesting that monocots benefit more from proline accumulation during drought stress than dicots (Bekka *et al*., 2018). This difference may be attributed to differences in the regulation of proline biosynthetic pathways and osmotic adjustment mechanisms between the two groups (Slama *et al*., 2015; Rai & Penna, 2013). Differences between monocot and dicot proline accumulation can also be influenced by AMF colonisation (Chandrasekaran, 2022), with variation in proline accumulation also dependent on AMF species (Chun *et al*., 2018). For example, host plants associated with *Rhizophagus fasciculatus* and *F. mosseae* were reported to experience higher accumulation of proline during drought stress than those associated with *Glomus deserticola* and *Claroideoglomus etunicatum* (Azcón *et al*.,

1996). Moreover, AM-*Z. mays* were demonstrated to have significantly lower accumulated proline than non-AM *Z. mays* plants, while other plant species such as *Allium sativum* and *Cicer arietinum* produced much more proline when associated with AMF (Chandrasekaran *et al*., 2014). It is important, then, to consider how symbiotic relationships or external conditions other than drought may impact proline accumulation.

Gaining species-specific knowledge about plant drought and nutrient stress adaptations and how they can be enhanced is vital if future food demands are to be met (van Dijk *et al*., 2021). Cereal crops account for over 50% of food intake globally, while legumes are important for both human consumption and as livestock feed (Wiebe *et al.,* 2015). Plants have evolved various root system adaptations to cope with drought by optimizing water uptake and minimizing water loss in challenging environments (Comas *et al*., 2013; Hodge *et al*., 2009). For example, dicotyledonous crop plants (e.g., *Daucus carots, Beta vulgaris L.*, and *V. faba*), produce deeply rooted taproots (Chiteri *et al.,* 2022). Deep root systems allow plants to access water stored in deeper soil layers which are more resistant to drying out during drought periods (Prince *et al.,* 2015). In contrast, monocots (e.g., *Z. mays*, *Hordeum vulgare* L., and *Avena sativa*) utilise fibrous root systems comprising of numerous fine roots that spread out horizontally nearer to the soil surface (Perkons *et al.,* 2014). The shallow depth and large surface area of fibrous roots in the soil enables the plant to take advantage of occasional light rain events or dew (Kou *et al*., 2022). However, fibrous root systems are perhaps more vulnerable to sudden drought periods than taproot systems as swift evaporation of water from the soil surface exposes them to more extreme fluctuations in soil moisture levels and they also have less water storage capacity compared to taproots (Ding *et al*., 2020; Castañeda *et al.*, 2019). Adventitious roots, which develop quickly from non-root tissues at the base of the stem, can emerge in response to drought stress to increase access to moisture (Fry *et al*., 2018; Atkinson *et al*., 2014). These additional root structures, also known as brace roots, can aid plants with fibrous root systems, such as *Z. mays*, to access water in deeper soil layers, although plants with taproots are also capable of producing adventitious roots when necessary (Coudert *et al.*, 2013).

Adaptations in root architecture alone may not be enough to protect plants from drought stress, thus many plants have adapted to cope with environmental stresses

through coevolution with soil microbes (Poudel *et al*., 2021). Associations with soil microbes can influence root system architecture, supporting the plant to gain water and nutrients from the soil (Zipfel & Olroyd, 2017; Sasse *et al*., 2018). Plants form complex associations with various soil microbes, such as bacteria and fungi, in their root zone (Artursson *et al*., 2006; Bulgarelli *et al*., 2015). These interactions, whether they have direct associations with the plant through symbiosis or have indirect benefits via improvement of soil fertility and structure, can significantly enhance nutrient uptake, disease resistance, and productivity in host plants (Bever *et al*., 2012; Bonfante & Anca, 2009). AMF associate with plants via host roots, where they can trade fixed C for enhanced access to water and nutrients in the soil (Wen *et al*., 2019). While the outcomes of arbuscular mycorrhizal fungi (AMF) colonization of plant roots vary due to factors such as plant and AMF species and environmental conditions (Wang *et al*., 2023), there is ample evidence of AMF contributing to enhanced plant drought tolerance and nutrient uptake (Chen *et al*., 2018). For example, inoculation with AMF was shown to improve grain nutrient profile in *Z. mays* and *Triticum aestivum* grown in nutrient deficient soils (Luo *et al*., 2021), while AM-colonised *Z. mays* plants grown under drought conditions experienced significantly less drought-induced oxidative damage and had significantly higher biomass compared to non-AM plants (Begum *et al*., 2019). Also, harvest yields in AM-leguminous crop species were increased by over 20% compared to non-AM plants (Wu *et al.,* 2022). The presence of both rhizobia and AMF in leguminous host plant roots synergistically increase soil and atmospheric N acquisition (Chen *et al*., 2018), thus improving N uptake of host plants (Ingraffia *et al*., 2019). Overall, plentiful evidence exists demonstrating the benefits of AMF for drought resilience of crop plants. However, integration of AMF biofertilizer into conventional agricultural practices has not yet been successfully achieved (Wahdan *et al*., 2024). Therefore, agricultural systems that enable the use of biofertilizers such as AMF could increase resilience to drought stress.

Organosilicone surfactants can be used as soil adjuvants to alter soil physical properties, improving soil structure, fertility, and moisture content (Lehrsch *et al*., 2011; Starr *et al*., 2005). The effects of an organosilicone surfactant recently developed by Momentive, SP, on plant drought tolerance and growth, are displayed in Figure 3.1.

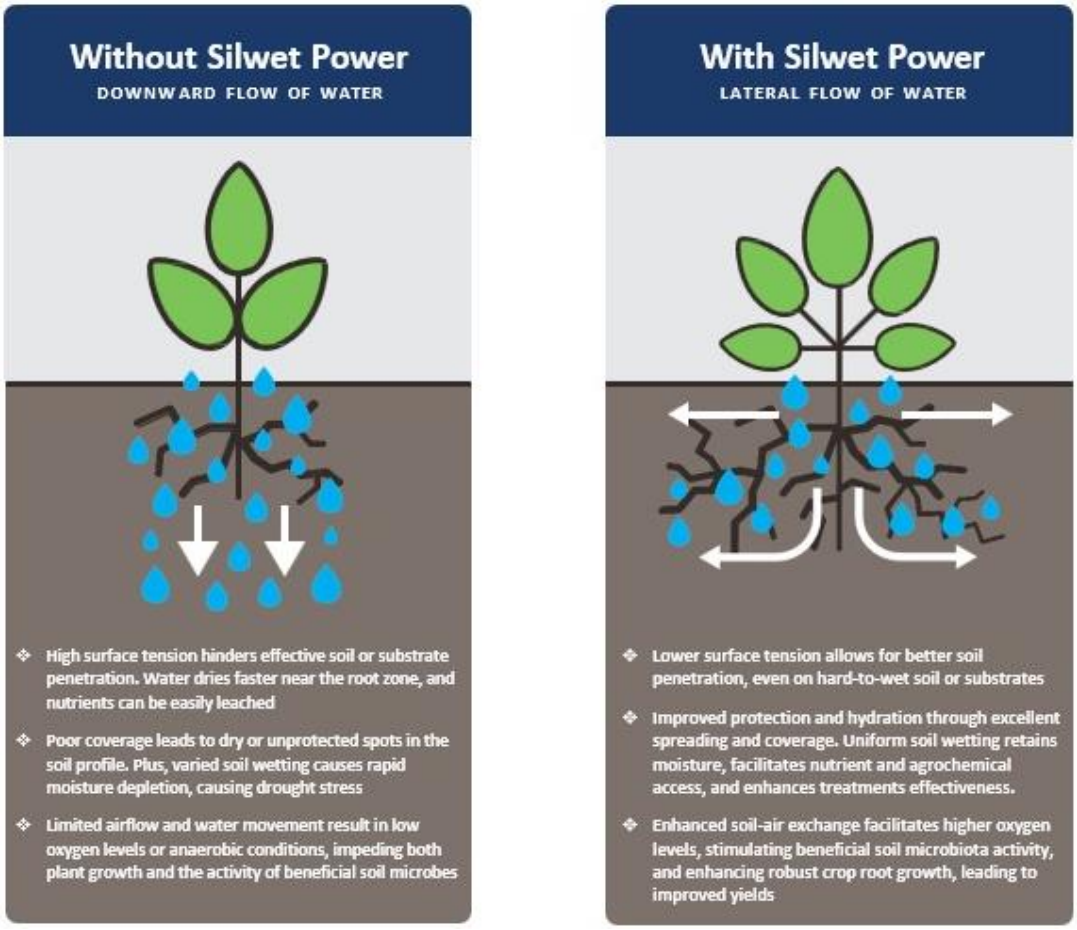

Figure 3.1. Silwet Power (SP) is an organosilicone surfactant designed for agricultural applications. When applied to the soil, it increases soil water infiltration and retention, leading to improved soil moisture content during periods of low precipitation or irrigation. These improvements to soil quality reduce drought stress experienced by cultivated plants. Diagram provided by Dr Benjamin Langendorf (Momentive Performance, London, UK).

Their ameliorating effects on soil moisture content have been reported to reduce drought stress and improve nutrient uptake in some crop species (Singh & Khan, 2012; Baratella & Trinchera, 2018), but have limited effects in others (Cooley *et al*., 2009). Direct application of organosilicone surfactants can cause phytotoxicity in some plant species, caused by interactions between the surfactant and epicuticular wax layer of the leaves, leading to alteration in cell permeability (John *et al*., 1974; Knoche et al., 1992). Knoche *et al*. (1992) found that oxyethylene chain length in the surfactants tested had a direct effect on phytotoxicity symptoms in *Brassica oleracea*, where low and high oxyethylene chain lengths caused less phytotoxicity than medium chain lengths. Moreover, limited *in vitro* studies suggest

variable impacts of organosilicone surfactants on bacteria (Nobels *et al*., 2011) and other soil organisms (Krogh *et al*., 2003). Nonetheless, careful use of organosilicone surfactants such as SP could prove highly beneficial for protecting a range of crops from drought stress.

The combined use of AMF and organosilicone surfactants in agricultural practices has only been reported in tomato, where it was used to reduce salinity stress (Chaichi *et al*., 2017). AM-*Medicago sativa* plants, in conjunction with an organosilicone surfactant, have been used to successfully decontaminate polluted soil (Wu *et al*., 2008), demonstrating potential synergistic benefits of AMF and organosilicone surfactants. In the latter study, combined use of AMF and the surfactant altered the rhizosphere microbial community structure. However, the impact of utilising organosilicone surfactants for improving drought tolerance of AM-plants has not been reported. Characterising the combined use of AMF and organosilicone surfactants for plants which represent the monocot and dicot evolutionary lineages could help to inform how the combined effects of AMF inoculation and organosilicone surfactant soil treatment might vary in different genetic models.

It is hypothesised that application of Silwet adjuvants to droughted sandy growth substrate will increase the SWHC and maintain a higher soil moisture content, directly proportional to the adjuvant concentration. SP (a powder adjuvant) will maintain a higher SMC in droughted growth substrate compared to S408 (a liquid adjuvant) due to its commercial design that tailors it for soil application. Rewetting of growth substrate treated with either adjuvant will lead to a reduction in adjuvant activity. In a plant system, SP is hypothesized to have no negative impacts on the ability of AMF to colonise host plant roots, due to compatibility between the two demonstrated in previous studies. Cultivating plants with AMF and SP will have a synergistic effect on nutrient and water availability for the plant, resulting in higher plant biomass and nutrient content. Given its extensive root branching, *Z. mays* is expected to better withstand drought conditions compared to *V. faba*. Therefore, the external hyphae from AMF associations and the improved water availability facilitated by SP are likely to benefit the less extensive root system of *V. faba* more than *Z. mays*. AMF will directly enhance osmolyte production in leaf cells, while SP

will not have any direct impact on proline accumulation relative to untreated controls. Therefore, drought-stressed *V. faba* will have enhanced proline accumulation compared to *Z. mays* L. due to its heightened reliance on AMF for water acquisition.

3.2 Materials and methods

3.2.1 Effect of two Silwet adjuvants on growth substrate water retention: pilot test

The effect of SP (a powder adjuvant) and S408 (a liquid adjuvant) on water retention of the growth substrate described in section 2.2.2.1 was examined in a pilot test. For each growth substrate treatment (SP, S408 and an untreated control), a column was prepared by placing a 12 cm Buchner funnel lined with 110 mm diameter grade 3 qualitative filter paper (Whatman, Kent, UK) above a 250 ml conical flask. A clear polyvinyl chloride (PVC) tube (10.2 cm outer diameter, 10.0 cm inner diameter x 16.7 cm length) was placed on top of the filter paper in each Buchner funnel and filled with 500 g of the growth substrate.

The growth substrate was sieved to 250 µm and used to prepare dilutions of SP and S408 at concentrations of 1 and 0.5 g kg^{-1} growth substrate, respectively. This process involved gradually adding each adjuvant to 100 g of growth substrate and thoroughly mixing to ensure even distribution. Following this, a 3.5 g aliquot of each adjuvant in sieved growth substrate was individually applied to the surface of the growth substrate resting in each column. A 500 ml capacity separating funnel clamped securely above each PVC tube was used to decant 300 ml dH_2O into each column (the approximate volume of water needed to saturate 500 g of the substrate). The setup is shown in Figure 3.2.

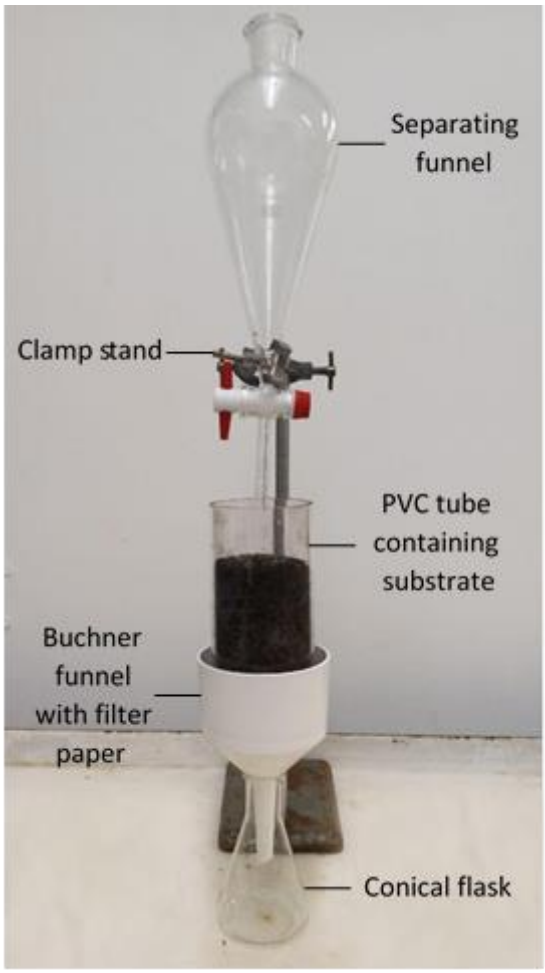

Figure 3.2. Experimental setup of the column used to evaluate the effect of two Silwet™ adjuvants on water retention and wetting behaviour of a loamy sandy growth substrate with an expected low water holding capacity. A separating funnel suspended from a clamp stand was used to decant water into the centre of a clear Polyvinyl Chloride (PVC) tube containing growth substrate treated with either Silwet™ Power, Silwet™ 408 (1 and 0.5 g kg^{-1} growth substrate, respectively), or untreated. The PVC tube was placed in a Buchner funnel lined with filter paper to prevent loss of growth substrate. An identical column was set up for each adjuvant treatment.

Both the time taken for the water to drain through the growth substrate and the amount of water drained from the column were measured. Any potential differences in growth substrate wetting resulting from the adjuvants were observed visually through the clear PVC tube. Each column, funnel and conical flask were then transferred to a growth chamber (AR-75 L Percival, Iowa, USA) set at the following conditions: 20:16 °C, 60:65% relative humidity and light:dark cycle of 16:8 h to

maintain a stable external environment. Illumination in the cabinet was provided by 8 fluorescent tubes (36 w, 4000 K) above each of the two shelves and light intensity in the cabinet was ca. 660 μmol^{-2} s^{-1} at shelf level. Data loggers were used to record temperature and relative humidity on each shelf every 10 min (View 2 TV-4500, TinyTag, Sussex, UK). Data collected from data loggers in the growth chamber were checked for anomalies in conditions throughout the experiment and showed that no unexpected changes in temperature or relative humidity occurred throughout the experimental period (Figure S4).

The % SMC was monitored with a capacitance probe (Delta-T ML3 ThetaKit, Cambridgeshire, UK) every 2 days over an 8-d period. The measurements concluded at this point, as it was anticipated that, beyond this timeframe, the adjuvants might start to lose their wetting properties due to hydrolysis in the alkaline substrate. Each % SMC measurement comprised the average of three measurements taken at different places in the growth substrate. Data collected on the day of watering were excluded from analysis because % SMC measurements were equal (50 or 20% of the SWHC), thus did not reflect change in the % SMC.

3.2.2 The impact of two Silwet adjuvants on growth substrate moisture content

The effect of adjuvant type and concentration on the moisture content of the growth substrate was investigated in a fully factorial pot trial. There were 10 treatment groups, thus: SP or S408 added at either a high or low concentration and an untreated control all subject to either an ambient or drought level of watering. There were 5 replicates for each treatment.

Pots were filled with 700 ml of growth substrate (described in section 2.2.2.1). SP and S408 were prepared as described in section 3.2.1 at the following concentrations: SP 0.05 g l^{-1}, SP 0.1 g l^{-1}, S408 0.025 g l^{-1} or S408 0.05 g l^{-1}, 0 g l^{-1} (untreated control) (pers. comms. Dr. Benjamin Langendorf, Momentive [London, UK], informed by previous field trials carried out by Momentive). As in section 2.2.1, these concentrations were chosen for each adjuvant based on the trisiloxane content

of each respective adjuvant. The adjuvants were applied to the growth substrate surface and then the pots were watered to either a drought (20% of the SWHC) or ambient (50% of the SWHC) level by the even distribution of 60 ml or 145 ml dH_2O, respectively, over the growth substrate surface. Pots were transferred to a growth chamber (as described in section 3.2.1) placed in a completely randomised, block design. Growth substrate moisture content was measured daily using a capacitance probe (Delta-T ML3 ThetaKit, Cambridgeshire, UK) for 28 d. After 14 d, 20 g subsamples were taken from each pot to calculate growth substrate moisture content by fresh and dried (105°C for 7 days) growth substrate weight and then pots were re-watered to return growth substrate moisture content to 50 or 20% of the SWHC. The % SMC was then recorded for another 14 d. After 28 d, further 20 g subsamples were taken from each pot to calculate the final % SMC by fresh and dried (105°C for 7 days) growth substrate weight. Again, data collected on the day of watering/ re-watering were excluded from analysis so that the model analysed change in % SMC only. See figure 3.3 for an overview of the experimental setup.

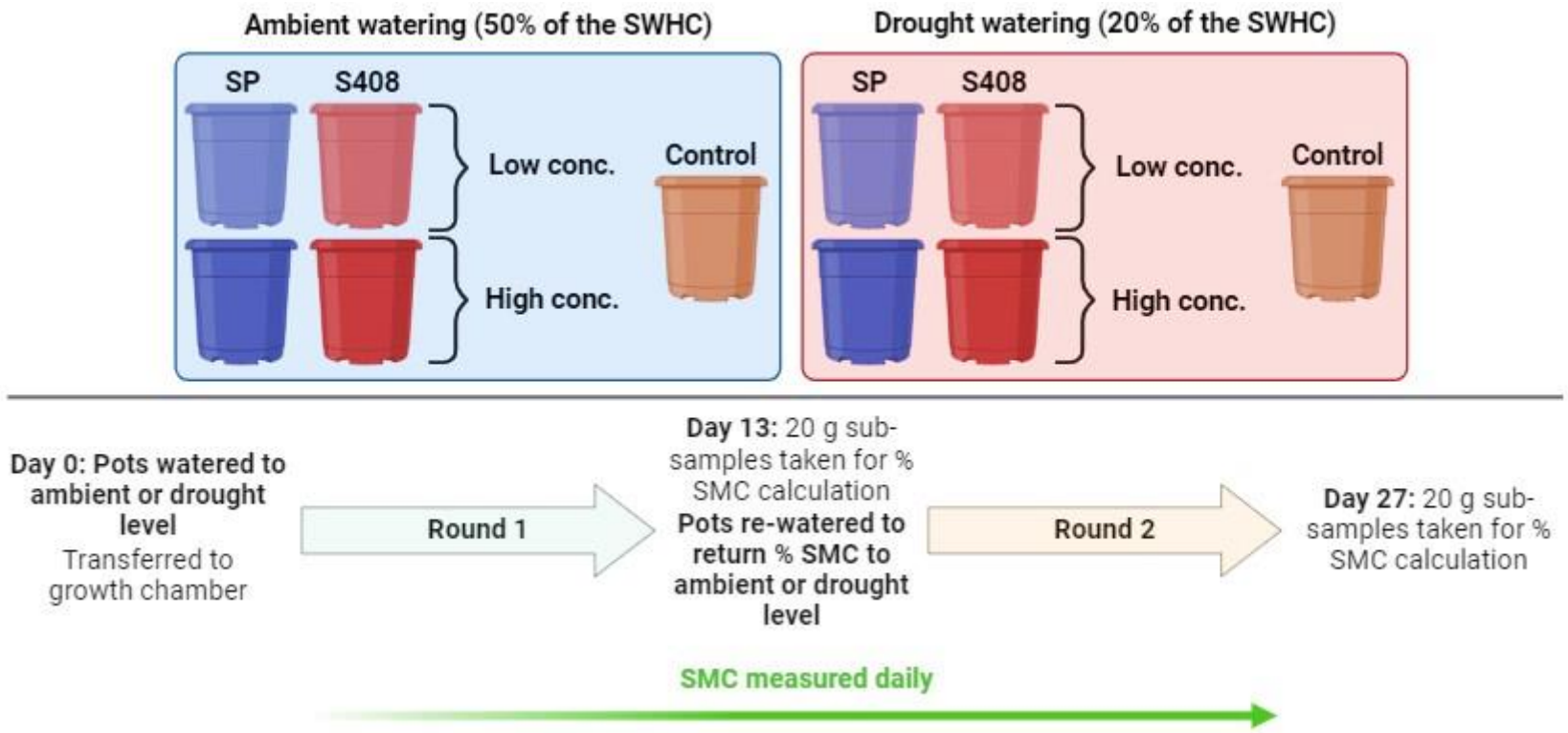

Figure 3.3. The experimental setup which was designed to test the effect of two organosilicone surfactants, namely Silwet™ Power (SP) and Silwet™ 408 (S408), on the growth substrate moisture content (% SMC) of a sandy loamy growth substrate with a low soil water holding capacity (SWHC). Growth substrate was treated with either SP or S408 at a low or high concentration (see section 3.2.2 for concentrations) or left untreated (control), and then watered to ambient (50% of the SWHC) or drought (20% of the SWHC) level (n = 5). The % SMC was monitored for 14 d (round 1), whereupon growth substrate was re-watered to return % SMC to ambient or drought level (round 2). The % SMC was then monitored for a further 14 d to observe how re-watering affected the active properties of the organosilicone surfactants in improving growth substrate water retention.

3.2.3 Effects of the combined use of a soil adjuvant, Silwet Power, and arbuscular mycorrhizal fungi on crop drought tolerance

3.2.3.1 Most Probable Number bioassay

Two species of AMF inoculum, *R. irregularis* and *Funneliformis mosseae* ([T.H. Nicolson & Gerd.] C. Walker & A. Schüßler), as an attapulgite clay/pumice/zeolite mix containing spores, mycelium, and colonised host plants root fragments were obtained from Plantworks Ltd (Kent, UK). These species were chosen due to their use in commercial biofertilizers and ease of cultivation with a range of host plants.

A most probable number (MPN) bioassay was carried out to estimate the number of infective propagules in each inoculum sample. The assay was carried out following a protocol by Alexander (1983). Briefly, for each AMF species, the inoculum was diluted to 1:10, 1:100 and 1:1000 in autoclaved (two cycles at 121°C for 15 min) attapulgite clay medium (AgSorb®, Oil-Dri Ltd, Cambridgeshire, UK) and split equally into 5 pots, so that there were 30 pots in total. *Zea mays* L. Rising Sun (Elsoms, Lincolnshire, UK) and ca. 30 *Trifolium repens* (Emorsgate Seeds, Cambridgeshire, UK) were used as trap plants. For each pot, three *Z. mays* and ca. 30 *T. repens* seeds were sown ca. 1 cm below the soil surface. Pots were kept in a glasshouse at ca. 15-20 °C and watered as required with dH_2O. Roots were harvested 11 weeks after sowing. Harvested roots were stained with an ink and vinegar solution (described in section 3.2.3.2.) and presence of AMF-specific structures (i.e., arbuscules and vesicles) was checked under a light microscope (Nikon Eclipse, Tokyo, Japan). AMF presence in the roots was then assessed using a dissecting microscope at 40x magnification for each sample. MPN for both AMF species was determined using an MPN table (Cochran, 1950) and both inoculum types were found to contain ca. 16,000 viable propagules ml^{-1}.

3.2.3.2 Root sampling and staining

To observe AMF structures in *Z. mays* and *V. faba* roots, roots were sampled randomly from the root system, rinsed in tap water to remove any adhering growth substrate and transferred to 50 ml universal tubes. Samples were then stained following the method of Wilkinson (2018). Briefly, cytoplasmic cell contents and secondary metabolites were cleared from samples by submerging in 10% w/v KOH for 45 min at 70°C. They were then rinsed 3 times with dH_2O and transferred to an ink and vinegar solution (5% v/v ink [Brilliant Black, Pelikan®, Gwent, UK] and 5% v/v acetic acid in dH_2O) for 30 min. Samples were then rinsed three times with 1% v/v acetic acid solution and destained in a 50% v/v glycerol solution overnight before mounting on microscope slides for microscopic observation.

3.2.3.3 Experimental design

The synergistic effects of SP and AMF inoculation on drought tolerance of *Z. mays* and *V. faba* were investigated in a fully factorial glasshouse trial. There were 8 treatment groups comprised of two watering regimes and four growth substrate treatments, with 5 replicates for each treatment group. The growth substrate (see section 2.2.2.1) was treated with AMF only, AMF and SP, SP only, or neither AMF or SP (control), and all treatments were subjected to two different watering regimes: ambient watering at 50% of the volume of the total SWHC (150 ml dH_2O), and drought watering at 30% of the volume of the total SWHC (90 ml dH_2O). For AMF treatments, *R. irregularis* and *F. mosseae* inoculum (source as described in section 3.2.3.1 prior to dilution) were combined in a 50:50 ratio.

For each treatment, five pots (section 2.2.2.3) were filled with 700 g growth substrate (see section 2.2.2.1) combined with either AMF, SP, or both depending on the treatment. The AMF inoculum was combined with the growth substrate at 20 ml l^{-1} (double the commercial dose recommended by Plantworks, Kent, UK to ensure successful colonisation of plant roots) and SP was combined with the growth substrate at 5 g kg^{-1} (pers comms. Dr Benjamin Langendorf, informed by previous

field trials by Momentive, London, UK). Control pots were filled with 700 g growth substrate combined with 20 ml l^{-1} autoclaved (two cycles at 121°C for 15 min) and dried AMF inoculum. A further 10 pots (5 for each plant species) were prepared with growth substrate treated with AMF only to be harvested for root colonisation assessment prior to full harvest.

Seeds from each species were prepared and sown a week apart to allow time for sample harvesting at the end of the growth period. As such, cultivation of each species was treated as a separate experiment. *V. faba* and *Z. mays* seeds were surface sterilised by submerging in 5 or 15% v/v bleach solution, respectively, for 15 min. The seeds were then stored at 4°C overnight and transferred to 90 mm diameter sealed Petri dishes containing damp filter paper soaked in ddH_2O. Seeds were kept in the dark at room temperature for 7 d to allow for radicle emergence. Three germinated seeds were initially transferred to each pot, which was later reduced to one plant per pot by cutting any extra plant stems at the growth substrate surface after 14 d.

Control and adjuvant only pots received 30 ml bacterial filtrate of the AMF by combining 140 ml of *R. irregularis* inoculum and 140 ml of *F. mosseae* inoculum with 600 ml autoclaved (one cycle, 121°C for 15 min) dH_2O. The solution was strained through a sieve to remove inoculum and the resulting solution collected and filtered through 11 µm filter paper (Whatman No. 1, Kent, UK) to remove AMF spores and propagules. The filtrate was stored at 4°C and used within 24 h after preparation. Initial watering quantity for control and adjuvant only pots was reduced accordingly.

Pots were watered every three days to maintain the ambient or drought watering level. The % SMC of each pot was monitored before and after each watering event using a capacitance probe inserted into the same part of the growth substrate each time to avoid disturbing roots and hyphae. After 14 d, plants were treated weekly with 10 ml of a 0.2% v/v nutrient solution (Thornton & Bausenwein, 2000) with phosphate concentration reduced by 10% (0.031 mmol l^{-1} of $NaH_2PO_4 \cdot 2H_2O$) to encourage AMF colonisation (Leigh *et al.,* 2009) while avoiding nutrient deficiency in the plants. Watering was reduced accordingly. The experimental setup is shown in Figure S7.

3.2.3.4 Pest and disease management

During the early growing stage (ca. 21 DPS), *V. faba* plants were infested with thrips (*Thysanoptera*), evident by silver, dry leaves that dropped prematurely (Figure 3.4). To control this pest, a mixed spray treatment containing 1 g l^{-1} Minecto One (Syngenta, Cambridgeshire, UK) and 1.2 ml l^{-1} Decis (Bayer Crop Science, Cambridgeshire, UK) was applied to the infested plants immediately after symptoms were noticed, followed by another treatment 7 d later. Subsequently, thrip predator mites (Thripex-Plus, Koppert, Ontario, Canada) were released onto the plants using a slow release sachet to control any remaining thrips until the end of the experiment.

Rolling and browning of leaves, large variation in plant height and leaf number were noticed in *V. faba* plants ca. 28 DPS across all treatment groups, including the control (Figure 3.4). Treatment of the thrips infestation did not ameliorate these symptoms, thus, at harvest, leaf samples were sent to FERA (Yorkshire, UK) for bean leaf roll virus analysis by ELIZA. However, the negative test result suggested another source for the symptoms, such as residual herbicide contamination in the growth substrate (Boutin *et al*., 2014). Possible presence of herbicide residues in the topsoil could not be disclosed by Rolawn® due to confidentiality restrictions, so the cause of the symptoms could not be verified. No pest management was required for the *Z. mays* plants.

Figure 3.4. Two examples of plants where pest and possible disease symptoms may be present in *Vicia faba* L. ca. 28 days post sowing. Newly developed leaves did not unfurl (indicated by yellow arrow), likely due to herbicide contamination in the topsoil used in the growth substrate. The lower leaves that had unfurled prior to symptoms becoming apparent became dry and brown (indicated by blue arrow), indicative of infestation with thrips. These symptoms were observed across plants from all treatments (including control plants) and watering levels. Notable variation in plant size and leaf number was observed; white scale bar is equal to 4 cm.

3.2.3.5 Measurements taken during plant growth period and at harvest

At 7-, 10-, and 14-DPS, and then every subsequent 2 weeks, plants were photographed, and plant height and leaf number were recorded. Signs of phytotoxicity caused by the adjuvant or other chemical contamination, such as leaf chlorosis, necrosis and plant stunting were also recorded. At 49 DPS, extra replicates grown for monitoring of root colonisation by AMF were destructively harvested. Roots were cleared and stained as described in section 3.2.3.2; presence of AMF in roots indicated that a full harvest could be carried out at 56 DPS.

3.2.3.6 Root sample processing

At 56 DPS, the entire root system for each plant was cleared of soil and then the total root system length was analysed using WinRHIZO™ software (Quebec, Canada). Presence of root nodules was recorded for *V. faba* plants. Each root system was then subsampled once; each subsample was weighed (for subsequent weight correction of whole plant biomass) and then cleared and stained as described in section 3.2.3.2.

Percentage root length colonisation (% RLC) was determined for each subsample, whereby stained roots were mounted on microscope slides using destain solution and viewed under a light microscope (Nikon Eclipse, Tokyo, Japan) fitted with an eyepiece graticule at 200x magnification. Occurrence of intraradical hyphae, arbuscules, and vesicles were recorded for each intersection between hyphal structure and graticule for a minimum of 100 fields of view for each root sample. Counts were recorded as percentage of root length colonised by the following equation:

$$\%\ RLC = \frac{Number\ of\ intersections\ with\ arbuscular\ hyphae}{Total\ number\ of\ intersections\ counted}\ x\ 100$$

The same equation was also used to calculate percentage of arbuscules, and vesicles present in the roots. Original root system samples were weighed and dried (105°C for 7 d) and dry weights recorded.

Extraradical hyphae were extracted from two 5 g subsamples of a soil sample collected from around the roots for each plant, following a modified membrane technique described by Staddon *et al.* (1999). The extracted hyphae were stained with 0.01% acid fuchsin solution (lactic acid, glycerol, deionised water 14:1:1 and 0.1 g l^{-1} acid fuchsin) and mounted on microscope slides with lactoglycerol (1:1:1 lactic acid, glycerol and water). Hyphal length was then determined microscopically by the gridline intersect method (Miller & Jastrow, 1992) using a 10 x 10 grid of 1 cm^2 slide lengths (Graticules Optics Ltd, Kent, UK). A minimum of 50 fields of view were assessed for each membrane at 100x magnification. Hyphal length density (HLD) was then calculated for each sample as follows:

$$h = \frac{\left[\text{counts} \times \frac{11}{14} \times \frac{1}{10}\right] \times (\pi \times 7.5^2)}{(\text{grid no.} \times 0.1)} \times 13.885$$

$$\text{HLD} = \left(h \times \frac{\text{initial solution volume}}{\text{sample volume}}\right) \Big/ \text{growth substrate sample DW}$$

Where:

h is hyphae

HLD is measured in m hyphae g^{-1} growth substrate fresh weight

DW is dry weight (g)

Initial solution volume is 500 ml

Sample volume is 5 ml

3.2.3.7 Aerial plant sampling and analysis

Plant height (cm) and number of leaves were recorded. Additionally, specific leaf area of each plant was measured (m^2 kg^{-1}) using a portable area meter coupled with a belt conveyer (LI-3000A and LI-3050A, LI-COR inc., Nebraska, USA). Any leaves that were not fully unfurled (either due to early growth stage or disease symptoms) were not measured for any analyses – only unfurled leaves were used. Leaf sub-samples of ca.100 mg were taken from each plant and stored in 2 ml Eppendorf tubes. The subsamples were weighed (exact weights recorded), submerged in liquid N, and stored at -80°C for proceeding proline extraction and quantification. The remainder of the original leaf samples were then weighed, dried (105°C for 7 d) and dry weights were recorded to determine aboveground water content, biomass, and specific leaf area.

Free proline content was assessed using a spectrophotometer following the method of Ábrahám *et al*. (2010). Briefly, leaf subsamples that were stored at -80°C were transferred to ice and then submerged in 3% w/v sulfosalicylic acid (5 µl mg^{-1} leaf fresh weight). Subsamples were centrifuged in a benchtop centrifuge (Sigma 3-16L, Eppendorf, Hamburg, Germany) at 13000 *g* for 5 min at room temperature. For each subsample, an aliquot of 100 µl of supernatant was transferred to a new 2 ml Eppendorf tube containing 100 ml 3% w/v sulfosalicylic acid, 200 µl glacial acetic acid and 200 µl 0.14 M acidic ninhydrin (1.25 g ninhydrin, 30 ml glacial acetic acid, 20 ml of 6 M orthophosphoric acid, dissolved by vortexing for ca. 5 min and incubating at 50°C for ca. 30 min). The solution was inverted 10 times and then incubated in a water bath (Thermo 12L, Grant Instruments, Cambridgeshire, UK) at 96°C for 60 min. After the elapsed time, the reaction was terminated on ice. Under a fume hood (Erlab, Massachusetts, USA), proline was extracted from the solution by adding 1 ml toluene to the reaction tube and vortexing (WM/250/FP/2 Whirlimixer, Fisons, Suffolk, UK) for 20 s. Samples were allowed to separate into organic and water phases for 5 min at room temperature before the organic phase red chromophore (containing proline) was removed using a P1000 micropipette and transferred to a new 2 ml Eppendorf tube. Chromophore extracts containing proline were assessed spectrophotometrically at 520 nm immediately after extraction, with toluene as a reference. A standard curve using pure L-Proline extracted by the same

method as above was produced to determine proline concentration by leaf fresh weight (mg g^{-1}).

3.2.3.8 Foliar macro- and micronutrient analysis

Dried plant material was milled (MM 400 Retsch, Haan, Germany) into a fine powder and 6-7 mg subsamples (exact weight recorded) from each plant were analysed for carbon (C) and N contents. The analysis was carried out by Dr. Matthew Pickering (Environment Department, University of York) using a Flash 1112 NC analyser (Thermo Scientific, Massachusetts, USA) fitted with an MAS autosampler and thermal conductivity detector. Details of instrument conditions are in Table S4.

Further subsamples of 18-22 mg (exact weight recorded) from each plant were analysed for micronutrient analysis (specifically, Ca, Si, P, K, B and Zn) by inductively coupled plasma optical emission spectroscopy (ICP-OES). Prior to analysis, the plant material subsamples were transferred to 2 ml screwcap Eppendorf reaction tubes and prepared for ICP-OES by nitric acid digestion. Each subsample was suspended in 500 µl concentrated analytical grade nitric acid by vortexing. Reaction tube lids were punctured using a syringe needle to prevent pressure build up and incubated in a heat block at 70°C for ca.16 h. After the elapsed time, reaction tubes were removed from the heat block and 1 ml ddH_2O was added to each tube. The resulting reaction mixtures were transferred to 15 ml conical centrifuge tubes and the 2 ml reaction tubes were rinsed twice with 2 ml ddH_2O to ensure minimal sample loss. All 15 ml centrifuge tubes were filled up to 10 ml with ddH_2O and then filtered into fresh centrifuge tubes using No. 540 12.5 cm diameter filter papers (Whatman, Buckinghamshire, UK). Digested filtrates were stored at 4°C until analysis, which was carried out by Blaine Hancock (Environment Department, University of York) using an iCAP 7000 (Thermo Scientific, Massachusetts, USA).

3.2.3.9 Data analysis

Analyses were performed in SPSS version 28.0.1.1 (SPSS Inc., Illinois, USA). The pilot study described in section 3.2.1 was not replicated and therefore no statistical analyses were carried out on this data set. For all other data, log10, square root and cube root transformations were carried out, followed by a Shapiro-Wilk normality test to determine distribution and a Levene's test to assess homogeneity of variance among groups to determine the best fit for the data. Significance was set at $P \leq 0.05$ for all analyses.

For the pot experiment testing the effect of two Silwet adjuvants on % SMC, proportion data were arcsine root transformed. Transformed data were not normally distributed however, so a generalised linear model with Gamma distribution (to account for proportion data), log link function (as the data follow a Poisson distribution) and heterogeneous compound symmetry covariance structure (to account for the correlation and variability among repeated measurements) was used to test the main and interactive effects of adjuvant type, adjuvant concentration and watering level on % SMC. Any potential block effect was also tested in the model initially, but where this was not significant it was subsequently removed to improve the statistical power of the model due to its increased precision and simplified interpretation. SP improved the growth substrate water retention more than S408, so it alone was used in the proceeding experiment.

To assess the synergistic effects of SP and AMF on crop drought tolerance, a two-way ANOVA was used to test the main and interactive effects of the growth substrate treatment (AMF only, SP only, AMF+SP, control) and watering level (ambient, drought) on plant biomass and water content, macro- and micronutrient content, RLC, including arbuscules and vesicles, and HLD at harvest. The model was unbalanced to allow for differences in the number of replicates within each treatment group where plants died before harvest. Block was included in the model as a random effect. If there was a significant block effect, Tukey post-hoc contrast tests were used to deduce which block was significantly different, so that it could be removed from analysis. Pairwise comparisons with Bonferroni corrections were

performed to examine significant interactions and Tukey post-hoc contrast tests were used to compare significant differences within treatments.

Aboveground Si contents of *V. faba* were not normally distributed, so a Mann Whitney U test was used to test the effect of watering, and a Kruskall-Wallis H test was used to test for block effect and the effect of growth substrate treatment. The impact of growth substrate treatment and watering level on presence of root nodules in *V. faba* root systems was analysed using a Chi-squared test.

3.3 Results

The following section presents the results from the two experiments which examined the effect of SP and S408 on water retention of the growth substrate at two different watering levels, and the impact of adding SP and AMF to the growth substrate on the ability of *Z. mays* and *V. faba* to tolerate drought.

3.3.1 Assessing the impact of two Silwet adjuvants on water retention of a sandy growth substrate

In the pilot study, both adjuvant treatments performed similarly in improving growth substrate water retention compared to the untreated control, with the growth substrate that was treated with S408 showing the greatest overall improvement. The growth substrate treated with S408 drained more slowly than either the growth substrate treated with SP or the untreated control growth substrate (i.e., ca. 19, 16, and 15 min, respectively). It also released a smaller amount of water from the column (i.e., 108, 116, and 118 ml, respectively). Furthermore, the % SMC of the growth substrate treated with S408 was slightly higher at the end of the 8d period compared to the growth substrate treated with SP and both greatly outperformed the untreated control growth substrate (25.5, 19.0 and 9.2%, respectively; Figure 3.5).

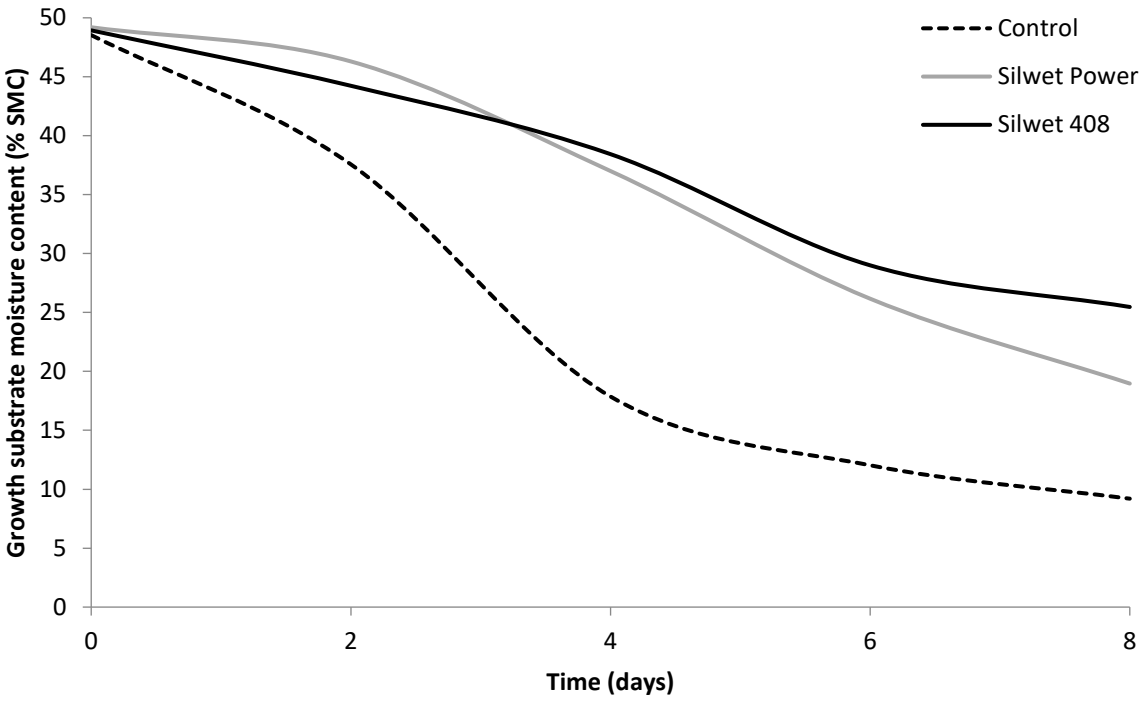

Figure 3.5. Moisture content of the growth substrate (% SMC) that was treated with Silwet™ Power, Silwet™ 408 or no adjuvant (control) and watered to saturation, then measured over an 8 d period (*n* = 1). No statistical analyses were applied due to the absence of replication.

3.3.2 The impact of two Silwet adjuvants on growth substrate water holding capacity

In the pot trial, the gravimetric data collected throughout the experiment showed high variability, possibly influenced by environmental conditions and/or equipment inaccuracies (see Figure S5). Therefore, these data were excluded from further analysis and statistical analyses were only carried out on % SMC data collected by capacitance probe.

In round 1 of the experiment (see Figure 3.3), % SMC was significantly improved by both adjuvants, although not by adjuvant concentration (Table 3.1). The full data set is shown in Figure S6.

Table 3.1. The results of a generalised linear model (GLM) with Gamma probability distribution and log link function comparing the effects of watering regime (ambient: 50%, drought: 20% of the soil water holding capacity), adjuvant type (Silwet™ Power [SP], Silwet™ 408 [S408], control with no adjuvant) and adjuvant concentration (SP 0.05 g l^{-1}, SP 0.1 g l^{-1}, S408 0.025 g l^{-1}, S408 0.05 g l^{-1}, 0 g l^{-1} [untreated control]) on growth substrate moisture content (% SMC) over 13 d. Significant *P* values are indicated in bold (*P* ≤ 0.05).

Source of variation	**Substrate moisture content (% SMC)**		
	Wald Chi-Square	***df***	***P***
Watering	1787	1	**< 0.001**
Adjuvant type	5.7	1	**0.017**
Adjuvant concentration	1.6	1	0.201
Watering * Adjuvant type	8.1	1	**0.004**
Watering * Adjuvant concentration	0.4	1	0.530
Adjuvant type * Adjuvant concentration	10.6	1	**0.001**
Watering * Adjuvant type * Adjuvant concentration	1.9	1	0.173

df = degrees of freedom *n* = 5.

The significant interaction between adjuvant type and watering (GLM, $X^2 = 8.1$, $P = 0.004$; Table 3.1) was due to SP being more effective than S408 in improving % SMC under ambient watering; however, neither adjuvant type differed from the untreated control. Under drought watering however, both adjuvants improved % SMC compared to the untreated control (Figure 3.6).

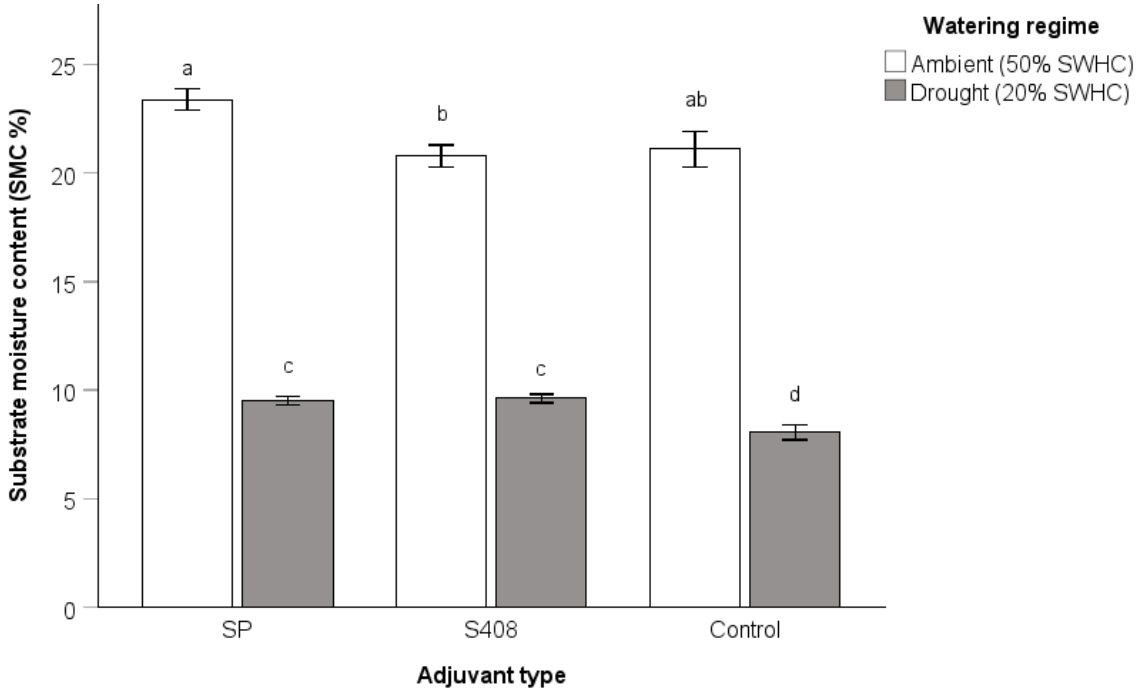

Figure 3.6. The graph shows the significant interaction between adjuvant type and watering on moisture content of growth substrate (% SMC; GLM, $X^2 = 8.1$, $P = 0.004$), determined by pairwise comparison with Bonferroni correction. Growth substrate was treated with Silwet™ Power (SP) or Silwet™ 408 (S408) and watered to drought or ambient level (20% and 50% of the soil water holding capacity, respectively). Different letters indicate significant differences at $P \leq 0.05$. Error bars are ± 1 standard error of the mean ($n = 10$).

To further examine the interaction between adjuvant type and concentration, the % SMC data was re-analysed excluding the untreated control data (see Figure 3.7). At low concentration SP significantly improved % SMC compared to when present at high concentration. Moreover, at low concentration SP improved % SMC compared to S408, whereas there was no difference between the two adjuvants at high concentration. There was no difference in % SMC between the low and high concentration of S408 (Figure 3.7).

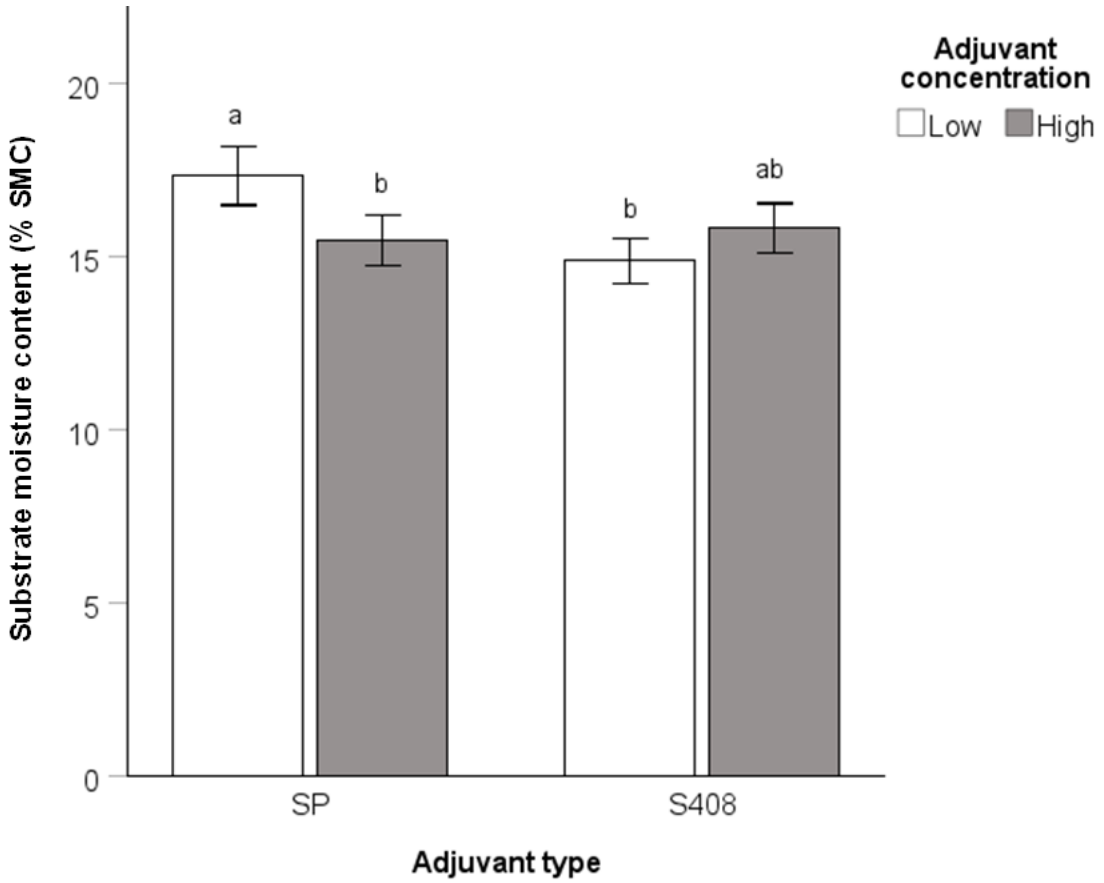

Figure 3.7. Average moisture content of growth substrate (% SMC) treated with Silwet™ Power (SP) or Silwet™ 408 (S408) at low or high concentration (see Table 2.1). Although concentration alone did not significantly affect moisture content of the growth substrate, there was a significant interaction between adjuvant concentration and adjuvant type (GLM, $X^2 = 10.6$, $P < 0.001$). The interaction was ascertained using pairwise comparisons with Bonferroni correction. Different letters indicate significant differences at $P \leq 0.05$. Error bars are ± 1 standard error of the mean ($n = 10$).

In round 2 of the experiment (see Figure 3.3), there was no significant difference in % SMC among treated and control pots or interaction terms; this effect was the same for both drought and ambient watering treatments (Table S5).

3.3.3 Drought tolerance of crops treated with Silwet soil adjuvants and arbuscular mycorrhizal fungi

3.3.3.1 Effect of Silwet Power and arbuscular mycorrhizal fungi on root length colonisation and hyphal length density in *Zea mays* and *Vicia faba*

Percentage root length colonisation (% RLC) of plants grown in substrate treated with AMF only, SP only, AMF+SP, or untreated (controls) and watered to either ambient (50% of the soil water holding capacity [SWHC]) or drought (30% of the SWHC) was assessed after 56 d growth. The frequency of intraradical hyphae, arbuscules and vesicles were also assessed (Figure 3.8).

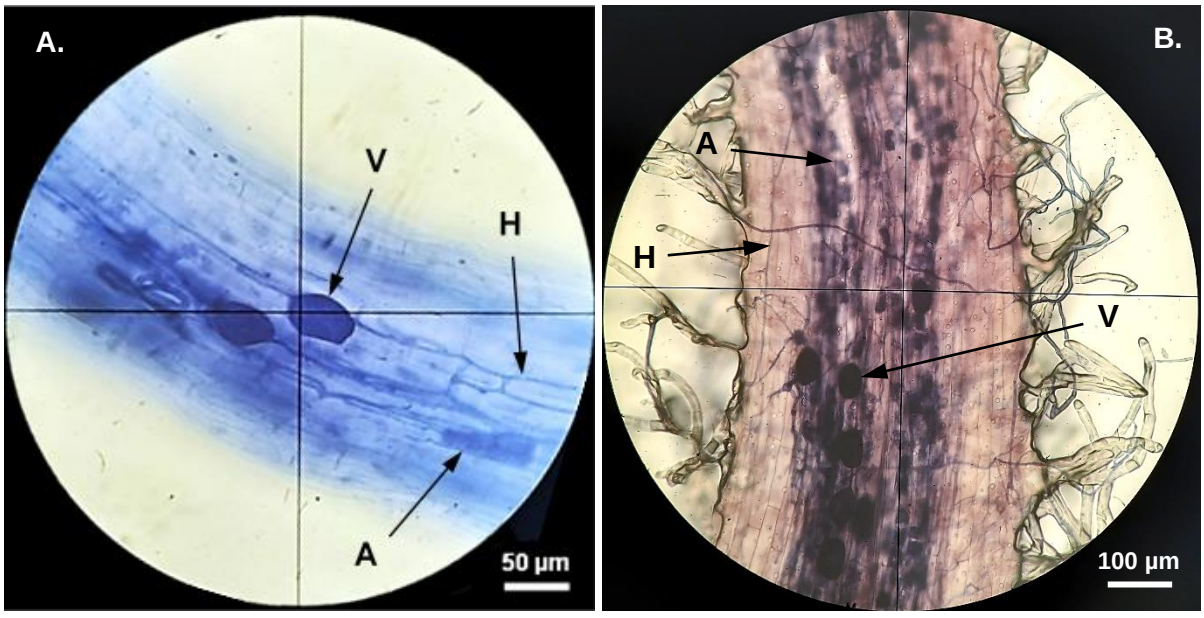

Figure 3.8. Examples of stained roots which were inoculated with the arbuscular mycorrhizal fungi (AMF) *Rhizophagus irregularis.* AMF structures present include intraradical hyphae (H), arbuscules (A), and vesicles (V). Figure 3.8A. A section of *Zea mays* L. root which was photographed after 8 weeks cultivation in a glasshouse. Figure 3.8B. A colonised section of *Vicia faba* L. root. White scale bar is equal to 100 µm.

For *Z. mays* roots, growth substrate treatment significantly ($F_{3,32}$ = 21.1, P < 0.001) increased % RLC (mean % RLC: 64 ± 11%) in those plants that had been treated with the AMF inoculum compared to those treated with SP only or the control plants (mean % RLC across treatments: 32 ± 13%) (Figure 3.9).

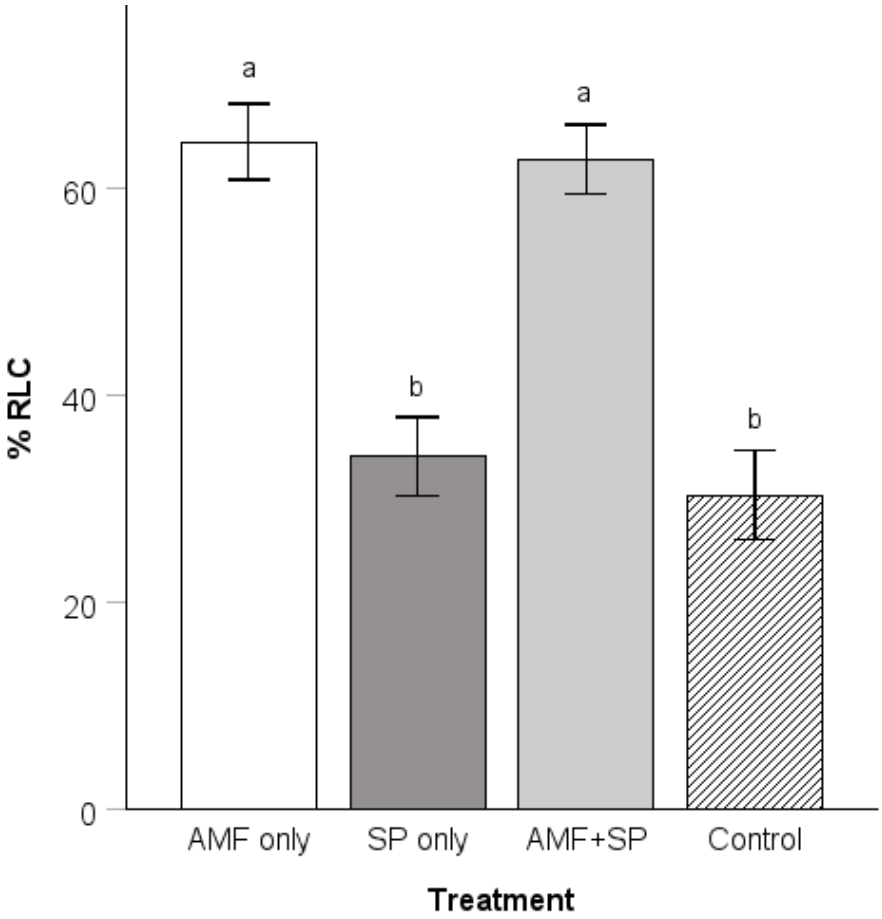

Figure 3.9. Average percentage root length colonisation (% RLC) of *Zea mays* L. plants grown in substrate treated with arbuscular mycorrhizal fungi (AMF) only, Silwet™ Power (SP) only or both (AMF+SP). Control plants were untreated. Addition of AMF increased % RLC in all cases and SP did not negatively impact % RLC, determined by a Tukey HSD post-hoc test with Bonferroni correction. Different letters indicate significant differences among treatments ($P \leq 0.05$) and error bars are ± 1 standard error of the mean (n = 10).

There were no significant differences in % RLC between *Z. mays* plants treated with AMF only and plants treated with AMF+SP, indicating that SP did not affect the ability of the added AMF inoculum to colonise *Z. mays* roots. Also, there were no significant differences in % RLC between plants treated with SP only and the no AMF added inoculum control plants, suggesting SP did not affect colonisation of *Z. mays* roots by background AMF populations present in the growth substrate either. Watering level did not affect ($F_{1,32}$ = 0.09, P = 0.771) % RLC of *Z. mays* plants (mean % RLC: 48 ± 3%).

In *V. faba* roots, % RLC values were lower than for *Z. mays* and neither the growth substrate treatment nor watering level significantly affected mean % RLC (overall mean: 36 ± <1%). In both *Z. mays* and *V. faba*, the frequency of arbuscules (mean across treatments: 9 ± 1% [*Z. mays*] and 4 ± 1% [*V. faba*]) and vesicles (4 ± 1% [*Z. mays*] and 1 ± <1% [*V. faba*]) in plant roots were low and not significantly affected by growth substrate treatment (*Z. mays*: $F_{3,32}$ = 1.89, P = 0.151 arbuscules and $F_{3,32}$ = 2.59, P = 0.070 vesicles; *V. faba*: $F_{3,28}$ = 1.16, P = 0.343 and $F_{3,28}$ = 1.24, P = 0.313, respectively) or watering level (*Z. mays*: $F_{1,32}$ = 0.07, P = 0.794, and $F_{1,32}$ = 0.03, P = 0.862, respectively; *V. faba*: $F_{3,28}$ = 0.33, P = 0.569 and $F_{3,28}$ = 1.03, P = 0.320, respectively).

There were significantly ($F_{3,70}$ = 18.3, P < 0.001) more HLD around the roots of *Z. mays* plants inoculated with AMF (mean HLD across treatments: 0.25 ± 0.02 mg g^{-1} soil DW) compared to plants that did not receive the AMF inoculum (SP only and control growth substrate treatments; mean HLD: 0.14 ± 0.02 mg g^{-1} soil DW). Watering level did not significantly affect HLD around *Z. mays* plants. There was a significant ($F_{3,70}$ = 6.58, P < 0.001) interaction between watering levels and growth substrate treatments on HLD because under drought conditions, HLD was always higher for plants from growth substrate treated with AMF (AMF only and AMF+SP) and lower for non-AMF treatments (SP only and control), but under ambient conditions the difference between AMF and non-AMF treatments was not significant (Figure 3.10).

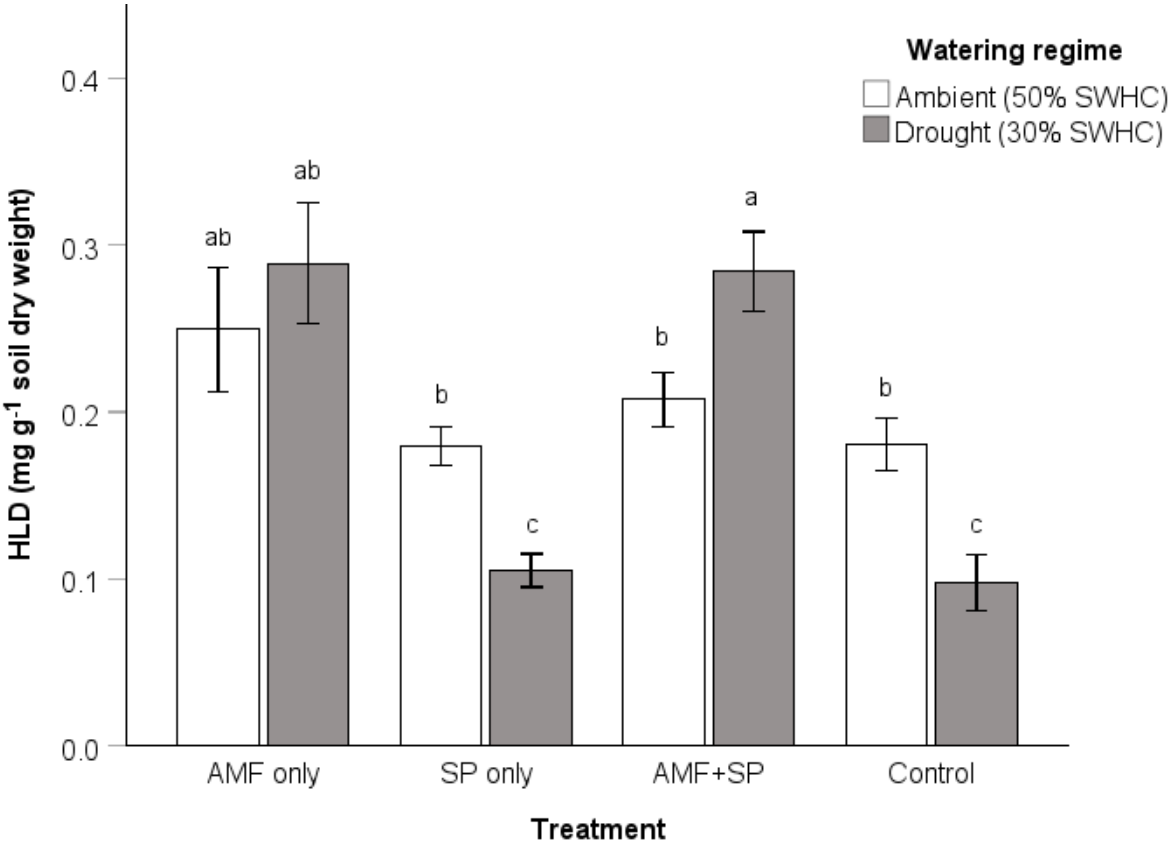

Figure 3.10. The significant ($F_{3,70}$ = 6.58, P < 0.001) interaction between watering and growth substrate treatment, which was tested by pairwise comparison with Bonferroni correction. Hyphal length density (HLD, mg g^{-1} soil dry weight) of *Zea mays* L. plants grown under ambient or drought watering conditions and treated with arbuscular mycorrhizal fungi (AMF), Silwet™ Power (SP), AMF+SP or untreated controls was measured, ± 1 standard error (n = 5). Ambient and drought watering was 50% and 30% of the soil water holding capacity (SWHC), respectively. For all variables, different letters represent significant differences ($P \leq$ 0.05).

In *V. faba*, the HLD was not significantly affected by watering level ($F_{1,28}$ = 0.37, P = 0.550) or growth substrate treatment ($F_{3,28}$ = 2.49, P = 0.081) and there was no interaction between the two (overall mean HLD: 0.12 ± 0.01 mg g^{-1} soil DW).

3.3.3.2 The impact of Silwet Power and arbuscular mycorrhizal fungi (AMF) biofertilizer on plant biomass, water content and nutrient levels

For both *Z. mays* and *V. faba* plants, there were no signs of phytotoxicity caused by exposure to SP. Growth substrate treatment did not affect the following variables for either plant species: specific leaf area; water content and biomass; aboveground-to-belowground water content ratio and aboveground-to-belowground biomass ratio and root system length. Of these variables, there were significant effects of watering level on mean biomass and water content for both plant species as shown in Table 3.2 while Table 3.3 gives the results of the statistical analysis. Although root nodules were not present on all *V. faba* root systems, growth substrate treatment nor watering level significantly impacted their presence ($X^2_{3,\, n\,=\,34}$ = 1.724, P = 0.632).

Table 3.2. Mean (± 1 SE) biomass and water content for aboveground and belowground parts of *Zea mays* L. and *Vicia faba* L. plants, the plants were cultivated under ambient (50% of the soil water holding capacity [SWHC]) or drought (30% of the SWHC) watering levels in a growth substrate that had been treated with arbuscular mycorrhizal fungi (AMF) only, Silwet™ Power (SP) only, AMF+SP or untreated (control). None of the growth substrate treatments had any significant impacts on the reported variables compared to control plants, but there were effects from watering level (see Table 3.3).

Variable	***Zea mays* L.**		***Vicia faba* L.**	
	Ambient	**Drought**	**Ambient**	**Drought**
Aboveground biomass (g)	1.3 ± 0.1	1.0 ± 0.1	1.2 ± 0.1	0.7 ± 0.1
Belowground biomass (g)	1.3 ± 0.1	1.0 ± 0.1	0.7 ± 0.1	0.5 ± 0.1
Root weight ratio (g g^{-1})	0.53 ± 0.03	0.47 ± 0.02	0.37 ± 0.02	0.41 ± 0.03
Aboveground water content (g)	9.1 ± 0.5	6.7 ± 1.6	8.2 ± 0.8	4.9 ± 0.4

Table 3.3. Results from the statistical analysis of aboveground and belowground biomass and water content of *Zea mays* L. and *Vicia faba* L. plants, in which there were significant watering effects but no growth substrate treatment effects. Plants were grown under two watering regimes (ambient: 50% and drought: 30% of the soil water holding capacity of the growth substrate) in growth substrate treated with arbuscular mycorrhizal fungi (AMF) only, Silwet™ Power (SP) only, AMF+SP or untreated controls. Analysis was carried out by 2-way ANOVA with Bonferroni correction. *P* values in bold are significant ($P \leq 0.05$).

Variable	***Zea mays* L.**				***Vicia faba* L.**			
	df	**Mean square**	***F***	***P***	***df***	**Mean square**	***F***	***P***
Aboveground biomass	1,31	0.74	8.73	**0.006**	1,28	2.13	16.3	**< 0.001**
Belowground biomass	1,32	1.14	6.22	**0.018**	1,28	0.20	2.82	0.104
Root weight ratio	1,32	0.11	0.97	0.332	1,28	0.27	4.47	**0.044**
Aboveground water content	1,32	67.4	38.7	**< 0.001**	1,28	88.6	11.3	**0.002**

df = degrees of freedom. For *Z. mays, n* = 10 and for *V. faba, n* = 7.

All the *Z. mays* leaves were N deficient (mean N concentration across all plants: 6 ± <1 mg g^{-1}) regardless of growth substrate treatment or watering level, evident by yellowing of new leaves which began at the tips and spread down the leaf (Figure 3.11).

Figure 3.11. *Zea mays* L. plants were nitrogen deficient across all growth substrate treatments and watering levels, apparent from yellowing of leaves starting at the leaf tips and spreading down the leaf as indicated by the white arrows.

Aboveground N content ($F_{3,32}$ = 0.47, P = 0.705; Figure 3.12B) and aboveground biomass (see Table 3.3 for P values) in *Z. mays* were unaffected by treatment of the growth substrate. However, N concentration of *Z. mays* leaves from the AMF only growth substrate was significantly ($F_{3,32}$ = 6.53, P = 0.001) higher (mean N concentration: 6.4 ± 0.2 mg g^{-1}) than plants from all other growth substrate treatments and controls (mean across treatments: 6 ± <1 mg g^{-1}; Figure 3.12A). Overall, this indicates that the AMF only treatment increased the total N per unit biomass, as explained by the significantly ($F_{3,32}$ = 7.27, P < 0.001) lower aboveground C:N ratio in AMF-only treated plants compared to all other growth substrate treatments (Figure 3.12C).

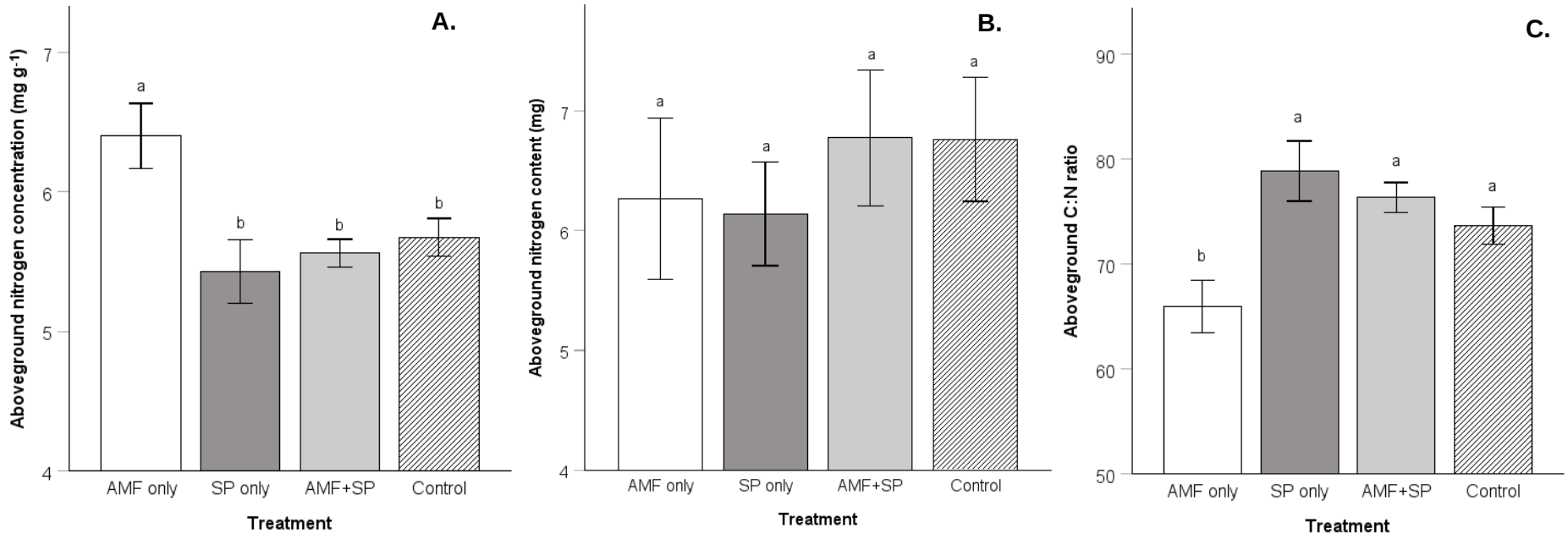

Figure 3.12. The aboveground nitrogen (N) concentration (mg g^{-1}), N content (mg) and carbon-to-nitrogen (C:N) ratio in *Zea mays* L. plants treated with arbuscular mycorrhizal fungi only (AMF), Silwet™ Power only (SP), AMF+SP or untreated (controls). Plants treated with AMF only had the highest N concentration compared to all other treatments ($F_{3,32}$ = 6.53, P = 0.001; Figure 3.12A), but there were no differences in aboveground N content among growth substrate treatments ($F_{3,32}$ = 0.47, P = 0.705; Figure 3.12B). The aboveground C:N ratio was significantly ($F_{3,32}$ = 7.27, P < 0.001; Figure 3.12C) reduced by AMF only compared to all other growth substrate treatments, suggesting that the AMF only treatment increased the total N per unit biomass in the aboveground parts of *Z. mays* plants. Please note the Y axis of all graphs does not start at 0 to demonstrate the differences among treatments more clearly. Error bars are ± 1 standard error (n = 10). For all variables, different letters represent significant differences ($P \leq 0.05$).

Watering did not affect N or C concentrations in *Z. mays* leaves. However, under drought conditions aboveground N ($F_{1,32}$ = 9.08, P = 0.005) and C content ($F_{1,32}$ = 9.46, P = 0.004) were significantly lower (mean aboveground C content: 422 ± 27 mg and mean aboveground N content: 6 ± 1 mg) than ambient conditions (mean aboveground C content: 541 ± 31 mg and mean aboveground N content: 7 ± <1 mg). This decrease was attributed to the lower aboveground biomass of *Z. mays* plants grown under drought conditions (see Table 3.2). There were no interactions between watering and growth substrate treatment for N or C.

There were significant impacts of both watering level and growth substrate treatment on P concentration ($F_{1,32}$ = 142.1, P < 0.001 and $F_{3,32}$ = 42.3, P < 0.001, respectively). There was also an interaction between watering and growth substrate treatment ($F_{3,32}$ = 10.5, P < 0.001), but the driver of this effect was unclear due to a significantly higher P concentration in control plants grown under ambient conditions (272 ± 10 µg g^{-1}) compared to all other growth substrate treatments and watering levels (Figure 3.13).

To interrogate whether there was a true interaction occurring between watering level and growth substrate treatment, the analysis was repeated excluding the control plant data, which removed the significant interaction term. Plants treated with AMF only had significantly (Tukey post-hoc: P < 0.001) higher aboveground P concentrations than plants that were treated with SP or the control plants (Figure 3.14A). Aboveground P content of *Z. mays* plants was also significantly ($F_{3,32}$ = 11.1, P < 0.001) impacted by the treatment of the growth substrate and was higher (Tukey post-hoc: P = 0.014) in the plants from the AMF only growth substrate treatment than the SP only growth substrate treatment. The aboveground P contents of *Z. mays* plants treated with both AMF and SP, however, did not differ from when these treatments were added singly (Figure 3.14B).

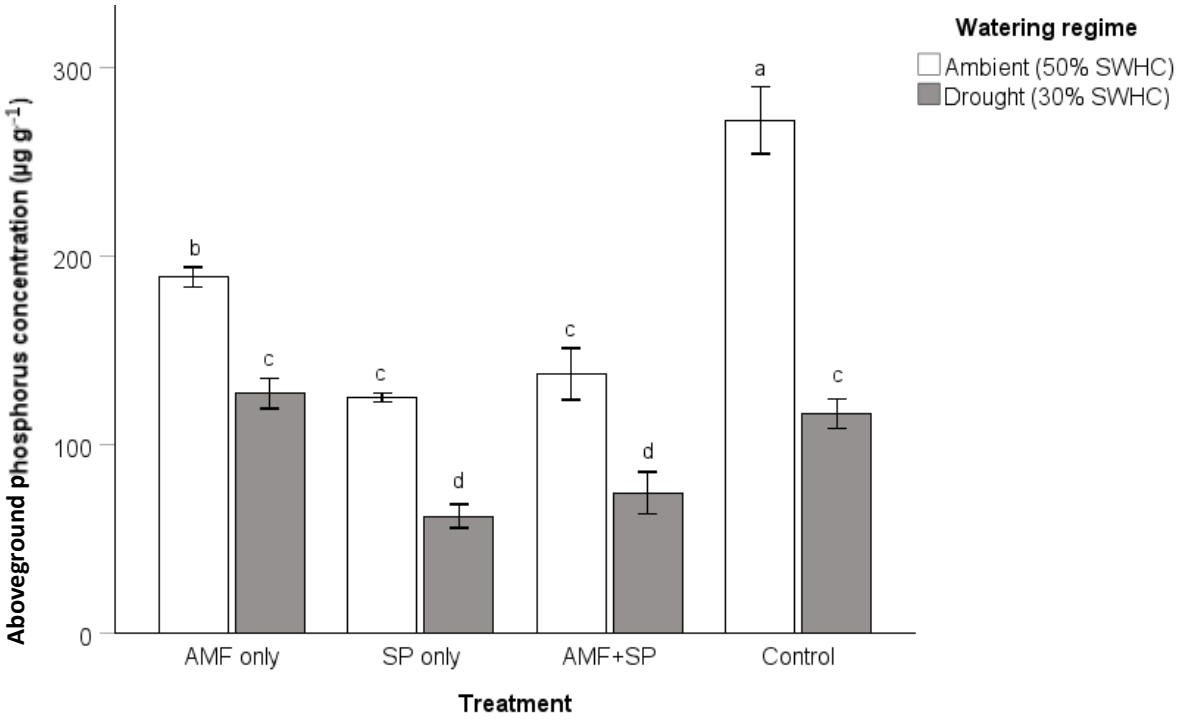

Figure 3.13. Aboveground phosphorus (P) concentration (µg g^{-1}) of *Zea mays* L. plants grown under ambient or drought watering conditions and cultivated in growth substrate treated with arbuscular mycorrhizal fungi (AMF) only, Silwet™ Power (SP) only, AMF+SP or untreated controls, ± 1 standard error (n = 5). Ambient and drought watering was 50% and 30% of the soil water holding capacity (SWHC), respectively. There was a significant interaction between watering and growth substrate treatment ($F_{3,32}$ = 10.5, P < 0.001) that was revealed by a pairwise comparison with Bonferroni correction test to be driven by two factors. The P concentrations in ambient watered control plants were higher compared to all other treatments and watering levels, and the aboveground P concentrations in plants from the treatments which included SP (SP only and AMF+SP) were reduced. Different letters represent significant differences among growth substrates and watering level treatments (P ≤ 0.05).

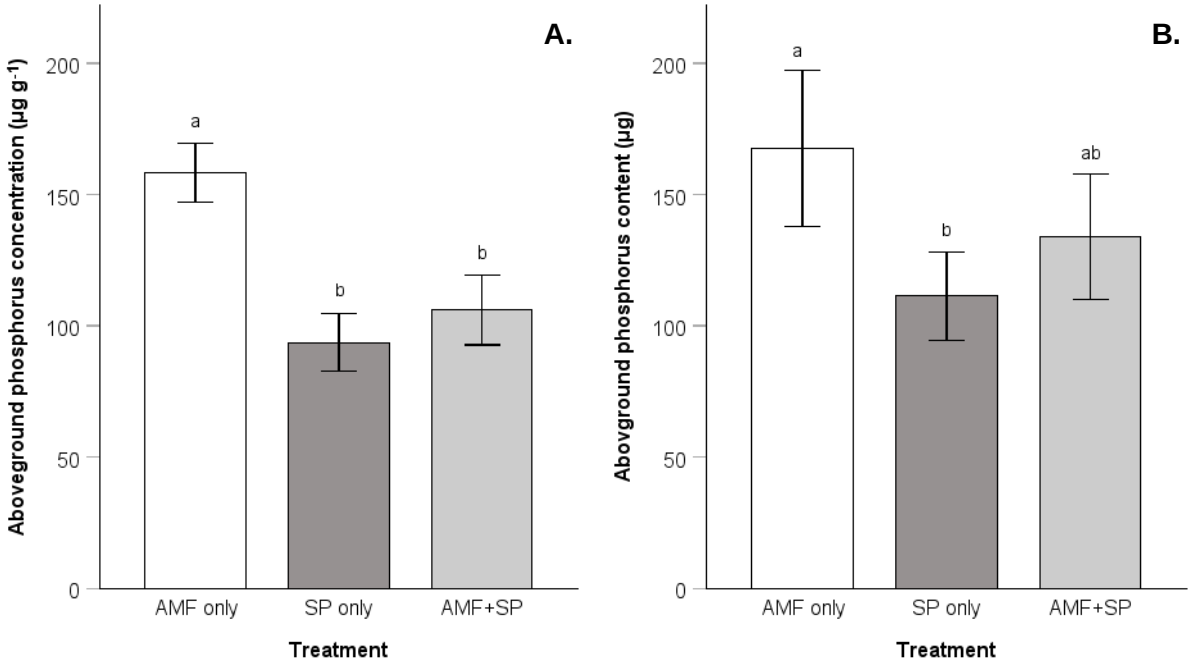

Figure 3.14. Aboveground phosphorus (P) concentration (µg g^{-1}; A) and aboveground P content (µg; B) of *Zea mays* L. plants cultivated in growth substrate treated with arbuscular mycorrhizal fungi (AMF) only, Silwet™ Power (SP) only or AMF+SP, ± 1 standard error (*n* =10). Aboveground parts of plants cultivated in growth substrate treated with AMF only had significantly (Tukey post-hoc: $P < 0.001$) higher P concentrations and P content (Tukey post-hoc: $P = 0.014$) than the foliage of plants grown with SP added to the growth substrate, either with AMF or without. Pairwise comparisons were tested by Tukey HSD with Bonferroni correction. Different letters represent significant differences among treatments ($P \leq 0.05$).

As aboveground biomass was unchanged by growth substrate treatment, this suggests that the AMF only treatment caused plants to accumulate more P per unit biomass than plants from all other growth substrate treatments. This is supported by the significantly lower aboveground C:P ratio ($F_{3,32} = 24.6$, $P < 0.001$; Figure 3.15A) and N:P ratio ($F_{3,32} = 12.4$, $P < 0.001$; Figure 3.15B) in plants from the AMF only growth substrate treatment compared to plants cultivated in growth substrate with SP.

Watering level significantly reduced the aboveground P content ($F_{3,32} = 64.8$, $P < 0.001$) and concentration ($F_{1,32} = 142.1$, $P < 0.001$) of *Z. mays* plants grown under drought conditions compared to under ambient conditions; the i.e., mean P content was 93 ± 15 µg *vs* 227 ± 15 µg, respectively and the mean P concentration was 95 ±

7 µg g^{-1} *vs* 181 ± 14 µg g^{-1}, respectively. This could be explained in part by the reduced aboveground biomass in drought watered *Z. mays* plants (see Table 3.2), but the lower P concentration suggests a change in the total P per unit biomass in drought watered *Z. mays* plants. There was a significant interaction ($F_{3,32}$ = 4.16, P = 0.013) between growth substrate treatment and watering on the C:P ratio in *Z. mays*, caused by a higher aboveground C:P ratio in SP-treated plants (SP only and AMF+SP) under drought watering. Drought watered plants had significantly ($F_{1,32}$ = 83.6, P < 0.001 and $F_{1,32}$ = 63.8, P < 0.001, respectively) higher aboveground C:P and N:P ratios (mean C:P ratio: 5021 ± 429 and mean N:P ratio: 70 ± 6) compared to ambient watered plants (mean C:P ratio: 2592 ± 189 and mean N:P ratio: 34 ± 6) (Figure 3.15). Together, this suggests that drought watered plants were storing less P than plants grown under ambient conditions.

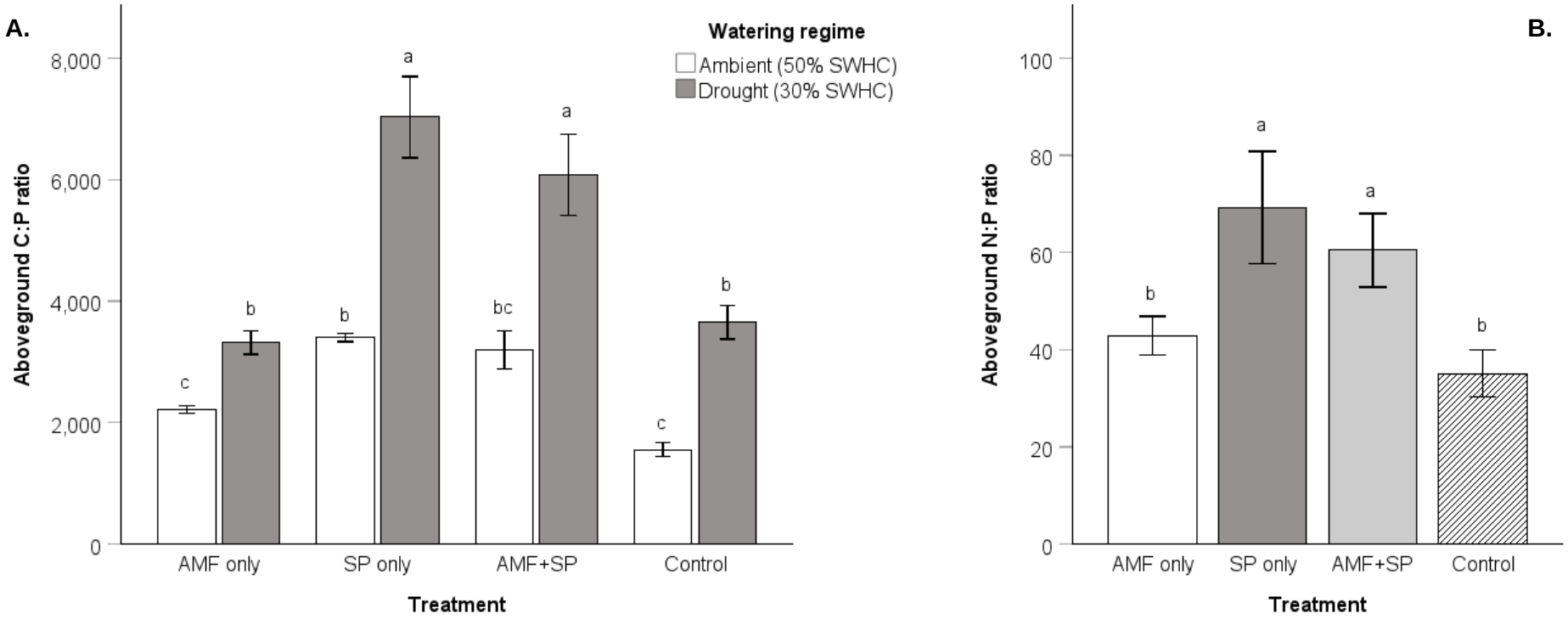

Figure 3.15. The carbon-to-phosphorus (C:P) ratio (A) and the nitrogen-to-phosphorus (N:P) ratio (B) of *Zea mays* L. plants grown under ambient (50% of the soil water holding capacity [SWHC]) or drought (30% of the SWHC) watering conditions in growth substrate treated with arbuscular mycorrhizal fungi (AMF) only, Silwet™ Power (SP) only, AMF+SP or untreated controls, ± 1 standard error (n = 5). There was a significant interaction between watering and growth substrate treatment ($F_{3,32}$ = 4.16, P = 0.013) on the C:P ratio, which was tested by pairwise comparison with Bonferroni correction. The N:P ratio was affected by growth substrate treatment ($F_{3,32}$ = 12.4, P < 0.001), with treatments containing Silwet™ Power (SP) causing a higher N:P ratio compared to treatments without SP (AMF only and untreated controls). Error bars are ± 1 standard error (n = 10). For all variables, different letters represent significant differences (P ≤ 0.05).

All growth substrate treatments significantly ($F_{3,29}$ = 3.46, P = 0.029) reduced aboveground N concentration in *V. faba* compared to control plants (Figure 3.16A), but the aboveground N content (mean: 35.4 ± 2.8 mg N; Figure 3.16B), biomass (mean: 948 ± 450 mg), and C:N ratio (mean: 0.1 ± <0.01) of *V. faba* plants were unaffected by growth substrate treatment. As there was no change in the aboveground C:N ratio, this suggests that all the growth substrate treatments caused a reduction in N accumulation per unit biomass compared to control plants.

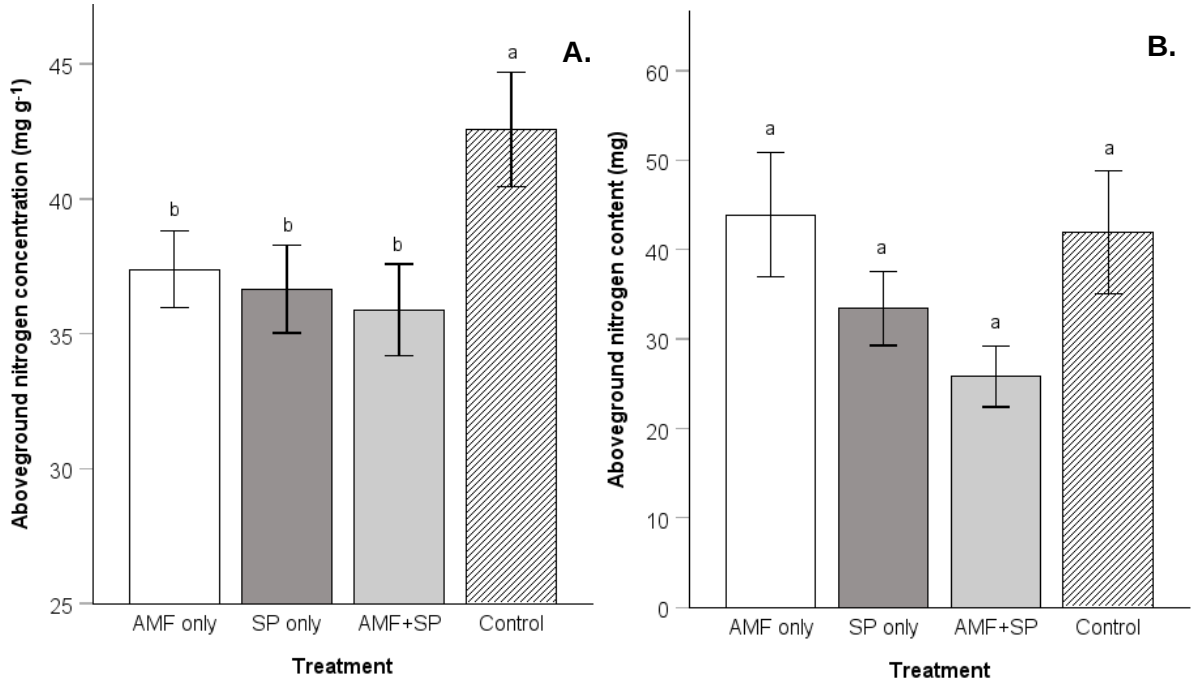

Figure 3.16. Nitrogen (N) concentration (mg g^{-1}; A) and N content (mg; B) in the leaves of *Vicia faba* L. plants cultivated in growth substrate which was treated with arbuscular mycorrhizal fungi (AMF) only, Silwet™ Power (SP) only, AMF+SP or left untreated (controls). Aboveground N concentration was shown by Tukey post-hoc test to be significantly ($F_{3,29}$ = 3.46, P = 0.029) lower in all growth substrate treatments compared to control plants, but the N content of *V. faba* plants was not significantly affected by growth substrate treatment ($F_{3,28}$ = 2.79, P = 0.059). Error bars are ± 1 standard error (n = 7). For all variables, different letters represent significant differences among treatments ($P \leq 0.05$).

There were no significant differences in P concentration among growth substrate treatments or watering levels (mean across treatments: 188 ± 26 µg g^{-1}). Aboveground N concentration was also unaffected by watering level, but N and P

content were both significantly (aboveground N content: $F_{1,28}$ = 13.5, P = 0.001 and aboveground P content: $F_{1,28}$ = 15.6, P < 0.001) lower (mean N content: 26.6 ± 9.7 mg and mean P content: 2657 ± 854 µg) in plants grown under drought conditions compared to those cultivated under ambient conditions (mean aboveground N content: 44 ± 4 mg and mean aboveground P content: 4789 ± 1927 µg). This can be explained by the aboveground biomass of *V. faba* plants being significantly reduced in drought watered plants compared to ambient watered plants (see Table 3.2 and Table 3.3).

Although aboveground water content was reduced by drought watering, it was not affected by growth substrate treatment in either *Z. mays* or *V. faba* (See Table 3.2 and Table 3.3). Also, the ratio of aboveground-to-belowground water content was unchanged by watering in *V. faba*, but it was increased by drought watering in *Z. mays*, thus indicating that drought watering increased allocation of water to the aboveground parts of the plant (Table 3.3).

3.3.3.3 Changes to proline and micronutrients in *Zea mays* and *Vicia faba* resulting from treatment with Silwet Power and arbuscular mycorrhizal fungi (AMF)

Proline, an amino acid normally associated with water stress, concentrations in leaf tissues were higher in *V. faba* (mean across treatments: 21 ± 1 mg g^{-1} FW) than in *Z. mays* (mean across treatments: 12 ± <1 mg g^{-1} FW). *Z. mays* plants from the growth substrate treated with AMF only had lower ($F_{3,32}$ = 40.9, P < 0.001) foliar proline concentrations (mean: 12 ± 1 mg g^{-1} FW) than plants treated with SP only and AMF+SP (mean: 13 ± 1 mg g^{-1} FW). There was no difference in the foliar proline concentrations of ambient *vs.* drought watered plants, except where there was a significant ($F_{3,32}$ = 7.55, P < 0.001) interaction in plants from the AMF only treatment which had lower proline concentrations under ambient watering (Figure 3.17). Control plants generally had lower foliar proline concentrations (mean: 9 ± 1 mg g^{-1}

FW) than all other treatments (13 ± <1 mg g^{-1} FW), with the exception of plants from the ambient watered AMF only treatment.

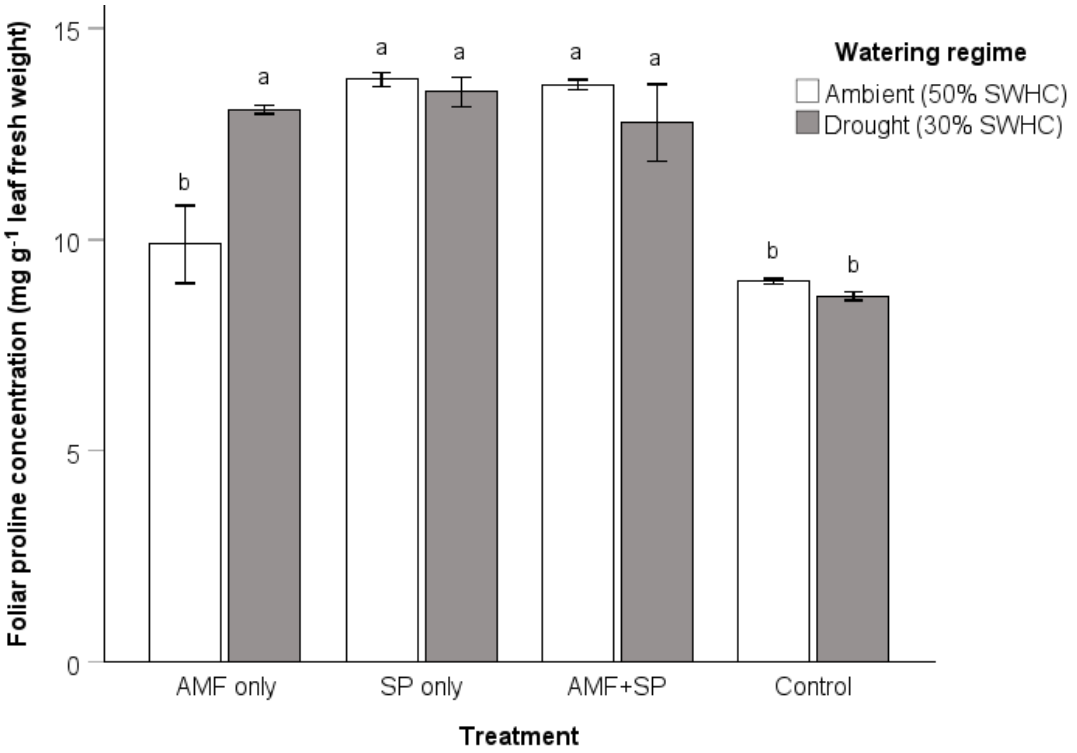

Figure 3.17. Foliar proline concentration (mg g^{-1} leaf fresh weight) of *Zea mays* L. plants that were grown under ambient (50% of the soil water holding capacity [SWHC]) or drought (30% of the SWHC) watering conditions in growth substrate treated with Silwet™ Power (SP) only, arbuscular mycorrhizal fungi (AMF) only, AMF+SP, or untreated (controls). There was a significant ($F_{3,32}$ = 7.55, P < 0.001) interaction between growth substrate treatment and watering level, as tested by a pairwise comparison with Bonferroni correction. Error bars are ± 1 standard error (n = 5). For all variables, different letters represent significant differences among treatments ($P \leq 0.05$).

Foliar proline concentration in *V. faba* was not affected by watering level, but plants from the AMF+SP treatment had significantly ($F_{3,28}$ = 4.64, P = 0.009) lower mean foliar proline concentrations (mean: 18 ± 1 mg g^{-1} FW) than plants from all other growth substrate treatments and controls (mean across treatments: 22 ± 1 mg g^{-1} FW; Figure 3.18).

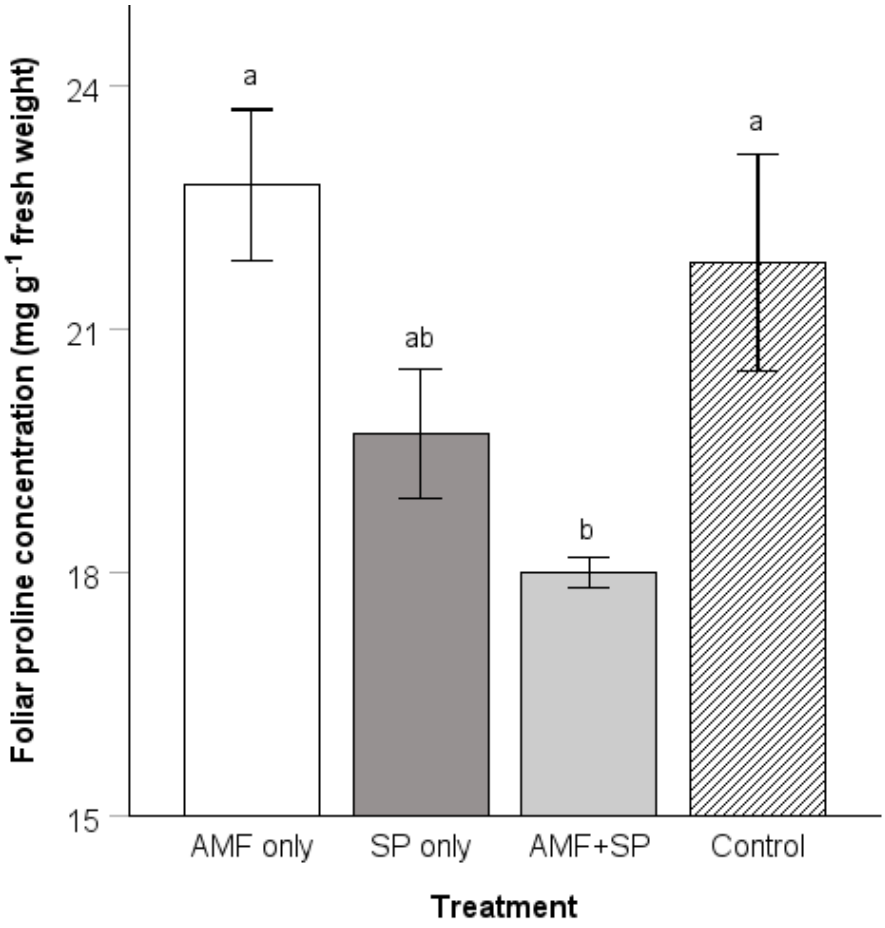

Figure 3.18. Foliar proline concentration (mg g^{-1} fresh weight) of *Vicia faba* L. plants was significantly ($F_{3,28}$ = 4.64, P = 0.009) lower in plants treated with both arbuscular mycorrhizal fungi (AMF) and Silwet™ Power (SP) compared to plants that were treated with AMF only, SP only, or control plants. Error bars are ± 1 standard error (n = 7). Different letters represent significant differences among treatments ($P \leq 0.05$), as determined a Tukey HSD post-hoc test.

Analysis of aboveground micronutrient concentration and content of K, Ca, Zn, B and Si revealed various impacts of watering level and growth substrate treatment upon both plant species. The effects of watering level are shown in Table 3.4, and significant differences and mean values for micronutrients from each growth substrate are shown in Table 3.5.

Table 3.4. Aboveground micronutrients were measured in *Zea mays* L. and *Vicia faba* L. plants which had been cultivated at two watering levels (ambient: 50% and drought: 30% of the growth substrate's soil water holding capacity) in growth substrate treated with arbuscular mycorrhizal fungi (AMF) only, Silwet™ Power (SP) only, AMF+SP or untreated controls. Analysis was carried out by two-way ANOVA with Bonferroni correction, with the exception of silicon concentration data from *V. faba* which, due to the non-normal distribution of the data, was tested by Mann-Whitney U-test.

Micronutrient Concentration (µg g^{-1})	***Zea mays* L.**				***Vicia faba* L.**			
	df	**Mean square**	***F***	***P***	***df***	**Mean square**	***F***	***P***
Potassium	1,32	66932	6.08	**0.021**	1,28	40511	1	0.327
Calcium	1,32	10015.5	10.8	**0.002**	1,28	0.008	0.42	0.524
Zinc	1,32	2.53×10^{-6}	0.35	0.558	1,26	2.58×10^{-7}	0.18	0.677
Boron	1,32	3.59×10^{-6}	1.01	0.324	1,28	1.83×10^{-7}	0.45	0.507
Silicon	1,32	8.01	3.93	0.056	1,28	*U* = 160		0.975
Micronutrient Content (µg)								
Potassium	1,32	615000	6.59	**0.015**	1,28	0.485	15.5	**< 0.001**
Calcium	1,32	3.42×10^{9}	0.77	0.386	1,28	122.9	6.41	**0.017**
Zinc	1,32	0.024	0.36	0.552	1,28	3.49	13.2	**0.001**
Boron	1,32	0.161	10.1	**0.003**	1,28	0.36	10.2	**0.003**
Silicon	1,32	0.012	0.34	0.562	1,28	3.94	7.36	**0.011**

P values in bold are significant ($P \leq 0.05$). For *Z. mays*, zinc and boron content data were log10 transformed. For *V. faba*, calcium and boron concentration, potassium and boron content data were log10 transformed and calcium, zinc and silicon content data were square root transformed.

Table 3.5. The mean values (± 1 SE) of aboveground micronutrients measured in *Zea mays* L. and *Vicia faba* L. plants that were cultivated in growth substrate treated with arbuscular mycorrhizal fungi (AMF) only, Silwet™ Power (SP) only, AMF+SP or untreated controls and watered at two watering levels (ambient: 50% and drought: 30% of the soil water holding capacity of the growth substrate). Significant differences between growth substrate treatments for each species were tested by a two-way ANOVA with Bonferroni correction, with the exception of silicon concentration data in *V. faba*, which were tested by Kruskal-Wallis given the data were not normally distributed. Significant differences between treatments for each micronutrient are indicated by different letters, but comparison is not between species or micronutrient.

Micronutrient Concentration (µg g^{-1})	***Zea mays* L.**				***Vicia faba* L.**			
	AMF only	**SP only**	**AMF+SP**	**Control**	**AMF only**	**SP only**	**AMF+SP**	**Control**
Potassium	1357 ± 34^{a}	1219 ± 37^{ab}	1197 ± 30^{b}	1262 ± 54^{ab}	1801 ± 53^{a}	1800 ± 67^{a}	1777 ± 66^{a}	1916 ± 68^{a}
Calcium	195 ± 16^{a}	133 ± 6^{b}	130 ± 8^{b}	187 ± 13^{a}	329 ± 43^{a}	247 ± 24^{a}	335 ± 38^{a}	260 ± 19^{a}
Zinc	2.7 ± 0.5^{a}	2.0 ± 0.3^{a}	3.1 ± 0.7^{a}	2.7 ± 0.7^{a}	4.2 ± 0.4^{a}	4.2 ± 0.3^{a}	4.1 ± 0.3^{a}	5.3 ± 0.4^{a}
Boron	1.8 ± 0.1^{a}	1.8 ± 0.1^{a}	1.7 ± 0.1^{a}	1.9 ± 0.1^{a}	2.8 ± 0.1^{a}	2.7 ± 0.1^{a}	3.0 ± 0.2^{a}	2.5 ± 0.1^{a}
Silicon	2.9 ± 0.3^{a}	5.0 ± 0.4^{b}	4.8 ± 0.8^{b}	3.1 ± 0.3^{a}	4.2 ± 1.8^{a}	12.7 ± 1.3^{b}	14.9 ± 1.0^{b}	2.5 ± 0.1^{a}
Micronutrient Content (µg)								
Potassium	1362 ± 147^{a}	1409 ± 116^{a}	1482 ± 102^{a}	1515 ± 78^{a}	1795 ± 287^{a}	2417 ± 436^{a}	2781 ± 292^{a}	2324 ± 445^{a}
Calcium	183 ± 14^{ab}	151 ± 13^{a}	157 ± 13^{a}	220 ± 18^{b}	346 ± 71^{a}	322 ± 61^{a}	492 ± 47^{a}	305 ± 50^{a}
Zinc	2.3 ± 0.3^{a}	2.3 ± 0.4^{a}	3.9 ± 1.1^{a}	3.3 ± 0.8^{a}	4.4 ± 0.8^{a}	5.4 ± 0.9^{a}	6.3 ± 0.8^{a}	6.5 ± 1.4^{a}
Boron	1.8 ± 0.2^{a}	2.1 ± 0.2^{a}	2.1 ± 0.2^{a}	2.3 ± 0.2^{a}	2.7 ± 0.4^{a}	3.7 ± 0.7^{a}	4.5 ± 0.4^{a}	3.1 ± 0.6^{a}
Silicon	3.0 ± 0.5^{a}	5.7 ± 0.7^{b}	5.6 ± 0.8^{b}	4.5 ± 0.4^{ab}	3.4 ± 0.9^{a}	17.1 ± 3.6^{b}	22.4 ± 2.2^{b}	2.9 ± 0.5^{a}

For *Z. mays*, zinc and boron content data were log10 transformed. For *V. faba*, calcium and boron concentration, potassium and boron content data were log10 transformed and calcium, zinc and silicon content data were square root transformed.

There was a significant ($F_{3,32}$ = 2.97, P = 0.047) interaction between growth substrate treatment and watering level affecting aboveground Ca concentration in *Z. mays*. Plants from any of the treated growth substrates (AMF only, SP only, AMF+SP) had a lower mean Ca concentration (153 ± 8 µg g^{-1}) than the control plants (mean: 187 ± 13 µg g^{-1}) regardless of watering level, with the exception of plants from the AMF only growth substrate treatment that were grown under drought conditions. Here, there was no significant difference compared to the control plants (Figure 3.19). Ca content (mean across treatments: 178 ± 8 µg) in *Z. mays* leaves was not impacted by growth substrate treatment or watering level, but aboveground biomass was significantly (*P* value in Table 3.3) lower (see Table 3.2 for mean value) in drought watered plants, overall suggesting a reallocation of Ca within plants treated with AMF only.

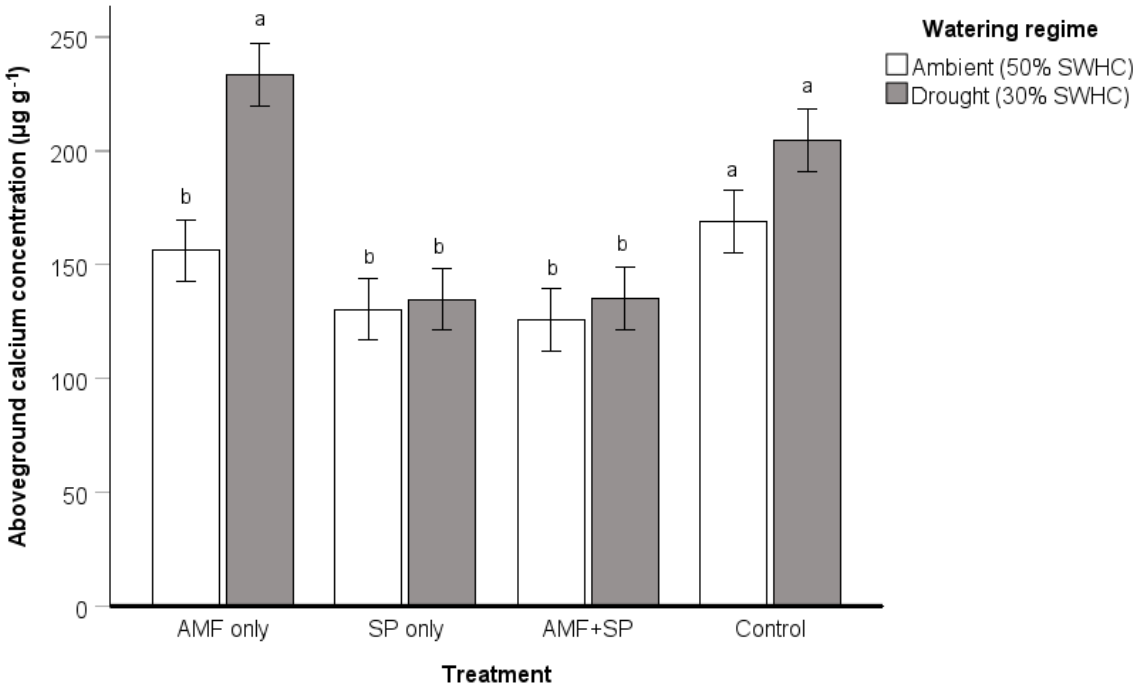

Figure 3.19. The interaction between watering level and growth substrate treatment on aboveground calcium (Ca) concentration (µg g^{-1}) of *Zea mays* L. plants was significant. Ca concentration was only impacted by watering level in plants from the arbuscular mycorrhizal fungi (AMF only) growth substrate treatment ($F_{3,32}$ = 2.97, P = 0.047), but not in those from all other growth substrate treatments (Silwet™ Power [SP], AMF+SP, or untreated controls). The interaction effect was tested by pairwise comparison with Bonferroni correction. Ambient and drought watering was 50% and 30% of the soil water holding capacity (SWHC), respectively. Error bars are ± 1 standard error (n = 5). For all variables, different letters represent significant differences among treatments ($P \leq 0.05$).

Aboveground K concentration in *Z. mays* was significantly ($F_{3,24}$ = 3.66, P = 0.026) higher in plants from the AMF only treatment compared to those from the SP treatments (SP only and AMF+SP; Table 3.5). However, neither treatment differed significantly from the control plants (Figure 3.20A). The lack of differences among aboveground K content (Figure 3.20B) or aboveground biomass among growth substrate treatments suggests that the higher K concentrations in plants from the AMF only growth substrate treatments were caused by a reallocation of plant K to aboveground plant tissues.

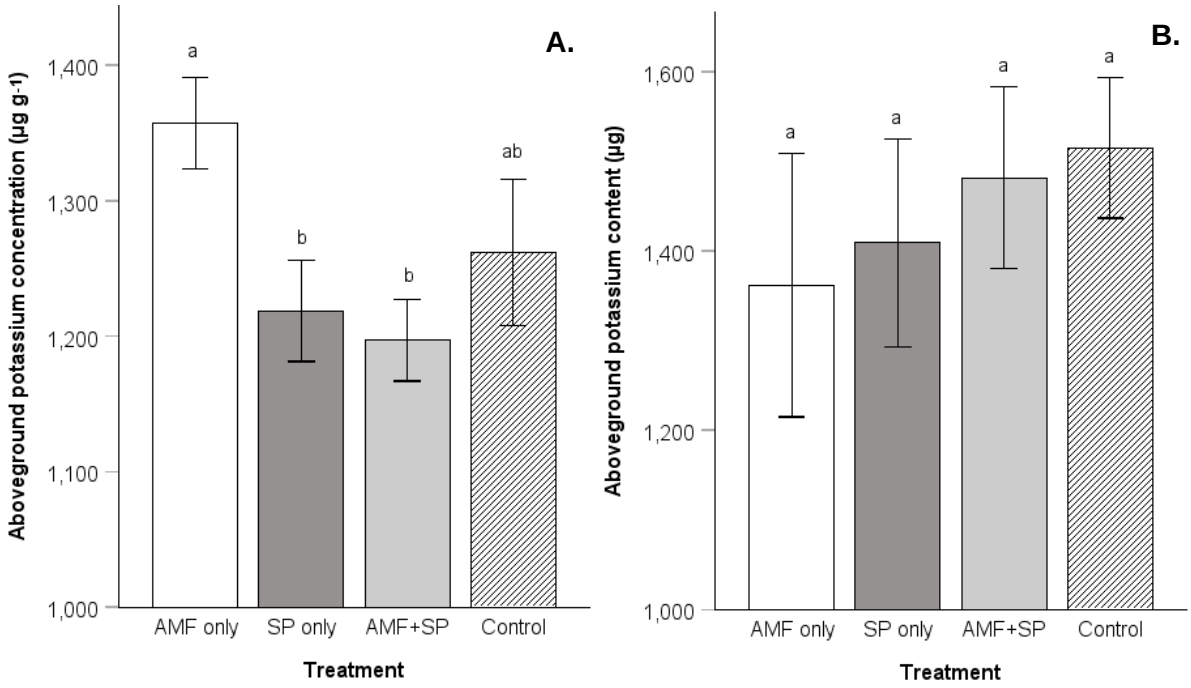

Figure 3.20. Aboveground potassium (K) concentration (µg g^{-1}; A) in *Zea mays* L. plants was significantly ($F_{3,24}$ = 3.66, P = 0.026) higher in plants from the arbuscular mycorrhizal fungi (AMF) only growth substrate treatment compared to the Silwet™ Power (SP) growth substrate treatment, or the combined growth substrate treatment (AMF+SP), as determined by Tukey HSD post-hoc comparison with Bonferroni correction. However, aboveground K content (µg; B) was unchanged by growth substrate treatment. Error bars are ± 1 standard error (n = 10). For all variables, different letters represent significant differences among treatments ($P \leq 0.05$).

Watering level significantly impacted both K concentration and K content in *Z. mays* leaves (see Table 3.4), but with opposite effects. Plants grown under drought watering conditions had a higher mean K concentration (mean aboveground K concentration under drought: 1304 ± 20 µg g^{-1} *vs* ambient: 1213 ± 21 µg g^{-1}), but a lower mean K content (mean aboveground K content under drought: 1318 ± 68 µg *vs* ambient: 1566 ± 68 µg) than plants grown under ambient conditions. The lower K content can be attributed to the reduced aboveground biomass in drought watered plants (see Table 3.2), but the higher K concentration suggests that plants grown under drought conditions accumulated more aboveground K per unit weight.

Growth substrate treatment did not affect either aboveground B concentration or content, but aboveground B content was significantly lower in *Z. mays* plants grown under drought conditions (mean aboveground B content: 1.8 ± 1.4 µg) compared to those grown under ambient conditions (mean aboveground B content: 2.4 ± 1.4 µg; Table 3.4). Aboveground B concentration remained unchanged by watering level, thus the change in aboveground B content can be explained by the reduced aboveground biomass in drought watered *Z. mays* plants (Table 3.2).

Aboveground Si was not impacted by watering level in *Z. mays* (Table 3.4). However, both aboveground Si concentration (Figure 3.21) and Si content were significantly ($F_{3,32}$ = 6.22, P = 0.002 and $F_{3,32}$ = 6.37, P = 0.002, respectively) higher (mean aboveground Si concentration: 4.9 ± 0.5 µg g^{-1} and mean aboveground Si content: 5.7 ± 0.8 µg) in plants from the growth substrate treatments with SP (SP only and AMF+SP) compared to plants from the AMF only growth substrate treatment and the control plants (mean aboveground Si concentration: 3.0 ± 0.3 µg g^{-1} and mean aboveground Si content: 4.5 ± 0.5 µg). The lack of impact of growth substrate treatment on aboveground biomass suggests that plants from the SP growth substrate treatments accumulated substantially more Si than plants treated with AMF only or the control plants.

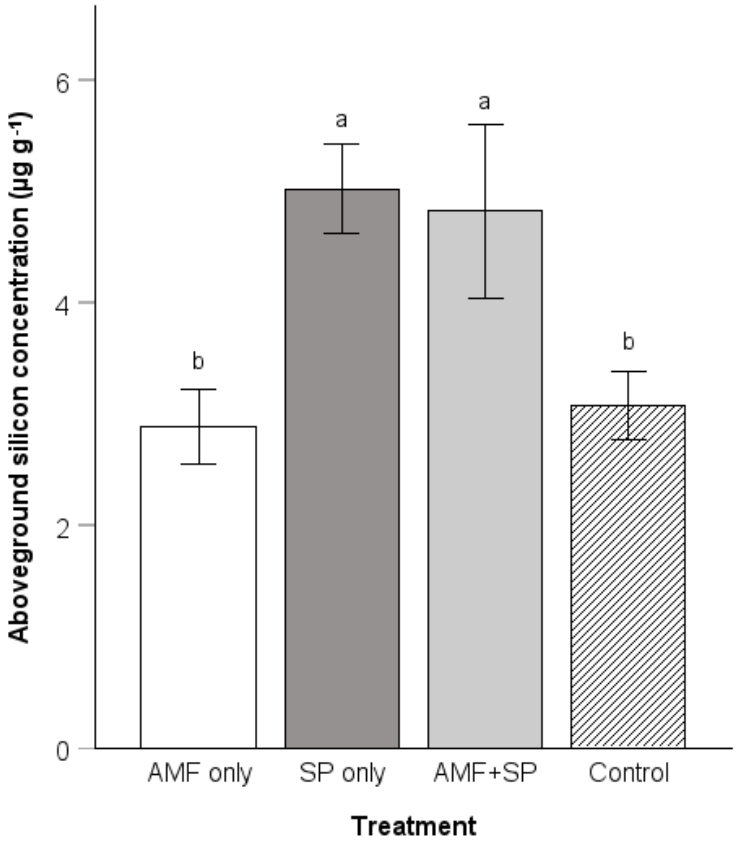

Figure 3.21. Aboveground silicon (Si) concentration (µg g^{-1}) in *Zea mays* L. was significantly ($F_{3,32}$ = 6.22, P = 0.002) higher when cultivated in growth substrate treated with Silwet™ Power (SP) only or arbuscular mycorrhizal fungi (AMF)+SP compared to plants treated with AMF only or the untreated control plants. Error bars are ± 1 standard error (n = 10). For all variables, different letters represent significant differences among treatments ($P \leq 0.05$), which were determined by a Tukey HSD post-hoc test.

In *V. faba*, the concentrations of aboveground K (1823 ± 33 µg g^{-1} across treatments), Ca (291 ± 17 µg g^{-1} across treatments), B (2.8 ± 0.1 µg g^{-1} across treatments) and Zn (4.5 ± 0.2 µg g^{-1} across treatments) were not affected by growth substrate treatment or watering level. The aboveground contents of these micronutrients were also unaffected by growth substrate treatment (Table 3.5). Plants grown under drought conditions had higher K (mean aboveground K content: 2970 ± 261 µg), Ca (mean aboveground Ca content: 444 ± 43 µg), B (mean aboveground B content: 4.3 ± 0.4 µg), and Zn (mean aboveground Zn content: 7.3 ± 0.7 µg) content than plants grown under ambient conditions. Considering the decrease in aboveground biomass in drought watered *V. faba* plants, this indicates

an overall increase in the amount of K, Ca, B and Zn present per unit biomass in *V. faba* leaves grown under drought conditions. Of all of the micronutrients measured, only aboveground Si was impacted by growth substrate treatment, as both aboveground Si concentration and content were significantly ($H_{(2, n = 37)}$ = 22.5, P < 0.001 and $F_{3,28}$ = 37.9, P < 0.001, respectively) higher in plants treated with SP (mean aboveground Si concentration: 14 ± 1 µg g^{-1} and Si content: 20 ± 2 µg across treatments) compared to those that were not (mean aboveground Si concentration: 3 ± 1 µg g^{-1} and Si content: 3 ± 2 µg across treatments; Figure 3.22).

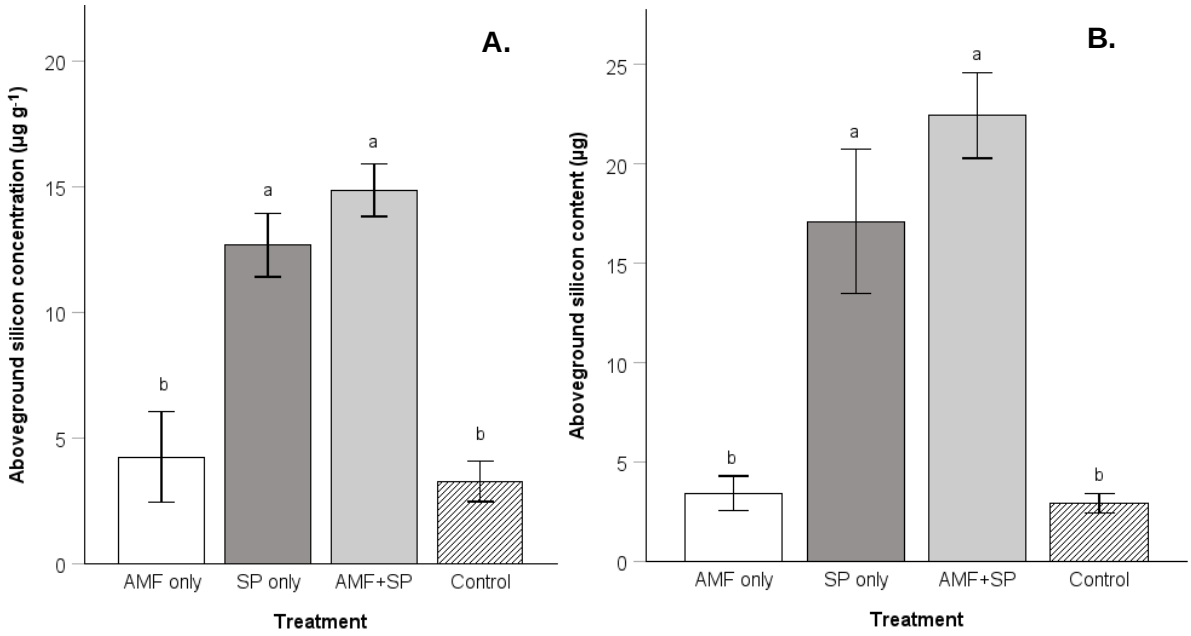

Figure 3.22. Both aboveground silicon (Si) concentration (µg g^{-1}; A) and aboveground Si content (µg; B) in *Vicia faba* L. were significantly ($X^2_{2,\ n = 37}$ = 22.5, P < 0.001 and $F_{3,28}$ = 37.9, P < 0.001) higher in plants from the growth substrates containing Silwet™ Power (SP) and arbuscular mycorrhizal fungi (AMF)+SP compared to plants from the growth substrate treated with AMF only or untreated control plants. The effect of growth substrate treatment on Si concentration was checked by a Kruskal-Wallis test and comparison among treatment effects on Si content was carried out on log10 transformed data using a Tukey post-hoc test. Error bars are ± 1 standard error (n = 7 for AMF only; n = 10 for SP only, AMF+SP and controls). For all variables, different letters represent significant differences among treatments ($P \leq 0.05$).

As with *Z. mays* plants, aboveground biomass was unaffected by growth substrate treatment. Thus, plants from the SP growth substrate treatments (SP only and AMF+SP) accumulated more Si than plants from the AMF only or control growth substrate treatments. Furthermore, Si content was significantly higher in plants grown under drought conditions (mean aboveground Si content: 14 ± 2 µg) compared to those grown under ambient conditions (mean aboveground Si content: 9 ± 1 µg), but Si concentration was unaffected by watering level (Table 3.4). The lower aboveground biomass in drought watered *V. faba* plants suggests then that the increased Si content was due to a redistribution of Si which localised it aboveground.

3.4 Discussion

3.4.1 The impact of two Silwet adjuvants on growth substrate moisture content

Soil adjuvants are often tailored for use in particular agricultural contexts, such as for combination with specific chemical formulations, application to different crop species or varying soil types (Krogh *et al*., 2003; Baratella *et al*., 2016; Chaichi *et al*., 2015; Lehrsch *et al*., 2011). Thus, it was hypothesized that the efficacy of SP in maintaining a higher SMC in droughted soils would be higher than that of S408 due to its commercial design that tailors it for soil application, but that this efficacy would also be directly proportional to the concentration of adjuvant applied. SP was in fact more effective at maintaining a higher SMC than S408, but, strikingly, only when present in the growth substrate at a low concentration. Considering the nonreplicated pilot study suggested that S408 was a more effective soil wetter, this result requires validation with a larger study. Generally, the concentration of an organosilicone surfactant improves water retention of a growth substrate in a directly proportional relationship (Baratella & Trinchera, 2018; Zhang *et al*., 2023). However, there is evidence counter to this trend. For example, Mobbs *et al*. (2012) found that four non-ionic surfactants (two of which were organosilicone) did not have any impact on infiltration rate, water holding capacity, water penetration or soil-water distribution and performance of the adjuvants varied across different experimental conditions. Also, Feng *et al*. (2002) determined that in some cases, a lower surfactant concentration can withstand rewetting more effectively than high concentrations. Furthermore, Abu-Zreig *et al*. (2003) demonstrated that very high surfactant concentrations can actually reduce water hydraulic conductivity. The impacts of organosilicone surfactants on hydraulic properties are, therefore, yet to be delimited. The current findings suggest that, within the range of concentrations tested, adjusting the concentration of the adjuvant may not be an effective strategy for manipulating SMC. Other factors, such as the choice of adjuvant itself or the timing of its application, may have a more significant impact on soil moisture management. Interactions between these factors should be established before replication in field

trials where environmental conditions make relationship dynamics harder to decipher.

Treatment of the growth substrate with a Silwet adjuvant did not impact SMC under ambient watering (50% of the SWHC), but under drought watering (30% of the SWHC) both adjuvants improved SMC compared to the controls that were adjuvant-free. This result supports the second hypothesis that adjuvant application to the growth substrate will increase SWHC and maintain a higher SMC compared to untreated growth substrate. These results reinforce literature demonstrating that organosilicone surfactants are effective tools for enhancing SMC under drought conditions due to their amphiphilic structure that bind water molecules to soil colloids (Lehrsch *et al*., 2012), reducing runoff and evaporation (Cooley *et al*., 2009; Li *et al*., 2019). The lack of improvement in SMC under ambient watering could be caused by saturation of the growth substrate such that pore spaces were reduced, leaving little room for the surfactant molecules to penetrate and interact with soil particles (Lehrsch *et al.,* 2011). Moreover, adjuvant breakdown may have occurred while SMC was still high enough to saturate pore spaces, reducing surfactant binding affinity, thus action on SMC (Ying, 2006). It should be noted that the results of the present study may vary quite significantly when translated to field conditions, where full saturation of the growth substrate may be limited due to increased evaporation and runoff capability (Biddoccu *et al*., 2016), or enhanced due to flooding events (Sefton *et al*., 2021). Thus, the possible benefits of the Silwet adjuvants tested may be limited by the nature of the study design. Nonetheless, the results of the present study develop previous knowledge by suggesting that while Silwet adjuvants such as SP and S408 are appropriate for improving water retention properties of low SWHC growth substrates under drought conditions, the benefits of using these adjuvants under ambient conditions are limited. Considering their relatively short persistence in soil, their use as a tool for improving SMC is therefore more appropriate to periods of short-term drought. Furthermore, this study tested a sandy growth substrate with a low SWHC; replication of the study using other low SWHC soil and growth substrates differing in composition could elucidate whether the benefits of Silwet adjuvants observed in the present study are substrate specific or a universal effect on growth substrates with a low SWHC. If the influence of Silwet adjuvants on the

water retention properties of different growth substrates varies, this could inform how organosilicone surfactant-based adjuvants are used in field contexts.

Following rewetting of the growth substrate, there were no significant differences among the moisture contents of treated and untreated growth substrates. This is consistent with the findings of Stevens (1995), which indicated that adsorption to soil particles and subsequent hydrolysis of organosilicones leads to their inactivation. The results of the present study therefore build upon the mechanism proposed by Stevens (1995) by demonstrating its occurrence in a specific growth substrate which has a high sand content, thus low SWHC. This opens the possibility for more tailored research which compares adjuvant inactivation in growth substrates of varying compositions, thus different properties. These results also confirmed the third hypothesis that rewetting of growth substrate treated with either adjuvant will lead to their degradation, thus reducing adjuvant action on SMC. Studies which determine the rate of degradation of organosilicone surfactants against drought in different growth substrates would help to inform the frequency in which these adjuvants should be applied to droughted soils to maintain a desired SMC for crop production. Used together with knowledge of adjuvant residual wetting properties could lead to a reduction in the frequency and quantity of adjuvant application and irrigation events necessary to maintain SMC and reduce crop drought stress over time.

Overall, this study demonstrated the effect of organosilicone surfactant-based adjuvants on SMC under drought conditions and was a direct comparison of the performance of two commercially available adjuvants. The study builds upon previously reported positive effects of organosilicone surfactants on SMC under drought conditions by providing a context which aligns with that of specific field conditions, enabling more tailored application of organosilicone surfactants to growth substrates. This has encouraging implications for their use in the management of SMC under drought conditions. While there were limitations with the study, it was effective in developing an intermediate method of testing the effect of adjuvants on SMC between laboratory and field testing and provides direction for future studies.

3.4.2 Drought tolerance of crops treated with Silwet soil adjuvants and arbuscular mycorrhizal fungi

This study hypothesized that the combined use of AMF biofertilizer and SP would improve the drought tolerance of two major crop species, namely *V. faba* and *Z. mays*. Initial observation of control plants from untreated growth substrate revealed that watering level had no significant impact on several measured variables at harvest, such as specific leaf area, root system length and some of the aboveground micronutrients measured. This indicates that the difference between the ambient and drought watering levels may not have been substantial enough to truly reflect the effects of drought on the plants tested. There is considerable debate regarding the best method of water application that adequately mimics natural soil water deficits in drought trials and, to date, no consensus has been determined (Poorter *et al*., 2012). However, the results suggest that under the conditions employed in this study, the droughted plants should have been cultivated with less water to more clearly observe the effects of AMF and SP on drought tolerance. Nonetheless, none of the individual nor combined growth substrate treatments appeared to improve water use efficiency in either crop species; only detrimental impacts of drought were reported. The literature suggests that AMF can improve drought tolerance in various host plants by enhancing water and nutrient acquisition (Smith & Read, 2010b), aiding photosynthesis (Gavito *et al*., 2019) and increasing stress resistance (Chandrasekaran, 2022), and adjuvants reduce drought conditions by increasing soil water retention (Karagunduz *et al.*, 2001). Therefore, the lack of drought ameliorating effects from either the AMF or SP is likely due to the restricted differences between the ambient and drought watering levels. Thus, the study is limited in its reflection of some of the potential benefits of the growth substrate treatments tested, such that hypotheses relating to their benefits could not be tested.

SP impacted nutrient uptake in *Z. mays* under drought conditions. Specifically, SP caused a reduction in total plant P per unit biomass in droughted *Z. mays* plants. Although the plants in a study by Giannakopoulos (2022) were not subjected to drought conditions, it was observed that surfactant application to soil had no impacts on nutrient acquisition in *Z. mays* or *H. vulgare*, demonstrating a contrast in the

impacts of surfactants on plant nutrient accumulation. In the present study, average P concentration was much lower than is expected in healthy plants for both plant species (138.0 ± 10.5 µg g^{-1} in *Z. mays* and 188.1 ± 26.3 µg g^{-1} in *V. faba vs.* ca. 2000 µg g^{-1} as reported by Fitter & Hay [2001]). This caused the C:P and N:P ratios to be unusually high despite evident nitrogen deficiency in *Z. mays* during cultivation. The low P concentrations reported for both plant species could be due to incomplete digestion of plant material during sample preparation, as signs of P deficiency during cultivation were not noticed in the plants (Khan *et al*., 2023). While this does reduce confidence in the results relating to P, the change in total P of plants grown in substrate treated with SP was notable. Previous studies by Momentive that tested foliar spray adjuvants observed that a higher volume of agricultural formulation applied to plants increased runoff, thus reducing the efficacy of the formulation. Reducing the volume of formulations applied to plants reduced runoff, therefore improving efficacy and efficiency of the formulation (pers. comms. Dr George Policello, Momentive, Tarrytown, USA). In the present study, the high sand content of the growth substrate may have reduced P availability in the presence of the organosilicone with the water volume applied. This would reduce the concentration of water soluble, thus bioavailable, P forms in the soil, leading to a reduction in P accumulation in plants. Reducing the watering level may therefore reduce runoff, diminishing impacts to P uptake. This is further indication that the drought watering level should be lower in future studies, particularly in sand-based or other low SWHC growth substrates. It should also be noted that, under ambient watering, the average aboveground P concentration of control plants was significantly higher than that of plants from all other growth substrate treatments. The increased aboveground P concentration of control plants could be due to the presence of the native AMF population in the growth substrate benefitting P accumulation in host plants. Alternatively, the growth substrate treatments may have negatively impacted the symbiosis between host plants and the native AMF, causing a comparative reduction in the aboveground P concentration. Considering this, phylogenetic analysis of the growth substrate may be helpful in future studies to improve understanding of interactions between the native microbiome and growth substrate treatments of interest.

Addition of SP also caused a decrease in total plant Ca in *Z. mays*. Strikingly, addition of SP caused both *Z. mays* and *V. faba* plants to accumulate significantly more Si compared to plants that were not cultivated with the adjuvant. This may have been contributed by the high Si content of both SP and the sandy growth substrate. The presence of Si in soil can improve availability of other vital nutrients via adsorption competition to soil minerals, releasing available nutrients for absorption by plants (Obihara & Russell, 1972). However, the decrease in plant P and Ca indicates an antagonistic impact of excessive Si uptake. Although uptake of Si, P, and Ca in plants is via distinct molecular mechanisms and pathways (Ma *et al*., 2001; Hu *et al*., 2020; Pathak *et al*., 2021), high concentrations of Si in the root zone may still compete with other essential nutrients for uptake sites on plant roots or within the plant. For example, excess Si accumulation in plants can lead to a decrease in plant P (Greger *et al*., 2018), attributed to physical apoplastic barriers in the roots formed through Si deposition (Ma, 2004). Si deposition in plant tissues, particularly in cell walls, can also influence membrane permeability and nutrient transport processes (Sahebi *et al*., 2015). Therefore, the high concentration of Si in the growth substrate may have impaired the uptake, translocation, and distribution of P and Ca within the plant (Kochian, 2018). This could also explain the reallocation of K and Si in *Z. mays* to aboveground plant tissues when exposed to SP, which occurred regardless of watering level. Alternatively, as plants undergo senescence or experience environmental stresses such as drought or nutrient deficiency, they may prioritize the allocation of resources towards maintaining essential physiological functions such as photosynthesis and respiration (Feller *et al*., 2018). Therefore, remobilizing nutrients away from roots and towards aboveground tissues can help plants to cope with stress and prioritize regrowth (Wang *et al*., 2021). This suggests the changes induced by cultivation with SP triggered stress responses in the plants. However, Si has numerous advantages in plants, including defence against pests and diseases (Samuels *et al*., 1991), resistance to drought (Zargar *et al*., 2019), structural integrity (Vaculík *et al*., 2009), and enhanced uptake of certain nutrients (Rizwan *et al*., 2015). Therefore, it is evident that more investigation into the changes to nutrient uptake in plants caused by organosilicone surfactants is needed to fully understand this result. In view of this, the competitive uptake of Si that occurs in the presence of SP should be investigated to confirm whether organosilicone surfactants indeed impact the uptake of essential and secondary nutrients in plants. The present

study did not investigate whether SP can actively release bioavailable forms of Si for absorption by plants. Thus, observation of the form of Si released by SP and that which is taken up by plants in the presence of SP would clarify whether the enhanced Si uptake observed in plants originates from SP or another environmental source. Revealing the molecular mechanisms responsible for these changes may also inform why there were different reactions to SP in the monocot and dicot plant models.

Foliar proline concentration increased following SP addition under drought conditions in *V. faba* and irrespective of watering level in *Z. mays*. Differences in the effects of SP on foliar proline concentration between *Z. mays* and *V. faba* may be due to the alternative pathways for proline biosynthesis regulation in response to stress that exists in monocots and dicots (Akbudak & Filiz, 2020). This contradicts the proposed hypothesis that SP will not have any direct impact on proline accumulation relative to untreated controls. Considering the reduced biomass and nutrient profile of plants from the SP treatment, this suggests an enhanced stress response (Essa *et al*., 2023), which is counter to other reports stating that surfactants reduce drought stress (Daneshnia *et al*., 2016; Sibley *et al*., 2018; Schiavon *et al*., 2014). However, there is emerging evidence that foliar-applied adjuvants can reduce drought tolerance by increasing epidermal transpiration (Räsch *et al*., 2018). Although relevant to a different mode of application, this raises questions about whether soil adjuvants could have indirect impacts on epidermal transpiration of treated plants. Moreover, SP had no effect on AMF colonisation of host plants, but it did impact the enhanced nutrient capture provided by AMF symbiosis in both *Z. mays* and *V. faba*, suggesting that colonisation may not equate to function (Treseder, 2013). Specifically, N and P in *Z. mays* and Ca in *V. faba* were reduced per unit biomass when cultivated with AMF and SP, compared to when grown with AMF only. These findings suggest that the interaction between organosilicone surfactants and AMF symbiosis can have nuanced effects on nutrient capture and allocation in host plants. This highlights the importance of considering both colonization and functional outcomes when assessing the impact of organosilicone surfactants on AMF-enhanced nutrient uptake in agricultural systems. Overall, these results infer that SP application heightened plant stress through alterations in AMF enhancement of micronutrient uptake mechanisms in both plant species and via impact to proline

production pathways in *V. faba*, but it did not hinder symbiotic associations between host plants and AMF in either species.

Independent of drought conditions, AMF improved aboveground N accumulation in *Z. mays*. Although, this was not apparent in plants from the combined growth substrate treatment (AMF+SP). AMF inoculation also led to increased P concentration per unit biomass in *Z. mays*. Colonisation with AMF has been established to improve foliar N, P, and K content of host plants (Rubio-Sanz & Jaizme-Vega, 2022; Shi *et al*., 2020). However, the reduction in aboveground N accumulation in plants from the combined growth substrate treatment compared to those cultivated with AMF only suggests that the adjuvant impedes AMF acquisition of essential nutrients. This is a contrasting result to that seen in *V. faba*, in which all of the growth substrate treatments seemed to reduce aboveground N accumulation, regardless of watering level. Root nodules formed through symbiosis with N_2-fixing rhizobial bacteria in *V. faba* allow for the fixation and storage of N in the root system (Chen *et al*., 2018). Co-colonisation with AMF and rhizobial bacteria has been reported to have synergistic effects on N and P concentration (Jia *et al*., 2004; Ingraffia *et al*., 2019). This may explain the reallocation of N from aboveground to belowground plant tissues in plants inoculated with AMF. Furthermore, recent evidence by Coquerel *et al*. (2023) proposed that enhanced Si supply may support nodulation and N_2 fixation by rhizobia. Therefore, the increased Si content of SP-treated soils may aid N accumulation in leguminous plants. Root nodule presence did not vary between growth substrate treatments, but this may be due to the existence of the original AMF population in the growth substrate synergistically aiding rhizobial bacteria in nutrient accumulation in host plants. Also, the impacts of pest and potential herbicide stress on *V. faba* in the present study should be considered when interpreting these results. Nonetheless, the presence of root nodules in *V. faba* could explain the differences in N accumulation between *V. faba* and *Z. mays* in the presence of AMF and SP.

Despite limitations in the present study which obscured some of the potential benefits of SP and AMF on drought tolerance, the results do enhance understanding in a number of areas. Evidence of the reduction and reallocation of important nutrients, particularly P, by SP in two major crop species that represent distinct evolutionary lineages within angiosperm crops has been demonstrated. The resulting

osmolytic changes in the two plant species in the presence of SP and/or AMF was also reported, which demonstrated a possible link between a stress response and alterations in the micronutrient profile of each crop species. Additionally, the study raised further questions about the complex relationship between AMF and organosilicone surfactants that will hopefully stimulate further investigation of this important area. These outcomes are informative for the development of sustainable agricultural practices that improve drought resilience without reliance on intensive synthetic inputs.

4 General discussion

4.1 Overview

Climate change is reducing global food security due to its increasingly unpredictable influence on global temperature and weather events which impact major crop yields (van Dijk *et al*., 2021). Drought is a significant outcome of climate change which is challenging crop cultivation and food production (Cohen *et al*., 2021). Finding the tools necessary to adapt current agricultural practices to cope with drought is paramount to protecting food security (Solh & van Ginkel, 2014). The main aim of this thesis was to investigate a novel approach utilising organosilicone surfactants together with AMF to improve the drought tolerance of crop plants. This was conducted through a number of experiments which explored the effect of Silwet™ (produced by Momentive) organosilicone surfactants on water retention of a hydrophobic growth substrate; the asymbiosis phase of AMF spores *in vitro;* the germination of *V. faba* and *Z. mays* seeds under drought conditions; and the drought tolerance of *V. faba* and *Z. mays* plants inoculated with AMF. With the exception of the *in vitro* study, all experiments were pot trials that were implemented under controlled conditions. Several knowledge gaps in the understanding of the interaction between organosilicone surfactants, AMF and plant drought tolerance were identified, including the variability of organosilicone surfactant action on soil moisture content; the evident, albeit minimal, ecotoxic effect of organosilicone surfactants on AMF; and the reduction and redistribution of nutrients in plants caused by exposure to SP.

4.2 Organosilicone surfactants benefit soil water retention but alter plant nutrient uptake

Silwet organosilicone surfactants were demonstrated to improve water retention of hydrophobic growth substrates, in agreement with other reports in this area

(Abagandura *et al*. 2021; Karagunduz *et al*., 2001; Starr *et al*., 2005). However, lower surfactant concentrations were more effective than higher concentrations at maintaining soil moisture content over time, demonstrating a dose-independent effect. Strikingly, the lower concentration of surfactant also had the biggest impact on the total length of hyphae produced by *R. irregularis* spores in the *in vitro* ecotoxicity study, which were shorter than that of all other surfactant concentrations and control spores. Together, these results indicate a higher overall efficacy of organosilicone surfactants when present at lower levels. Feng *et al*. (2002) observed that a lower concentration of surfactant could better withstand rewetting than the higher concentration tested, though further investigation into this effect has not yet been carried out. The prolonged activity of organosilicone surfactants at low concentrations may have a greater impact on asymbiotic hyphae production, although the mechanism of this effect is not yet clear. Depending on application rate, ecotoxicity may be reduced in the field due to dilution of adjuvants in a greater soil volume and more rapid surfactant hydrolysis in the environment. These factors would reduce exposure of soil organisms and plants to organosilicone surfactants. Also, the presence of a more complex soil microbial community in the field may improve resilience of AMF to organosilicone surfactant exposure. For example, the more diverse range of metabolic capabilities present in the microbial community could break down any persistent ecotoxic components of the surfactants (Jia *et al*., 2023). Furthermore, the two surfactants tested in the soil moisture content experiment (SP, S408) produced different outcomes regarding their improvement of soil water retention; this reinforces the need to thoroughly assess under which environmental contexts organosilicone surfactants may be useful. Therefore, use of organosilicone surfactants in the environment should be carefully considered and risk assessed for environmental impacts prior to application.

To the best of this author's knowledge, the use of organosilicone surfactants in combination with AMF biofertilizer to improve drought tolerance of crop plants is a novel approach. An interaction between Si uptake and P and Ca uptake was observed in the presence of SP, in which increased Si uptake coincided with reduced P and Ca uptake. Also, a redistribution of K and Si in host plants was observed. While there are potential drawbacks to this outcome with regard to plant growth, it could also be utilised in soils with high mineral fertilizer inputs. P toxicity

can affect enzymatic reactions and osmotic pressure, thus decreasing essential metal element availability in plant cells (Yoneyama, 1988). Si has been shown to reduce P toxicity (Ma & Takahashi, 1990). Therefore, the high Si content of SP might help to reduce fertilizer toxicity in soils whose nutrient profiles have become unbalanced through overuse of artificial fertilizers. Also, should the mechanisms responsible for nutrient redistribution in the presence of SP be elucidated, this could also be utilised for specific plant nutrition requirements.

There were contradictory results regarding the effect of exposure to SP on the symbiosis between AMF and host plants. Enhancement of nutrient acquisition and distribution by inoculation with AMF biofertilizer appeared to be negatively impacted by SP in AM plants, but colonisation of host plant roots and subsequent growth of hyphae in the mycorrhizosphere were not. This also suggests that the reduced length of hyphae caused by exposure to low concentrations of organosilicone surfactants did not detriment host colonisation by AMF. This brings question to whether AMF colonisation of host plant roots is an appropriate measurement of AM-plant symbiosis, as colonisation does not always equal function (Treseder, 2013). However, this result could also be explained by the small pot size used in the experiment. Qin *et al*. (2022) found that pot-bound roots and reduced photosynthesis of plants grown in small pots could reduce the role of AMF for host plants. Therefore, pot size may have impacted quantification of AM colonisation of host plant roots, such that the effects of exposure to SP were not reflected in these results. Nonetheless, Si accumulation in the monocot *Brachypodium distachyon* has been associated with lower AMF colonisation (Johnson *et al*., 2022). The results from the present study suggest it may operate in dicots as well. Si accumulation may also be enhanced in AM-plants due to the promotion of Si accumulation in plant roots by AMF (Etesami *et al*., 2022), thus self-perpetuating any impacts of high Si accumulation to AMF symbiosis. Further investigation of the impacts of organosilicone surfactants on soil Si, alongside the impacts of plant Si accumulation on AMF colonisation in different plant models, is required to elucidate the impact of SP on AM symbiosis.

4.3 Recommendations and future work

The current study presented evidence that organosilicone surfactants impeded AMF hyphae production *in vitro*. Elucidating the biological significance of these findings is a vital proceeding step, as the 0.1 cm average difference in hyphal length between organosilicone-treated and untreated *R. irregularis* spores may not prevent hypha from successfully locating and colonising plant roots. Nonetheless, it would be helpful to delimit the maximum tolerance of AMF to the surfactant before growth of hyphae is inhibited entirely. Future studies might also address the molecular and physiological processes behind organosilicone ecotoxicity in AMF, enhancing our understanding of the complex relationship between the two. This would inform the parameters in which organosilicone surfactants can be used in field contexts to benefit crop plants. Discerning the impact of organosilicone surfactants on soil microbial community composition and abundance would be a vital following step prior to a transition from pot to field trials. The soil microbial community plays a fundamental role in increasing the drought resilience of crop plants through their various functions including nutrient cycling (Toberman *et al*., 2008), soil structure formation (Evans & Wallenstein, 2014), disease suppression (Yin *et al*., 2021a) and overall ecosystem functioning (Osburn *et al*., 2023). Any disruptions to this community could therefore impact plant-bacterial relationships (DiLegge *et al*., 2022). The presence of AMF can influence soil microbial community composition and dynamics through alteration of plant root exudates (Mitra *et al*., 2021), changes in soil nutrient dynamics (Clark & Zeto, 2000) and regulation of the proliferation of certain microbial taxa, leading to changes in microbial diversity and abundance (Xu *et al*., 2018). Therefore, impacts of organosilicones to AMF could have cascading effects on the surrounding soil microbial community, reducing their ecosystem services thus plant resilience to drought (Seitz *et al*., 2021). Conversely, should the ecotoxicity of organosiliones be reduced in field conditions, benefits gained from the improved moisture content and structure of the soil may benefit plants experiencing drought. Exploring impacts of organosilicones on the soil microbiome, potentially via meta-genomic and meta-transcriptomic analysis is therefore important for the evaluation of the organosilicone benefits for plant drought resilience.

There are limited reports in which AMF and surfactants have been used in conjunction in an agricultural context, but these have focused on salinity stress (Chaichi *et al*., 2017), a related problem to drought but one which this thesis did not address. Salinity stress occurs when soil contains high levels of soluble salts, increasing the osmotic pressure of the soil solution, thus limiting water availability to plants (Zhao *et al*., 2021). This leads to reduced water uptake by plant roots, resulting in water stress and dehydration. Additionally, high salt concentrations in the soil can disrupt ion balance within plant cells, leading to ion toxicity and nutrient imbalances (Park *et al*., 2016). These physiological disturbances can impair plant growth (van Zelm *et al*., 2020), reduce photosynthetic efficiency (Chen & Hoehenwarter, 2015), inhibit nutrient uptake (Gong, 2021), and ultimately result in decreased crop yields and quality (Parida & Das, 2005). Salinity stress can arise from natural causes such as the weathering of rocks, sea spray, or saline groundwater intrusion (Stavi *et al*., 2021), human activities including irrigation practices (Shahid, 2013) and excessive fertilizer use (de Almeida Silva *et al*., 2019). There is growing evidence to suggest that non-ionic surfactants can aid plants in coping with salinity stress. Chaichi *et al*. (2016) found that non-ionic surfactant application to soil improved *Z. mays* resilience under salinity and drought stress, producing higher yields. Also, Dadresan *et al*. (2015) reported enhancement of total photosynthetic and carotenoid activities in *Trigonella foenum-graecum* grown in high salinity conditions with surfactant treatment. Furthermore, Chaichi (2018) related improvement in growth and development of *Allium cepa* when cultivated in surfactant-treated soil. Of the reports mentioned, surfactants other than organosilicones were utilised to treat salinity stress. However, due to the similar properties of non-ionic surfactants, this opens avenues for enquiry as to whether organosilicone surfactants could be potential tools for mitigating the effects of salinity stress in agricultural environments. Furthermore, considering the promising report of Chaichi *et al*. (2017) regarding the synergistic effects of non-ionic surfactants and AMF in relieving salinity stress in *Solanum lycopersicum*, this could suggest a more appropriate context in which organosilicone surfactants could be used alongside AMF to provide stress resilience for crop plants.

All of the experiments reported in this thesis that studied AMF used *F. mosseae* and/or *R. irregularis*, both of which are from the *Glomeraceae* family (Smith & Smith,

1997). *Gigaspora* and *Scuttelospora* from the *Gigasporaceae* family are genetically and physiologically distinct from *R. irregularis* and *F. mosseae*. They do not form vesicle storage organs (Godbold, 2004) and have different mechanisms for hyphal injury recovery compared to members of the *Glomeraceae* family. Specifically, *Glomaraceae* are able to reconnect damaged hyphal networks by anastomosis (interconnection of different mycelial networks belonging to the same isolate; Voets *et al*., 2006), whereas *Gigasporaceae* such as *Scutellospora reticulata* and *Gigaspora gigantea* produce new hyphal tips away from the damage site to survive adverse conditions (de la Providencia *et al*., 2005). The hyphal network repair mechanism of *Glomeraceae* makes them better adapted to survive in regularly disturbed soils such as arable land (Oehl *et al*., 2003), whereas *Gigasporaceae* are found in lower abundance at these sites (Jansa *et al*., 2003). Nonetheless, there are reported benefits of inoculating crop plants with *Gigasporaceae* species. *Gigaspora margarita* improved Ca and Mg concentration in *Z. mays* plants, while *Gigaspora rosea* increased *Z. mays* seedling biomass, *Cucurbita moschata* Ca uptake (Carrara & Heller, 2022) and *Olea europaea* seedling biomass (Ferreira *et al*., 2015). Also, P uptake in *Glycine max* and *Trifolium pratense* were improved by colonisation with *Gigaspora gigantea* (Stürmer, 2004). There are also synergistic effects of inoculating crop plants with AMF species from both the *Glomaraceae* and *Gigasporaceae* families. Joint inoculation with *Glomus deserticola* and *G. gigantea* led to increased tolerance to drought and charcoal rot disease in *Vigna unguiculata* (Oyewole *et al*., 2017), while a mixed inoculum containing *G. gigantea, Glomus manihotis,* and *Scutellospora heterograma* alleviated transplantation shock in micropropagated *Vitis vinifera* plants (Krishna *et al*., 2006). Therefore, exploring how organosilicone surfactants might be utilised to reduce soil disturbance could open avenues in which *Gigasporaceae* biofertilizer species can be exploited for their benefits to crop plants.

Could the project have been extended, it would have been beneficial to compare the effect of SP and S408 on the water retention properties of other growth substrates which differed in composition. This would inform how these surfactants could be used to benefit a range of crop species that require specific growth substrates. For example, soilless substrates such as coconut coir and green-waste composts are becoming increasingly popular for cultivation of a range of crops, including

vegetables, fruits, ornamentals, and herbs due to their superior water retention and aeration properties (Yin *et al*., 2021) and sustainable production (Mariotti *et al*., 2020; Inghels *et al*., 2016). They are more commonly utilised in small-scale horticultural contexts rather than in the field (Mariotti *et al*., 2020; van der Gaag *et al*., 2007), but have an increasing variety of uses (Feng *et al*., 2021). However, coir lacks its own source of nutrients, relying solely on fertilizer addition for successful plant cultivation (Tuckeldoe *et al*., 2023). Furthermore, the use of green-waste composts is limited by a number of factors including high compaction and unbalanced nutrient contents (Massa *et al*., 2018). The limitations with these more sustainable growth substrates could potentially be ameliorated with careful use of organosilicone surfactants. Therefore, delineating the effect of SP and S408 on the water retention properties and nutrient availability of these growth substrates could open avenues for more sustainable crop production which reduces impact to the global soil stock. Also, due to their breakdown in soil, it is unclear whether organosilicone surfactants might help plants to cope with intermittent drought periods. Due to the implication that low surfactant concentrations may be more tolerant of rewetting than higher concentrations (Feng *et*., 2002), it could be possible that low surfactant concentrations might help to retain soil moisture during period of no precipitation. This should be explored in pot trials to determine if benefits of organosilicone surfactants can be gained from single applications or if organosilicone application must be regular to gain the benefits of improved water retention of soil. Also, further experiments should focus on determining appropriate drought conditions of the growth substrate used. For example, reducing water availability for droughted plants would enable a clearer demonstration of the impacts of drought on plant physiology and growth, including the amelioratory effects of the growth substrate treatments (SP and/or AMF) on drought stress. The positive impacts of the treatments tested in the present study were hidden by the lack of initial drought conditions. Furthermore, in the seed germination trial, drought conditions were too severe to be able to observe whether SP improved soil moisture conditions enough to aid germination under drought. Future studies should then aim to carry out more controlled drought trials prior to treatment with SP and/or AMF to determine drought conditions that are tolerable but not optimal for target plants. These conditions would need to be growth substrate specific.

4.4 Conclusion

Overall, this thesis gathered novel information concerning the effects of Silwet adjuvants with AMF on two model plant species. While there were limitations with the drought aspects of the studies, a number of impacts of organosilicone surfactants on AMF asymbiotic growth and nutrient uptake in both plant species tested were demonstrated. Indications of impacts from organosilicones on AM symbiosis were also presented that require further investigation. Application of Silwet surfactants in the environment should be preceded by thorough investigation into which field contexts are the most appropriate to gain benefits from these adjuvants, considering growth substrate composition, plant species cultivated, and the frequency and severity of drought periods. The impacts of organosilicone surfactants on soil microbial communities were not assessed, but there were indications that they induce toxicity in AMF and trigger alterations in plant nutrient uptake pathways. Therefore, sound evidence of synergy between these surfactants and AMF would be needed before this author would recommend the use of Silwet adjuvants and AMF together as an alternative sustainable tool for improving drought resilience in agricultural systems.

5 References

Abagandura GO, Park D, & Bridges WC Jr. 2021. Surfactant and irrigation impacts on soil water content and leachate of soils and greenhouse substrates. *Agrosystems, Geosciences & Environment* **4**: e20153.

Ábrahám E, Hourton-Cabassa C, Erdei L, & Szabados L. 2010. Methods for determination of proline in plants. In *Methods in Molecular Biology* (Humana Press, Clifton, New Jersey) 317–331.

Abu-Zreig M, Rudra RP & Dickinson WT. 2003. Effect of application of surfactants on hydraulic properties of soils. *Biosystems Engineering* **84**: 363–372.

Akbari S, Nour AH, Yunus RM & Farhan AH. 2018. Biosurfactants as promising multifunctional agent: A mini review. *SSRN Electronic Journal* **1**: 1-6.

Akbudak MA & Filiz E. 2020. Genome-wide investigation of proline transporter (ProT) gene family in tomato: Bioinformatics and expression analyses in response to drought stress. *Plant Physiology and Biochemistry* **157**: 13–22.

Akiyama K, Matsuzaki KI & Hayashi H. 2005. Plant sesquiterpenes induce hyphal branching in arbuscular mycorrhizal fungi. *Nature* **435**: 824–827.

Alexander M. 1983. Most probable number method for microbial populations. In A. L. Page (Ed.), *Methods of Soil Analysis: Part 2 Chemical and Microbiological Properties* **9.2.2**: 815-820, Amer Society of Agronomy.

Alguacil MM, Lumini E, Roldán A, Salinas-García JR, Bonfante P & Bianciotto V. 2008. The impact of tillage practices on arbuscular mycorrhizal fungal diversity in subtropical crops. *Ecological Applications* **18**: 527–536.

de Almeida Silva JL, Duarte SN, da Silva DD & Miranda NDO. 2019. Reclamation of salinized soils due to excess of fertilizers: evaluation of leaching systems and equations. *Dyna* **86**: 115–124.

Andrino A, Guggenberger G, Kernchen S, Mikutta R, Sauheitl L & Boy J. 2021. Production of organic acids by arbuscular mycorrhizal fungi and their contribution in the mobilization of phosphorus bound to iron oxides. *Frontiers in Plant Science* **12**: 661842.

Arriaga FJ, Lowery B & Kelling KA. 2009. Surfactant impact on nitrogen utilization and leaching in potatoes. *American Journal of Potato Research* **86**: 383–390.

Arteaga S, Yabor L, Díez MJ, Prohens J, Boscaiu M & Vicente O. 2020. The use of proline in screening for tolerance to drought and salinity in common bean (*Phaseolus vulgaris* L.) genotypes. *Agronomy* **10**: 817.

Artursson V, Finlay RD & Jansson JK. 2006. Interactions between arbuscular mycorrhizal fungi and bacteria and their potential for stimulating plant growth. *Environmental Microbiology* **8**: 1–10.

Atkinson JA, Rasmussen A, Traini R, Voß U, Sturrock C, Mooney SJ, Wells DM & Bennett MJ. 2014. Branching out in roots: Uncovering form, function, and regulation. *Plant Physiology* **166**: 538–550.

Avila-Flores R, Ceballos G, de Villa-Meza A, List R, Marcé E, Pacheco J, Sánchez-Azofeifa GA & Boutin S. 2012. Factors associated with long-term changes in distribution of black-tailed prairie dogs in northwestern Mexico. *Biological Conservation* **145**: 54–61.

Azcón R, Gómez M & Tobar R. 1996. Physiological and nutritional responses by *Lactuca Sativa* L. to nitrogen sources and mycorrhizal fungi under drought conditions. *Biology and Fertility of Soils* **22**: 156–16.

Bago B, Pfeffer PE, Douds DD, Brouillette J, Bécard G & Shachar-Hill Y. 1999. Carbon metabolism in spores of the arbuscular mycorrhizal fungus *Glomus intraradices* as revealed by nuclear magnetic resonance spectroscopy. *Plant Physiology* **121**: 263–272.

Bahn Y-S, Xue C, Idnurm A, Rutherford JC, Heitman J, Cardenas ME. 2007. Sensing the environment: lessons from fungi. *Nature Reviews Microbiology* **5**: 57– 69.

Bailey FE & Koleske JV. 1990. Physical Properties of Poly(alkylene oxide)s. In *Alkylene Oxides and Their Polymers*: 153-204. CRC Press.

Banks ML, Kennedy AC, Kremer RJ & Eivazi F. 2014. Soil microbial community response to surfactants and herbicides in two soils. *Applied Soil Ecology: A Section of Agriculture, Ecosystems & Environment* **74**: 12–20.

Baratella V, Renzaglia M & Trinchera A. 2016. Effect of surfactant as adjuvant for irrigation/ fertigation in vegetables production: preliminary results on lettuce. *Acta Horticulturae* **1123**: 157–164.

Baratella V & Trinchera A. 2018. Organosilicone surfactants as innovative irrigation adjuvants: Can they improve water use efficiency and nutrient uptake in crop production? *Agricultural Water Management* **204**: 149–161.

Barker L, Hannaford J, Svensson C, & Tanguy M. 2015. A preliminary assessment of meteorological and hydrological drought indicators for application to catchments across the UK. In *Drought: Research and Science-Policy Interfacing* (CRC Press, Florida, USA): 231–236.

Barnabás B, Jäger K & Fehér A. 2008. The effect of drought and heat stress on reproductive processes in cereals. *Plant, Cell & Environment* **31**: 11–38.

Baumhardt RL & Blanco-Canqui H. 2014. Soil: Conservation Practices. In *Encyclopedia of Agriculture and Food Systems* (Academic Press, Massachusetts, USA): 153–165.

Bauters TWJ, Steenhuis TS, DiCarlo DA, Nieber JL, Dekker LW, Ritsema CJ, Parlange J-Y & Haverkamp R. 2000. Physics of water repellent soils. *Journal of Hydrology* **231–232**: 233–243.

Bekka S, Abrous-Belbachir O & Djebbar R. 2018. Effects of exogenous proline on the physiological characteristics of *Triticum aestivum* L. and *Lens culinaris Medik*. under drought stress. *Acta Agriculturae Slovenica* **111**: 477.

Begum N, Ahanger MA, Su Y, Lei Y, Mustafa NSA, Ahmad P & Zhang L. 2019. Improved drought tolerance by AMF inoculation in maize (*Zea mays*) involves physiological and biochemical implications. *Plants* **8**: 579.

Besserer A, Bécard G, Jauneau A, Roux C & Séjalon-Delmas N. 2008. GR24, a synthetic analog of strigolactones, stimulates the mitosis and growth of the arbuscular mycorrhizal fungus *Gigaspora rosea* by boosting its energy metabolism. *Plant Physiology* **148**: 402–413.

Besserer A, Puech-Pagès V, Kiefer P, Gomez-Roldan V, Jauneau A, Roy S, Portais JC, Roux C, Bécard G & Séjalon-Delmas N. 2006. Strigolactones stimulate arbuscular mycorrhizal fungi by activating mitochondria. *PLoS Biology* **4**: e226.

Bever JD, Platt TG & Morton ER. 2012. Microbial population and community dynamics on plant roots and their feedbacks on plant communities. *Annual Review of Microbiology* **66**: 265–283.

Bi Y, Li X, Wang H & Christie P. 2004. Establishment of monoxenic culture between the arbuscular mycorrhizal fungus *Glomus sinuosum* and Ri T-DNA-transformed carrot roots. *Plant and Soil* **261**: 239–244.

Biddoccu M, Ferraris S, Opsi F & Cavallo E. 2016. Long-term monitoring of soil management effects on runoff and soil erosion in sloping vineyards in Alto Monferrato (North–West Italy). *Soil & Tillage Research* **155**: 176–189.

Bitterlich M, Jansa J, Graefe J, Pauwels R, Sudová R, Rydlová J & Püschel D. 2024. Drought accentuates the role of mycorrhiza in phosphorus uptake, part II – The intraradical enzymatic response. *Soil Biology & Biochemistry* **193**: 109414.

Bonfante P & Anca IA. 2009. Plants, mycorrhizal fungi, and bacteria: a network of interactions. *Annual Review of Microbiology* **63**: 363–383.

Bonnington LS. 2000. Analysis of organosilicone surfactants and their degradation products. PhD thesis, The University of Waikato, Hamilton, New Zealand.

Boutin C, Strandberg B, Carpenter D, Mathiassen SK & Thomas PJ. 2014. Herbicide impact on non-target plant reproduction: what are the toxicological and ecological implications? *Environmental Pollution* **185**: 295–306.

Brown MF, Thurmond RL, Dodd SW, Otten D & Beyer K. 2002. Elastic deformation of membrane bilayers probed by deuterium NMR relaxation. *Journal of the American Chemical Society* **124**: 8471–8484.

Bruijn FJ. 2020. Signalling and infection events in the arbuscular mycorrhiza– *Medicago truncatula* symbiosis: introduction. In *The Model Legume Medicago Truncatula* (Wiley-Blackwell, New Jersey, USA): 467–470.

Bulgarelli D, Garrido-Oter R, Münch PC, Weiman A, Dröge J, Pan Y, McHardy AC & Schulze-Lefert P. 2015. Structure and function of the bacterial root Microbiota in wild and domesticated barley. *Cell Host & Microbe* **17**: 392–403.

Butcher KR, Nasto MK, Norton JM & Stark JM. 2020. Physical mechanisms for soil moisture effects on microbial carbon-use efficiency in a sandy loam soil in the western United States. *Soil Biology & Biochemistry* **150**: 107969.

Buysens C, Dupré de Boulois H, & Declerck S. 2015. Do fungicides used to control *Rhizoctonia solani* impact the non-target arbuscular mycorrhizal fungus *Rhizophagus irregularis? Mycorrhiza* **25**: 277–288.

Cabot C, Martos S, Llugany M, Gallego B, Tolrà R & Poschenrieder C. 2019. A role for zinc in plant defense against pathogens and herbivores. *Frontiers in Plant Science* **10**: 1171.

Campagnac E, Fontaine J, Sahraoui A L-H, Laruelle F, Durand R & Grandmougin-Ferjani A. 2008. Differential effects of fenpropimorph and fenhexamid, two sterol biosynthesis inhibitor fungicides, on arbuscular mycorrhizal development and sterol metabolism in carrot roots. *Phytochemistry* **69**: 2912–2919.

Carrara JE & Heller WP. 2022. Arbuscular mycorrhizal species vary in their impact on nutrient uptake in sweet corn (*Zea mays*) and butternut squash (*Cucurbita moschata*). *Frontiers in Agronomy* **4**: 1040054.

Cassia R, Nocioni M, Correa-Aragunde N, & Lamattina L. 2018. Climate change and the impact of greenhouse gasses: CO2 and NO, friends and foes of plant oxidative stress. *Frontiers in Plant Science,* **9**: 273.

Castañeda V, de la Peña M, Azcárate L, Aranjuelo I & Gonzalez EM. 2019. Functional analysis of the taproot and fibrous roots of *Medicago truncatula*: Sucrose and proline catabolism primary response to water deficit. *Agricultural Water Management* **216**: 473–483.

Chaichi MR. 2018. Surfactant application improves growth and development of onion (crystal white var.) in saline soil. *Advances in Plants & Agriculture Research* **8**: 241-245.

Chaichi MR, Keshavarz-Afshar R, Lu B & Rostamza M. 2017. Growth and nutrient uptake of tomato in response to application of saline water, biological fertilizer, and surfactant. *Journal of Plant Nutrition* **40**: 457–466.

Chaichi MR, Nurre P, Slaven J & Rostamza M. 2015. Surfactant application on yield and irrigation water use efficiency in corn under limited irrigation. *Crop Science* **55**: 386–393.

Chaichi MR & Keshavarz-Afshar R, Saberi M, Rostamza M & Falahtabar N. 2016. Alleviation of salinity and drought stress in corn production using a non-ionic surfactant. *Journal of Animal and Plant Sciences* **26**: 1042-1047.

Chandrasekaran M. 2022. Arbuscular mycorrhizal fungi mediated alleviation of drought stress via non-enzymatic antioxidants: A meta-analysis. *Plants* **11**: 2448.

Chandrasekaran M, Boughattas S, Hu S, Oh S-H & Sa T. 2014. A meta-analysis of arbuscular mycorrhizal effects on plants grown under salt stress. *Mycorrhiza* **24**: 611–625.

Chapman JS, Diehl MA, Lyman RC. 1993. Biocide susceptibility and intracellular glutathione in Escherichia coli. *Journal of Industrial Microbiology* **12**: 403–407.

Chaves MM. 2002. How plants cope with water stress in the field? Photosynthesis and growth. *Annals of Botany* **89**: 907–916.

Chen J, Fine JD & Mullin CA. 2018. Are organosilicon surfactants safe for bees or humans? *The Science of the Total Environment* **612**: 415–421.

Chen J-C, Ma Z-Z, Gong Y-J, Cao L-J, Wang J-X, Guo S-K & Wei S-J. 2022. Toxicity and control efficacy of an organosilicone to the two-spotted spider mite *Tetranychus urticae* and its crop hosts. *Insects* **13**: 341.

Chen M, Arato M, Borghi L, Nouri E & Reinhardt D. 2018. Beneficial services of arbuscular mycorrhizal fungi - from ecology to application. *Frontiers in Plant Science* **9**: 1270.

Chen Y & Hoehenwarter W. 2015. Changes in the phosphoproteome and metabolome link early signaling events to rearrangement of photosynthesis and central metabolism in salinity and oxidative stress response in Arabidopsis. *Plant Physiology* **169**: 3021–3033.

Chen YX, Zou L, Penttinen P, Chen Q, Li QQ, Wang CQ & Xu KW. 2018. Faba bean (*Vicia faba* L.) nodulating rhizobia in panxi, China, are diverse at species, plant growth promoting ability, and symbiosis related gene levels. *Frontiers in Microbiology* **9**: 1338.

Chiteri KO, Jubery TZ, Dutta S, Ganapathysubramanian B, Cannon S & Singh A. 2022. Dissecting the root phenotypic and genotypic variability of the Iowa Mung Bean Diversity Panel. *Frontiers in Plant Science* **12**: 808001.

Chun SC, Paramasivan M & Chandrasekaran M. 2018. Proline accumulation influenced by osmotic stress in arbuscular mycorrhizal symbiotic plants. *Frontiers in Microbiology* **9**: 2525.

Civeira G. 2019. Introductory chapter: Soil moisture. In *Soil Moisture* (IntechOpen, London, UK): 3-5.

Clark RB & Zeto SK. 2000. Mineral acquisition by arbuscular mycorrhizal plants. *Journal of Plant Nutrition* **23**: 867–902.

Cochran WG. 1950. Estimation of bacterial densities by means of the 'most probable number'. *Biometrics* **6**: 105–116.

Cohen I, Zandalinas SI, Huck C, Fritschi FB & Mittler R. 2021. Meta-analysis of drought and heat stress combination impact on crop yield and yield components. *Physiologia Plantarum* **171**: 66–76.

Comas LH, Becker SR, Cruz VM, Byrne PF & Dierig DA. 2013. Root traits contributing to plant productivity under drought. *Frontiers in Plant Science* **4**: 422.

Cook BI, Miller RL & Seager R. 2009. Amplification of the North American 'Dust Bowl' drought through human-induced land degradation. *Proceedings of the National Academy of Sciences of the United States of America* **106**: 4997–5001.

Cooley ET, Lowery B, Kelling KA, Speth PE, Madison FW, Bland WL & Tapsieva A. 2009. Surfactant use to improve soil water distribution and reduce nitrate leaching in potatoes. *Soil Science* **174**: 321–329.

Cope KR, Bascaules A, Irving TB, Venkateshwaran M, Maeda J, Garcia K, Rush TA, Ma C. Labbé J, Jawdy S. 2019. The ectomycorrhizal fungus *Laccaria bicolor* produces lipochitooligosaccharides and uses the common symbiosis pathway to colonize *Populus* roots. *The Plant Cell* **31**: 2386–2410.

Coquerel R, Arkoun M, Dupas Q, Leroy F, Laîné P & Etienne P. 2023. Silicon supply improves nodulation and dinitrogen fixation and promotes growth in *Trifolium incarnatum* subjected to a long-term sulfur deprivation. *Plants* **12**: 2248.

Corradi N & Bonfante P. 2012. The arbuscular mycorrhizal symbiosis: origin and evolution of a beneficial plant infection. *PLoS Pathogens* **8**: e1002600.

Coudert Y, le Thi V & Gantet P. 2013. Lateral Root Development. In *Plant Roots (4th Edition* (CRC Press, Florida, USA): 112–125.

Czajka A, Hazell G & Eastoe J. 2015. Surfactants at the design limit. *Langmuir: The ACS Journal of Surfaces and Colloids* **31**: 8205–8217.

Dadresan M, Luthe DS, Reddivari L, Chaichi MR & Yazdani D. 2015. Effect of salinity stress and surfactant treatment on physiological traits and nutrient absorption of fenugreek plant. *Communications in Soil Science and Plant Analysis* **46**: 2807–2820.

Daneshnia F, Amini A & Chaichi MR. 2016. Berseem clover quality and basil essential oil yield in intercropping system under limited irrigation treatments with surfactant. *Agricultural Water Management* **164**: 331–339.

Daniel AI, Fadaka AO, Gokul A, Bakare OO, Aina O, Fisher S, Burt AF, Mavumengwana V, Keyster M & Klein, A. 2022. Biofertilizer: The future of food security and food safety. *Microorganisms* **10**: 1220.

Davis SJ, Caldeira K & Matthews HD. 2010. Future CO2 emissions and climate change from existing energy infrastructure. *Science* **329**: 1330–1333.

Deja-Sikora E, Werner K & Hrynkiewicz K. 2023. AMF species do matter: *Rhizophagus irregularis* and *Funneliformis mosseae* affect healthy and PVY-infected *Solanum tuberosum* L. in a different way. *Frontiers in Microbiology* **14**: 1127278.

Deng Z, Huang M, Zhang W, Wang G, Huang X, Liang G & Li N. 2023. Effects of five years conservation tillage for hedging against drought, stabilizing maize yield, and improving soil environment in the drylands of northern China. *PloS One* **18**: e0282359.

van Dijk M, Morley T, Rau ML & Saghai Y. 2021. A meta-analysis of projected global food demand and population at risk of hunger for the period 2010–2050. *Nature Food* **2**: 494–501.

DiLegge MJ, Manter DK & Vivanco JM. 2022. Soil microbiome disruption reveals specific and general plant-bacterial relationships in three agroecosystem soils. *PloS One* **17**: e0277529.

Ding Y, Schiestl-Aalto P, Helmisaari H-S, Makita N, Ryhti K & Kulmala L. 2020. Temperature and moisture dependence of daily growth of Scots pine (*Pinus sylvestris* L.) roots in Southern Finland. *Tree Physiology* **40**: 272–283.

Donovan BJ & Elliott G. 2001. Honey bee response to high concentrations of some new spray adjuvants. *New Zealand Plant Protection* **54**: 51–55.

Du Q, Guan Q, Sun Y, Wang Q, Zhang J, Xiao X, Liu H, Yang X & Zhang E. 2024. Legacy effects of extreme drought and wetness events on mountain grassland ecosystems and their elevation dependence. *Journal of Hydrology* **630**: 130757.

Duan S, Zhang C, Song S, Ma C, Zhang C, Xu W, Bondada B, Wang L & Wang S. 2022. Understanding calcium functionality by examining growth characteristics and structural aspects in calcium-deficient grapevine. *Scientific Reports* **12** : 3233.

Dumenu WK & Obeng EA. 2016. Climate change and rural communities in Ghana: Social vulnerability, impacts, adaptations and policy implications. *Environmental Science & Policy* **55**: 208–217.

Essa SM, Wafa HA, Mahgoub E-SI, Hassanin AA, Al-Khayri JM, Jalal AS, El-Moneim D, Alshamrani SM, Safhi FA & Eldomiaty AS. 2023. Assessment of eight faba bean (*Vicia faba* L.) cultivars for drought stress tolerance through molecular, morphological, and physiochemical parameters. *Sustainability* **15**: 3291.

Etesami H, Li Z, Maathuis FJM & Cooke J. 2022. The combined use of silicon and arbuscular mycorrhizas to mitigate salinity and drought stress in rice. *Environmental and Experimental Botany* **201**: 104955.

Evans SE & Wallenstein MD. 2014. Climate change alters ecological strategies of soil bacteria. *Ecology Letters* **17**: 155–164.

Falk RH, Guggenheim R & Schulke G. 1994. Surfactant-Induced Phytotoxicity. *Weed Technology* **8**: 519–525.

Farkas A, Maróti G, Kereszt A & Kondorosi É. 2017. Comparative analysis of the bacterial membrane disruption effect of two natural plant antimicrobial peptides. *Frontiers in Microbiology* **8**: 51.

Feller U, Kopriva S & Vassileva V. 2018. Plant nutrient dynamics in stressful environments: Needs interfere with burdens. *Agriculture* **8**: 97.

Feng GL, Letey J & Wu L. 2002. The influence of two surfactants on infiltration into a water-repellent soil. *Soil Science Society of America Journal* **66**: 361–367.

Feng X, Sun X, Zhou W, Zhang W, Che F & Li S. 2021. The effects of green waste compost on soil N, P, K, and organic matter fractions in forestry soils: elemental analysis evaluation. *RSC Advances* **11**: 31983–31991.

dos reis Ferreira GM, Melloni R, de Oliveira da Silva LF, Martins FB & Gonçalves ED. 2015. Arbuscular mycorrhizal fungi in seedling development of olive (*Olea europaea* L.) in the south of Minas Gerais, Brazil. *Revista Brasileira de Ciencia Do Solo* **39**: 361–366.

Fitter AH & Hay RKM. 2001. Mineral Nutrients: Introduction. In *Environmental Physiology of Plants* (3rd ed.) (Academic Press, London, UK): 74-82.

Flexas J & Medrano H. 2002. Drought-inhibition of photosynthesis in C3 plants: stomatal and non-stomatal limitations revisited. *Annals of Botany* **89**: 183–189.

Floss DS, Levy JG, Lévesque-Tremblay V, Pumplin N & Harrison MJ. 2013. DELLA proteins regulate arbuscule formation in arbuscular mycorrhizal symbiosis. *Proceedings of the National Academy of Sciences of the United States of America* **110**: E5025-34.

Foley JA, Ramankutty N, Brauman KA, Cassidy ES, Gerber J, Johnston ML, Mueller ND, O'Connell C, Ray DK, West P, *et al.* 2011. Solutions for a cultivated planet. *Nature* **478**: 337–342.

Fournier C, Durand JL, Ljutovac S, Schäufele R, Gastal F & Andrieu B. 2005. A functional-structural model of elongation of the grass leaf and its relationships with the phyllochron. *The New Phytologist* **166**: 881–894.

Friel S, Berry H, Dinh H, O'Brien L & Walls HL. 2014. The impact of drought on the association between food security and mental health in a nationally representative Australian sample. *BMC Public Health,* **14**: 1102.

Fry EL, Evans AL, Sturrock CJ, Bullock JM & Bardgett RD. 2018. Root architecture governs plasticity in response to drought. *Plant and Soil* **433**: 189–200.

van der Gaag DJ, van Noort FR, Stapel-Cuijpers LHM, de Kreij C, Termorshuizen AJ, van Rijn E, Zmora-Nahum S & Chen Y. 2007. The use of green waste compost in peat-based potting mixtures: Fertilization and suppressiveness against soilborne diseases. *Scientia Horticulturae* **114**: 289–297.

Gálvez A, López-Galindo A & Peña A. 2018. Effect of different surfactants on germination and root elongation of two horticultural crops: implications for seed coating. *New Zealand Journal of Crop and Horticultural Science* **47**: 1–16.

Gavito ME, Jakobsen I, Mikkelsen TN & Mora F. 2019. Direct evidence for modulation of photosynthesis by an arbuscular mycorrhiza-induced carbon sink strength. *The New Phytologist* **223**: 896–907.

Genre A, Chabaud M, Faccio A, Barker DG & Bonfante P. 2008. Prepenetration apparatus assembly precedes and predicts the colonization patterns of arbuscular mycorrhizal fungi within the root cortex of both *Medicago truncatula* and *Daucus carota*. *The Plant Cell* **20**: 1407–1420.

Genre A, Chabaud M, Timmers T, Bonfante P & Barker DG. 2005. Arbuscular mycorrhizal fungi elicit a novel intracellular apparatus in *Medicago truncatula* root epidermal cells before infection. *The Plant Cell* **17**: 3489–3499.

Giannakopoulos V. 2022. *Effects of surfactant-based wetting agents on cereal growth and physiology*. PhD thesis, Lancaster University.

Giovannetti M. 2000. Spore germination and pre-symbiotic mycelial growth. In *Arbuscular Mycorrhizas: Physiology and Function* (Springer, Dordrecht): 47–68.

Giovannetti M, Sbrana C, Strani P, Agnolucci M, Rinaudo V & Avio L. 2003. Genetic diversity of isolates of *Glomus mosseae* from different geographic areas detected by vegetative compatibility testing and biochemical and molecular analysis. *Applied and Environmental Microbiology* **69**: 616–624.

Giovannini L, Palla M, Agnolucci M, Avio L, Sbrana C, Turrini A & Giovannetti M. 2020. Arbuscular mycorrhizal fungi and associated Microbiota as plant biostimulants: Research strategies for the selection of the best performing inocula. *Agronomy* **10**: 106.

Godbold DL. 2004. Tree physiology | Mycorrhizae. In *Encyclopedia of Forest Sciences* (Academic Press, Massachusetts, USA): 1633–1639.

Gong Z. 2021. Plant abiotic stress: New insights into the factors that activate and modulate plant responses. *Journal of Integrative Plant Biology* **63**: 429–430.

Görlach B, Landgrebe-Trinkunaite R & E Interwies. 2004. Assessing the Economic Impacts of Soil Degradation. Volume I: Literature Review. Study commissioned by the European Commission, DG Environment, Study Contract ENV.B.1/ETU/2003/0024. Berlin: Ecologic.

Gosling P, Hodge A, Goodlass G & Bending GD. 2006. Arbuscular mycorrhizal fungi and organic farming. Agriculture, *Ecosystems & Environment* **113**: 17–35.

Greger M, Landberg T & Vaculík M. 2018. Silicon influences soil availability and accumulation of mineral nutrients in various plant species. *Plants* **7**: 41.

Groh J, Vanderborght J, Pütz T, Vogel H-J, Gründling R, Rupp H, Rahmati M, Sommer M, Vereecken H & Gerke HH. 2020. Responses of soil water storage and crop water use efficiency to changing climatic conditions: a lysimeter-based space-for-time approach. *Hydrology and Earth System Sciences* **24**: 1211–1225.

Gruber BD, Giehl RFH, Friedel S & von Wirén N. 2013. Plasticity of the Arabidopsis root system under nutrient deficiencies. *Plant Physiology* **163**: 161–179.

Gutjahr C & Paszkowski U. 2013. Multiple control levels of root system remodeling in arbuscular mycorrhizal symbiosis. *Frontiers in Plant Science* **4**: 204.

Harrison RD. 2000. Repercussions of El Niño: drought causes extinction and the breakdown of mutualism in Borneo. *Proceedings. Biological Sciences* **267:** 911–915.

Hatfield JL & Prueger JH. 2015. Temperature extremes: Effect on plant growth and development. *Weather and Climate Extremes,* **10**: 4–10.

Hazen JL. 2000. Adjuvants—Terminology, Classification, and Chemistry. *Weed Technology* **14**: 773-784.

Hertel TW. 2011. The global supply and demand for agricultural land in 2050: A perfect storm in the making? *American Journal of Agricultural Economics* **93**: 259–275.

Hijri M & Sanders I. 2005. Low gene copy number shows that arbuscular mycorrhizal fungi inherit genetically different nuclei. *Nature* **433**: 160–163.

Hill RM. 2003. Silicone (Siloxane) Surfactants. In *Encyclopedia of Physical Science and Technology* (Academic Press, Massachusetts, USA): 793–804.

Hodge A, Berta G, Doussan C, Merchan F & Crespi M. 2009. Plant root growth, architecture and function. *Plant and Soil* **321**: 153–187.

Hodge A, Campbell CD & Fitter AH. 2001. An arbuscular mycorrhizal fungus accelerates decomposition and acquires nitrogen directly from organic material. *Nature* **413**: 297–299.

Howe JA & Smith AP. 2021. The soil habitat. In *Principles and Applications of Soil Microbiology* (Prentice Hall, New Jersey, USA): 23–55.

Hu AY, Xu SN, Qin DN, Li W & Zhao XQ. 2020. Role of silicon in mediating phosphorus imbalance in plants. *Plants* **10**: 51.

Humphrey V, Zscheischler J, Ciais P, Gudmundsson L, Sitch S & Seneviratne SI. 2018. Sensitivity of atmospheric CO_2 growth rate to observed changes in terrestrial water storage. *Nature* **560**: 628–63.

Ingensiep HW. 2004. The History of the Plant Embryo. Terminology and Visualization from Ancient until Modern Times. *History and Philosophy of the Life Sciences* **26**: 309–331.

Inghels D, Dullaert W & Bloemhof J. 2016. A model for improving sustainable green waste recovery. *Resources, Conservation, and Recycling* **110**: 61–73.

Ingraffia R, Amato G, Frenda AS & Giambalvo D. 2019. Impacts of arbuscular mycorrhizal fungi on nutrient uptake, N_2 fixation, N transfer, and growth in a wheat/faba bean intercropping system. *PloS One* **14**: e0213672.

Intergovernmental Panel on Climate Change. 2014. Climate change 2014: Synthesis report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Core Writing Team, Pachauri RK & Meyer LA. (Eds.).

IPCC. 2022. Climate Change 2022: Impacts, Adaptation and Vulnerability. Contribution of Working Group II to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change [Pörtner HO, Roberts DC, Tignor M, Poloczanska ES, Mintenbeck K, Alegría A, Craig M, Langsdorf S, Löschke S, Möller V, Okem A, Rama B (eds.)]. Cambridge University Press. Cambridge University Press, Cambridge, UK and New York, NY, USA.

Ishiguro M & Koopal LK. 2016. Surfactant adsorption to soil components and soils. *Advances in Colloid and Interface Science* **231**: 59–102.

Jacoby R, Peukert M, Succurro A, Koprivova A & Kopriva S. 2017. The role of soil microorganisms in plant mineral nutrition-current knowledge and future directions. *Frontiers in Plant Science* **8**: 1617.

Jakab G, Madarász B, Masoudi M, Karlik M, Király C, Zacháry D, Filep T, Dekemati I, Centeri C, Al-Graiti T & Szalai, Z. 2023. Soil organic matter gain by reduced tillage intensity: Storage, pools, and chemical composition. *Soil & Tillage Research* **226**: 105584.

Janker J, Mann S & Rist S. 2018. What is sustainable agriculture? Critical analysis of the international political discourse. *Sustainability* **10**: 4707.

Jansa J, Mozafar A, Kuhn G, Anken T, Ruh R, Sanders IR & Frossard E. 2003. Soil tillage affects the community structure of mycorrhizal fungi in maize roots. *Ecological Applications* **13**: 1164–1176.

Jia W, Cheng L, Tan Q, Liu Y, Dou J, Yang K, Yang Q, Wang S, Li J & Niu G. 2023. Response of the soil microbial community to petroleum hydrocarbon stress shows a threshold effect: research on aged realistic contaminated fields. *Frontiers in Microbiology* **14**: 1188229.

Jia Y, Gray VM & Straker CJ. 2004. The influence of Rhizobium and arbuscular mycorrhizal fungi on nitrogen and phosphorus accumulation by *Vicia faba*. *Annals of Botany* **94**: 251–258.

Jibrin MO, Liu Q, Jones JB & Zhang S. 2021. Surfactants in plant disease management: A brief review and case studies. *Plant Pathology* **70**: 495–510.

John JBS, Bartels PG & Hilton JL. 1974. Surfactant effects on isolated plant cells. *Weed Science* **22**: 233–237.

Johnson NC, Rowland DL, Corkidi L, Egerton-Warburton LM & Allen EB. 2003. Nitrogen enrichment alters mycorrhizal allocation at five Mesic to semiarid grasslands. *Ecology* **84**: 1895–1908.

Johnson SN, Powell JR, Frew A & Cibils–Stewart X. 2022. Silicon accumulation suppresses arbuscular mycorrhizal fungal colonisation in the model grass *Brachypodium distachyon*. *Plant Soil* **477**: 219–232.

Kakouridis A, Hagen JA, Kan MP, Mambelli S, Feldma, LJ, Herman DJ, Weber PK, Pett-Ridge J & Firestone MK. 2022. Routes to roots: direct evidence of water transport by arbuscular mycorrhizal fungi to host plants. *The New Phytologist* **236**: 210–221.

Kallenbach C, Frey S & Grandy A. 2016. Direct evidence for microbial-derived soil organic matter formation and its ecophysiological controls. *Nature Communications* **7**: 13630.

Karagunduz A, Pennell KD & Young MH. 2001. Influence of a nonionic surfactant on the water retention properties of unsaturated soils. *Soil Science Society of America* **65**: 1392–1399.

Karpouzas DG, Papadopoulou E, Ipsilantis I, Friedel I, Petric I, Udikovic-Kolic N, Djuric S, Menkissoglu-Spiroudi U & Martin-Laurent F. 2014. Effects of nicosulfuron on the abundance and diversity of arbuscular mycorrhizal fungi used as indicators of pesticide soil microbial toxicity. *Ecological Indicators* **39**: 44–53.

Kassie M, Jaleta M, Shiferaw B, Mmbando F & Mekuria M. 2013. Adoption of interrelated sustainable agricultural practices in smallholder systems: Evidence from rural Tanzania. *Technological Forecasting and Social Change* **80**: 525–540.

Kéfi S, Rietkerk M, Alados C, Pueyo Y, Papanastasis VP, ElAich A & de Ruiter PC. 2007. Spatial vegetation patterns and imminent desertification in Mediterranean arid ecosystems. *Nature* **449**: 213–217.

Keymer A, Pimprikar P, Wewer V, Huber C, Brands M, Bucerius SL, Delaux P-M, Klingl V, Röpenack-Lahaye von E, Wang TL *et al*. 2017. Lipid transfer from plants to arbuscular mycorrhiza fungi. *eLife* **6**: e29107.

Khan F, Siddique AB, Shabala S, Zhou M & Zhao C. 2023. Phosphorus plays key roles in regulating plants' physiological responses to abiotic stresses. *Plants* **12**: 2861.

Kibbou F, El Bouhmadi K, Marrou H, Sinclair TR & Ghanem ME. 2022. Impact of drought and temperature constraints on development and growth of faba bean (*Vicia faba L.*). *Journal of Crop Improvement* **36**: 57–72.

Kijowska-Oberc J, Dylewski Ł & Ratajczak E. 2023. Proline concentrations in seedlings of woody plants change with drought stress duration and are mediated by seed characteristics: a meta-analysis. *Scientific Reports* **13**: 15157.

Kim K-H & Lee B-M. 2023. Effects of climate change and drought tolerance on maize growth. *Plants* **12**: 3548.

Kim SJ, Eo J-K, Lee E-H, Park H & Eom A-H. 2017. Effects of arbuscular mycorrhizal fungi and soil conditions on crop plant growth*. Mycobiology* **45**: 20–24.

Kirk JL, Moutoglis P, Klironomos J, Lee H & Trevors JT. 2005. Toxicity of diesel fuel to germination, growth and colonization of *Glomus intraradices* in soil and *in vitro* transformed carrot root cultures. *Plant and Soil* **270**: 23–30.

Kochian LV. 2018. Mechanisms of Micronutrient Uptake and Translocation in Plants. In *Micronutrients in Agriculture* (Wiley, New Jersey, USA): 229–296.

Kokkoris V, Banchini C, Paré,L, Abdellatif L, Séguin S, Hubbard K, Findlay W, Dalpé Y, Dettman J, Corradi N *et al*. 2023. *Rhizophagus irregularis*, the model fungus in arbuscular mycorrhiza research, forms dimorphic spores. *The New Phytologist* **242**: 1771–1784.

Kopittke PM, Menzies NW, Wang P, McKenna BA & Lombi E. 2019. Soil and the intensification of agriculture for global food security. *Environment International* **132**: 105078.

Kostka SJ, Cisar JL, Mitra S, Park DM, Ritsema CJ, Dekker LW & Franklin MA. 2007. Irrigation efficiency. Soil surfactants can save water and help maintain turfgrass quality. *Golf Course Industry*: 91-95.

Kosuta S, Chabaud M, Lougnon G, Gough C, Dénarié J, Barker DG & Bécard G. 2003. A diffusible factor from arbuscular mycorrhizal fungi induces symbiosis-specific MtENOD11 expression in roots of *Medicago truncatula*. *Plant Physiology* **131**: 952–962.

Kou X, Han W & Kang J. 2022. Responses of root system architecture to water stress at multiple levels: A meta-analysis of trials under controlled conditions. *Frontiers in Plant Science* **13**: 1085409.

Kozlowski TT & Pallardy SG. 1997. Seed germination and seedling growth. In *Growth Control in Woody Plants* (Academic Press, Massachusetts, USA): 14–72.

Knoche M, Noga G & Lenz F. 1992. Surfactant-induced phytotoxicity: evidence for interaction with epicuticular wax fine structure. *Crop Protection* **11**: 51–56.

Krajinski F, Biela A, Schubert D, Gianinazzi-Pearson V, Kaldenhoff R & Franken P. 2000. Arbuscular mycorrhiza development regulates the mRNA abundance of Mtaqp1 encoding a mercury-insensitive aquaporin of *Medicago truncatula*. *Planta* **211**: 85–90.

Krishna H, Singh SK, Minakshi, Patel VB, Khawale RN, Deshmukh PS & Jindal PC. 2006. Arbuscular-mycorrhizal fungi alleviate transplantation shock in micropropagated grapevine (*Vitis vinifera* L.). *The Journal of Horticultural Science & Biotechnology* **81**: 259–263.

Krogh KA, Halling-Sørensen B, Mogensen BB & Vejrup KV. 2003. Environmental properties and effects of nonionic surfactant adjuvants in pesticides: a review. *Chemosphere* **50**: 871–901.

Lal R. 2003. Soil erosion and the global carbon budget. *Environment International* **29**: 437–450.

Lee J. 2011. The distribution of cytoplasm and nuclei within the extra-radical mycelia in *Glomus intraradices*, a species of arbuscular mycorrhizal fungi. *Mycobiology* **39**: 79–84.

Lee KS, Starov VM, Muchatuta TJP & Srikantha SIR. 2009. Spreading of trisiloxanes over thin aqueous layers. *Colloid Journal* **71**: 365–369.

Lehrsch GA, Sojka, RE & Koehn AC. 2012. Surfactant effects on soil aggregate tensile strength. *Geoderma* **190**: 199–206.

Lehrsch GA, Sojka RE, Reed JL, Henderson RA & Kostka SJ. 2011. Surfactant and irrigation effects on wettable soils: runoff, erosion, and water retention responses. *Hydrological Processes* **25**: 766–777.

Lei ZY, Han JM, Yi XP, Zhang WF & Zhang YL. 2018. Coordinated variation between veins and stomata in cotton and its relationship with water-use efficiency under drought stress. *Photosynthetica* **56**: 1326–1335.

Leigh J, Hodge A & Fitter AH. 2009. Arbuscular mycorrhizal fungi can transfer substantial amounts of nitrogen to their host plant from organic material. *The New Phytologist* **181**: 199–207.

Li J. 2017. Endosperm Differentiation. In *Encyclopedia of Applied Plant Sciences* (Academic Press, Massachusetts, USA): 497–503.

Li H, Travlos I, Qi L, Kanatas P & Wang P. 2019. Optimization of herbicide use: Study on spreading and evaporation characteristics of glyphosate-organic silicone mixture droplets on weed leaves. *Agronomy* **9**: 547.

Lin F, Mao Y, Zhao F, Idris AL, Liu Q, Zou S, Guan X & Huang T. 2023. Towards sustainable green adjuvants for microbial pesticides: Recent progress, upcoming challenges, and future perspectives. *Microorganisms* **11**: 364.

Lloret A, Conejero A, Leida C, Petri C, Gil-Muñoz F, Burgos L, Badenes ML & Ríos G. 2017. Dual regulation of water retention and cell growth by a stress-associated protein (*SAP*) gene in prunus. *Scientific Reports* **7**: 2017.

Lobell DB, Schlenker W & Costa-Roberts J. 2011. Climate trends and global crop production since 1980. *Science* **333**, 616–620.

Lu X, Lu X & Liao Y. 2018. Effect of tillage treatment on the diversity of soil arbuscular mycorrhizal fungal and soil aggregate-associated carbon content. *Frontiers in Microbiology* **9**: 2986.

Lu Y, Liu, H, Chen Y, Zhang L, Kudusi K & Song J. 2022. Effects of drought and salt stress on seed germination of ephemeral plants in desert of northwest China. *Frontiers in Ecology and Evolution* **10**: 1026095.

Luo X, Shi S, Liu Y, Yang H, Li N, Dong Z, Zhu B & He X. 2021. Arbuscular mycorrhizal fungal communities of topsoil and subsoil of an annual maize-wheat rotation after 15-years of differential mineral and organic fertilization. *Agriculture, Ecosystems & Environment* **315**: 107442.

Lv L, Gao Z, Liao K, Zhu Q & Zhu J. 2023. Impact of conservation tillage on the distribution of soil nutrients with depth. *Soil & Tillage Research* **225**: 105527.

Lynas M, Houlton BZ & Perry S. 2021. Greater than 99% consensus on human caused climate change in the peer-reviewed scientific literature. *Environmental Research Letters* **16**: 114005.

Ma JF. 2004. Role of silicon in enhancing the resistance of plants to biotic and abiotic stresses. *Soil Science and Plant Nutrition* **50**: 11–18.

Ma J, Bair E & Motsinger-Reif A. 2020. Nonlinear dose-response modeling of high-throughput screening data using an evolutionary algorithm. *Publication of International Hormesis Society* **18**: 1559325820926734.

Ma J, Takahashi E. 1990. Effect of silicon on the growth and phosphorus uptake of rice. *Plant Soil* **126**: 115–119.

Ma JF, Miyake Y & Takahashi E. 2001. Chapter 2 Silicon as a beneficial element for crop plants. In *Studies in Plant Science: Silicon in Agriculture* (Elsevier, Amsterdam, Netherlands): 17–39.

Ma X, Geng Q, Zhang H, Bian C, Chen H YH, Jiang D & Xu X. 2021. Global negative effects of nutrient enrichment on arbuscular mycorrhizal fungi, plant diversity and ecosystem multifunctionality. *The New Phytologist* **229**: 2957–2969.

Magor E, Wilson MD, Wong H, Cresswell T, Sánchez-Palacios JT, Bell RW & Penrose B. 2023. Selected adjuvants increase the efficacy of foliar biofortification of iodine in bread wheat (*Triticum aestivum* L.) grain. *Frontiers in Plant Science* **14**: 1246945.

Manabe S & Wetherald RT. 1967. Thermal Equilibrium of the Atmosphere with a Given Distribution of Relative Humidity. *Journal of Atmospheric Sciences* **24**: 241-259.

Manteghi A, Danesh YR, Hesar AM, Demir S, Boyno G, Catani L & Semprucci F. 2022. Is the arbuscular mycorrhizal fungus *Funneliformis mosseae* a suitable agent to control criconematid populations? *Diversity* **14**: 898.

Mariotti B., Martini S, Raddi S, Tani A, Jacobs DF, Oliet JA & Maltoni A. 2020. Coconut coir as a sustainable nursery growing media for seedling production of the ecologically diverse *Quercus* species. *Forests* **11**: 522.

Marro N, Grilli G, Soteras F, Caccia M, Longo S, Cofré N, Borda V, Burni M, Janoušková M & Urcelay C. 2022. The effects of arbuscular mycorrhizal fungal species and taxonomic groups on stressed and unstressed plants: a global meta-analysis. *The New Phytologist* **235**: 320–332.

Martin T, Rothwell S & Stevens C. 2022. An investigation into the principal modes of action of surfactants and how a novel formulation may improve turfgrass quality by increasing the dominance of *Agrostis* spp. in golf greens. *International Turfgrass Society Research Journal* **14**: 1010–1015.

Martinez-Ghersa MA, Satorre EH & Ghersa CM. 1997. Effect of soil water content and temperature on dormancy breaking and germination of three weeds. *Weed Science* **45**: 791–797.

Masroor M, Sajjad H, Rehman S, Singh R, Hibjur Rahaman M, Sahana M, Ahmed R & Avtar R. 2022. Analysing the relationship between drought and soil erosion using vegetation health index and RUSLE models in Godavari middle sub-basin, India. *Geoscience Frontiers* **13**: 101312.

Massa D, Malorgio F, Lazzereschi S, Carmassi G, Prisa D & Burchi G. 2018. Evaluation of two green composts for peat substitution in geranium (*Pelargonium zonale* L.) cultivation: Effect on plant growth, quality, nutrition, and photosynthesis. *Scientia Horticulturae* **228**: 213–221.

Mbarki Y, Gumiere SJ, Celicourt P & Brédy J. 2023. Study of the effect of the compaction level on the hydrodynamic properties of loamy sand soil in an agricultural context. *Frontiers in Water* **5**: 1255495.

McMillan M, Daroub S, Moore K, Erickson J, Kostka S & Fidanza M. 2023. Surfactant-coated seed emergence and establishment under deficit irrigation in hydrophilic and hydrophobic soils. *Journal of Environmental Horticulture* **41**: 33–39.

Meehl GA & Tebaldi C. 2004. More intense, more frequent, and longer lasting heat waves in the 21st century. *Science* **305**: 994–997.

Meena M, Divyanshu K, Kumar S, Swapnil P, Zehra A, Shukla V, Yadav M & Upadhyay RS. 2019. Regulation of L-proline biosynthesis, signal transduction, transport, accumulation and its vital role in plants during variable environmental conditions. *Heliyon* **5**: e02952.

Miller RM, Jastrow JD. 1992. Extraradical hyphal development of vesicular–arbuscular mycorrhizal fungi in a chronosequence of prairie restoration. In *Mycorrhizas in ecosystems.* (CAB International, Wallingford, UK): 171–176.

Mitra D, Rad KV, Chaudhary P, Ruparelia J, Sagarika MS, Boutaj H, Das Mohapatra, PK & Panneerselvam P. 2021. Involvement of strigolactone hormone in root development, influence and interaction with mycorrhizal fungi in plant: Mini-review. *Current Research in Microbial Sciences* **2**: 100026.

Mobbs TL, Peters RT, Davenport JR, Evans MA & Wu JQ. 2012. Effects of four soil surfactants on four soil-water properties in sand and silt loam. *Journal of Soil and Water Conservation* **67**: 275–283.

Moffett JO & Morton HL. 1975. Repellency of surfactants to honey bees. *Environmental Entomology* **4**: 780–782.

Mohammad C, Afshar RK, Saberi M, Rostamza M, Falahtabar N. 2016. Alleviation of salinity and drought stress in corn production using a non-ionic surfactant. *Journal of Animal and Plant Sciences* **26**: 1042-1047.

Mojsiewicz-Pieńkowska K, Jamrógiewicz M, Szymkowska K & Krenczkowska D. 2016. Direct human contact with siloxanes (silicones) - safety or risk part 1. Characteristics of siloxanes (silicones). *Frontiers in Pharmacology* **7**: 132.

Moore D, Kostka S, Boerth T, Franklin M, Ritsema C, Dekker L, Oostindie K, Stoof C & Wesseling J. 2010. The effect of soil surfactants on soil hydrological behavior, the plant growth environment, irrigation efficiency and water conservation. *Journal of Hydrology and Hydromechanics* **58**: 142-148.

Munjonji L, Ayisi KK, Mudongo EI, Mafeo TP, Behn K, Mokoka MV & Linstädter A. 2020. Disentangling drought and grazing effects on soil carbon stocks and CO_2 fluxes in a semi-arid African Savanna. *Frontiers in Environmental Science* **8**: 590665.

Muller B, Pantin F, Génard M, Turc O, Freixes S, Piques M & Gibon Y. 2011. Water deficits uncouple growth from photosynthesis, increase C content, and modify the relationships between C and growth in sink organs. *Journal of Experimental Botany* **62**: 1715–1729.

Navazio L, Moscatiello R, Genre A, Novero M, Baldan B, Bonfante P & Mariani P. 2007. A diffusible signal from arbuscular mycorrhizal fungi elicits a transient cytosolic calcium elevation in host plant cells. *Plant Physiology* **144**: 673–681.

Nelissen H, Gonzalez N & Inzé D. 2016. Leaf growth in dicots and monocots: so different yet so alike. *Current Opinion in Plant Biology* **33**: 72–76.

Newman EI. 1966. A Method of Estimating the Total Length of Root in a Sample. *Journal of Applied Ecology* **3**: 139–145.

Nobels I, Spanoghe P, Haesaert G, Robbens J & Blust R. 2011. Toxicity ranking and toxic mode of action evaluation of commonly used agricultural adjuvants on the basis of bacterial gene expression profiles. *PloS One,* **6**: e24139.

Obihara CH & Russell EW. 1972. Specific adsorption of silicate and phosphate by soils. *Journal of Soil Science* **23**: 105–117.

Oehl F, Sieverding E, Ineichen K, Mader P, Boller T & Wiemken A. 2003. Impact of land use intensity on the species diversity of arbuscular mycorrhizal fungi in agroecosystems of Central Europe. *Applied Environmental Microbiology* **69**: 2816–2824.

Osburn ED, Yang G, Rillig MC & Strickland MS. 2023. Evaluating the role of bacterial diversity in supporting soil ecosystem functions under anthropogenic stress. *ISME Communications* **3**: 66.

Osuna-Canizalez FJ, De Datta SK & Bonman JM. 1991. Nitrogen form and silicon nutrition effects on resistance to blast disease of rice. *Plant Soil* **135**: 223–231.

Otkin JA, Svoboda M, Hunt ED, Ford TW, Anderson MC, Hain C & Basara JB. 2018. Flash droughts: A review and assessment of the challenges imposed by rapid-onset droughts in the United States. *Bulletin of the American Meteorological Society* **99**: 911–919.

Oyewole BO, Olawuyi OJ. Odebode AC & Abiala MA. 2017. Influence of Arbuscular mycorrhiza fungi (AMF) on drought tolerance and charcoal rot disease of cowpea. *Biotechnology Reports* **14**: 8–15.

Palma-Bautista C, Vazquez-Garcia JG, Travlos I, Tataridas A, Kanatas P, Domínguez-Valenzuela JA & Prado RD. 2020. Effect of Adjuvant on Glyphosate Effectiveness, Retention, Absorption and Translocation in Lolium rigidum and Conyza canadensis. *Plants* **9**: 297.

Panday D & Nkongolo NV. 2021. No tillage improved soil pore space indices under cover crop and crop rotation. *Soil Systems* **5**: 38.

Parida AK & Das AB. 2005. Salt tolerance and salinity effects on plants: a review. *Ecotoxicology and Environmental Safety* **60**: 324–349.

Park HJ, Kim W-Y & Yun D-J. 2016. A new insight of salt stress signaling in plant. *Molecules and Cells* **39**: 447–459.

Parniske M. 2008. Arbuscular mycorrhiza: the mother of plant root endosymbioses. *Nature Reviews. Microbiology* **6**: 763–775.

Pathak RK, Singh DB, Sharma H Pandey D & Dwivedi S. 2021. Calcium uptake and translocation in plants. In *Calcium Transport Elements in Plants* (Academic Press, Massachusetts, USA): 373–386.

Pauwels R, Graefe J & Bitterlich M. 2023. An arbuscular mycorrhizal fungus alters soil water retention and hydraulic conductivity in a soil texture specific way. *Mycorrhiza* **33**: 165–179.

Perico C, Tan S & Langdale JA. 2022. Developmental regulation of leaf venation patterns: monocot versus eudicots and the role of auxin. *The New Phytologist* **234**: 783–803.

Perkons U, Kautz T, Uteau D, Peth S, Geier V, Thomas K, Lütke Holz K, Athmann M, Pude R & Köpke U. 2014. Root-length densities of various annual crops following crops with contrasting root systems. *Soil and Tillage Research* **137**: 50–57.

le Pioufle O & Declerck S. 2018. Reducing water availability impacts the development of the arbuscular mycorrhizal fungus *Rhizophagus irregularis* MUCL 41833 and its ability to take up and transport phosphorus under in vitro conditions. *Frontiers in Microbiology* **9**: 1254.

Plaza-Bonilla D, Arrúe JL, Cantero-Martínez C. Fanlo R, Inglesias A & Álvaro-Fuentes J. 2015. Carbon management in dryland agricultural systems. A review. *Agronomy for Sustainable Development* **35**: 1319–1334.

Poorter H, Fiorani F, Stitt M, Schurr U, Finck A, Gibon Y, Usadel B, Munns R, Atkin OK, Tardieu F *et al*. 2012. The art of growing plants for experimental purposes: a practical guide for the plant biologist. *Functional Plant Biology* **39**: 821–838.

Poirier F, Boursier C, Mesnage R, Oestreicher N, Nicolas V & Vélot C. 2017. Proteomic analysis of the soil filamentous fungus *Aspergillus nidulans* exposed to a Roundup formulation at a dose causing no macroscopic effect: a functional study. *Environmental Science and Pollution Research International* **24**: 25933–25946.

Polavarapu BKK & Nese S. 2014. Is proline accumulation per se correlated with stress tolerance or is proline homeostasis a more critical issue? *Plant, Cell & Environment* **37**: 300–311.

Policello GA, Stevens PJG, Forster WA & Murphy GJ. 1995. The influence of pH on the Performance of Organosilicone Surfactants. In *Pesticide Formulations and Application Systems* (ASTM International, Pennsylvania, USA) **14**: 313–315.

Poudel M, Mendes R, Costa LAS, Bueno CG, Meng Y, Folimonova SY, Garrett KA & Martins SJ. 2021. The role of plant-associated bacteria, fungi, and viruses in drought stress mitigation. *Frontiers in Microbiology* **12**: 743512.

Prince SJ, Song L, Qiu D, Maldonado dos Santos JV, Chai C, Joshi T, Patil G, Valliyodan B, Vuong TD, Murphy M *et al*. 2015. Genetic variants in root architecture-related genes in a glycine soja accession, a potential resource to improve cultivated soybean. *BMC Genomics* **16**: 132.

Prodhan FA, Zhang J, Pangali Sharma TP, Nanzad L, Zhang D, Seka AM, Ahmed AM, Hasan SS, Hoque MZ & Mohana HP. 2022. Projection of future drought and its impact on simulated crop yield over South Asia using ensemble machine learning approach. *The Science of the Total Environment* **807**: 151029.

de la Providencia IE, de Souza FA, Fernández F, Delmas NS & Declerck S. 2005. Arbuscular mycorrhizal fungi reveal distinct patterns of anastomosis formation and hyphal healing mechanisms between different phylogenic groups. *The New Phytologist* **165**: 261–271.

Qin M, Li L, Miranda J-P, Tang Y, Song B, Oosthuizen MK & Wei W. 2022. Experimental duration determines the effect of arbuscular mycorrhizal fungi on plant biomass in pot experiments: A meta-analysis. *Frontiers in Plant Science* **13**: 1024874.

Quintana JR, Martín-Sanz JP, Valverde-Asenjo I & Molina JA. 2023. Drought differently destabilizes soil structure in a chronosequence of abandoned agricultural lands. *Catena* **222**: 106871.

Raeisi F, Mousavi SM, Hashemi SA, Malekpour L, Bahrani S, Lai CW., Chiang W-H, Babapoor A, Mazraedoost S & Esmaeili H. 2021. Application of biosurfactant as a demulsifying and emulsifying agent in the formulation of petrochemical products. In *Green Sustainable Process for Chemical and Environmental Engineering and Science* (Elsevier, Amsterdam, Netherlands): 399–422.

Rai AN & Penna S. 2013. Molecular evolution of plant P5CS gene involved in proline biosynthesis. *Molecular Biology Reports* **40**: 6429–6435.

Rajjou L, Duval M, Gallardo K, Catusse J, Bally J, Job C & Job D. 2012. Seed germination and vigor. *Annual Review of Plant Biology* **63**: 507–533.

Räsch A, Hunsche M, Mail M, Burkhardt J, Noga G & Pariyar S. 2018. Agricultural adjuvants may impair leaf transpiration and photosynthetic activity. *Plant Physiology and Biochemistry* **132**: 229–237.

Reinikainen O & Herranen M. 1997. The influence of wetting agent on physical properties of peat. *Acta Horticulturae* **450**: 375–380.

Rivero RM, Kojima M, Gepstein A, Sakakibara H, Mittler R, Gepstein S & Blumwald E. 2007. Delayed leaf senescence induces extreme drought tolerance in a flowering plant. *Proceedings of the National Academy of Sciences of the United States of America* **104**: 19631–19636.

Rizwan M, Ali S, Ibrahim M, Farid M, Adrees M, Bharwana SA, Zia-ur-Rehman M, Qayyum MF & Abbas F. 2015. Mechanisms of silicon-mediated alleviation of drought and salt stress in plants: a review*. Environmental Science and Pollution Research International* **22**: 15416–15431.

Robertson BC, Han Y, Li C. 2023. A comparison of different stomatal density phenotypes of *Hordeum vulgare* under varied watering regimes reveals superior genotypes with enhanced drought tolerance. *Plants* **12**: 2840.

Rockström J, Williams J, Daily GC, Noble A, Matthews N, Gordon L, Wetterstrand H, DeClerck F, Shah M, Steduto P, *et al*. 2016. Sustainable intensification of agriculture for human prosperity and global sustainability. *AMBIO: A Journal of the Human Environment* **46**: 4–17.

Rosas-Moreno J, Walker C, Duffy K, Krüger C, Krüger M, Robinson CH & Pittman JK. 2023. Isolation and identification of arbuscular mycorrhizal fungi from an abandoned uranium mine and their role in soil-to-plant transfer of radionuclides and metals. *The Science of the Total Environment* **876**: 162781.

Rubel F & Kottek M. 2010. Observed and projected climate shifts 1901-2100 depicted by world maps of the Köppen-Geiger climate classification. *Meteorologische Zeitschrift* **19**: 135–141.

Rubio-Sanz L & Jaizme-Vega MC. 2022. Mycorrhization of *Moringa oleifera* improves growth and nutrient accumulation in leaves. *Journal of Plant Nutrition* **45**: 1765–1773.

Ruemmele BA & Amador JA. 1999. Turfgrass and Soil Responses to Soil Wetting Agents. *Journal of Turfgrass Management* **2**: 71-82.

Sahebi M, Hanafi MM, Siti Nor Akmar A, Rafii MY, Azizi P, Tengoua FF, Nurul Mayzaitul Azwa J & Shabanimofrad M. 2015. Importance of silicon and mechanisms of biosilica formation in plants. *BioMed Research International* **2015**: 396010.

Sakuraba Y. 2022. Molecular basis of nitrogen starvation-induced leaf senescence. *Frontiers in Plant Science* **13**: 1013304.

Salmeron-Santiago IA, Martínez-Trujillo M, Valdez-Alarcón JJ, Pedraza-Santos ME, Santoyo G, López PA, Larsen J, Pozo MJ & Chávez-Bárcenas AT. 2023. Carbohydrate and lipid balances in the positive plant phenotypic response to arbuscular mycorrhiza: increase in sink strength. *Physiologia Plantarum* **175**: e13857.

Samuels AL, Glass ADM, Ehret DL & Menzies JG. 1991. Mobility and deposition of silicon in cucumber plants. *Plant, Cell & Environment* **14**: 485–492.

Sasse J, Martinoia E & Northen T. 2018. Feed your friends: Do plant exudates shape the root microbiome? *Trends in Plant Science* **23**: 25–41.

Schiavon M, Leinauer B, Serena M, Maier B & Sallenave R. 2014. Plant growth regulator and soil surfactants' effects on saline and deficit irrigated warm-season grasses: II. Pigment content and superoxide dismutase activity. *Crop Science* **54**: 2827–2836.

Scholtz M & Bidleman T. 2007. Modelling of the long-term fate of pesticide residues in agricultural soils and their surface exchange with the atmosphere: Part II. Projected long-term fate of pesticide residues. *The Science of the Total Environment* **377**: 61–80.

Sefton C, Muchan K, Parry S, Matthews B, Barker LJ, Turner S & Hannaford J. 2021. The 2019/2020 floods in the UK: a hydrological appraisal. *Weather* **76**: 378–384.

Seitz TJ, Schütte UME & Drown DM. 2021. Soil disturbance affects plant productivity via soil microbial community shifts. *Frontiers in Microbiology* **12**: 619711.

Shahid S. 2013. Irrigation-Induced Soil Salinity Under Different Irrigation Systems – Assessment and Management: Short Technical Note. *Climate Change Outlook and Adaptation* **1**: 19-24.

Shi ZY, Xu SX, Yang M, Zhang MG, Lu SC, Chang HQ, Wang XN & Chen XN. 2020. Leaf nitrogen and phosphorus stoichiometry are closely linked with mycorrhizal type traits of legume species. *Legume Research* **44**: 81-87.

Sibley JL, Yang X, Lu W, Eakes DJ, Gilliam CH, Foshee WG III, Pickens JM & Zinner B. 2018. Effects of a nonionic surfactant on growth, photosynthesis, and transpiration of New Guinea Impatiens in the Greenhouse. *Journal of Environmental Horticulture* **36**: 73–81.

Siebrecht N. 2020. Sustainable agriculture and its implementation gap—overcoming obstacles to implementation. *Sustainability* **12**: 3853.

Singh JP, Singh M & Malhotra P. 2020. Impact of Adjuvant Application on Soil Infiltration Rate, Moisture Content and Yield of Pea Crop. *Journal of Soil Salinity and Water Quality* **12**: 225-233.

Singh Z & Khan AS. 2012. Surfactant and Nutrient Uptake in Citrus. In *Advances in Citrus Nutrition* (Springer, Berlin, Germany): 157–167.

Slama I, Abdelly C, Bouchereau A, Flowers T & Savouré A. 2015. Diversity, distribution and roles of osmoprotective compounds accumulated in halophytes under abiotic stress. *Annals of Botany* **115**: 433–447.

Smith FA & Smith SE. 1997. Structural diversity in (vesicular)-arbuscular mycorrhizal symbioses. *The New Phytologist* **137**: 373–388.

Smith SE, Smith FA & Jakobsen I. 2003. Mycorrhizal fungi can dominate phosphate supply to plants irrespective of growth responses. *Plant Physiology* **133**: 16–20.

Smith SE & Read DJ. 2010a. Arbuscular mycorrhizas. In *Mycorrhizal Symbiosis (3rd ed.)* (Academic Press, Massachusetts, USA): 11-13.

Smith SE & Read DJ. 2010b. Functioning of mycorrhizas in broader contexts. In *Mycorrhizal Symbiosis (3rd ed.)* (Academic Press, Massachusetts, USA): 611-636.

Solh M & van Ginkel M. 2014. Drought preparedness and drought mitigation in the developing world's drylands. *Weather and Climate Extremes* **3**: 62–66.

Song E, Schneider JG, Anderson SH, Goyne KW & Xiong X. 2014. Wetting agent influence on water infiltration into hydrophobic sand: I. rewettability. *Agronomy Journal* **106**: 1873–1878.

Spinoni J, Vogt JV, Naumann G, Barbosa P & Dosio A. 2018. Will drought events become more frequent and severe in Europe? *International Journal of Climatology* **38**: 1718–1736.

Springmann M, Clark M, Mason-D'Croz D, Wiebe K, Bodirsky BL, Lassaletta L, de Vries W, Vermeulen SJ, Herrero M, Carlson KM *et al*. 2018. Options for keeping the food system within environmental limits. *Nature* **562**: 519–525.

Staddon PL, Fitter AH & Graves JD. 1999. Effect of elevated atmospheric CO2 on mycorrhizal colonization, external mycorrhizal hyphal production and phosphorus inflow in *Plantago lanceolata* and *Trifolium repens* in association with the arbuscular mycorrhizal fungus *Glomus mosseae. Global Change Biology* **5**: 347–358.

Stark JM & Firestone MK. 1995. Mechanisms for soil moisture effects on activity of nitrifying bacteria. *Applied and Environmental Microbiology* **61**: 218–221.

Starr GC, Cooley ET, Lowery B, Kelling K. 2005. Soil water fluctuations in a loamy sand under irrigated potato. *Soil Science* **170**: 77-89.

Stavi I, Thevs N & Priori S. 2021. Soil salinity and sodicity in drylands: A review of causes, effects, monitoring, and restoration measures. *Frontiers in Environmental Science* **9**: 712831.

Stevens PJ. 1995. Fate of Silwet L-77 organosilicone surfactant in soil and water. In *Fourth International Symposium on Adjuvants for Agrochemicals* (New Zealand Forest Research Institute, Rotorua, New Zealand): 345-349.

Stürmer SL. 2004. Effect of different fungal isolates from the same mycorrhizal community on plant growth and phosphorus uptake in soybean and red clover. *Revista Brasileira de Ciencia Do Solo* **28**: 611–622.

Sumberg J & Giller K. 2022. What is 'Conventional' Agriculture? *Global Food Security* **32**: 100617.

Sun R, Li W, Dong W, Tian Y, Hu C & Liu B. 2018. Tillage changes vertical distribution of soil bacterial and fungal communities. *Frontiers in Microbiology* **9**: 699.

Svitova T, Hoffmann H & Hill RM. 1996. Trisiloxane surfactants: Surface/interfacial tension dynamics and spreading on hydrophobic surfaces. *Langmuir: The ACS Journal of Surfaces and Colloids* **12**: 1712–1721.

Syamsiyah J, Herawati A & Mujiyo. 2018. The potential of arbuscular mycorrhizal fungi application on aggregrate stability in alfisol soil. IOP Conference Series. *Earth and Environmental Science* **142**: 012045.

Szatanik-Kloc A, Horn R, Lipiec J, Siczek A & Szerement J. 2018. Soil compaction-induced changes of physicochemical properties of cereal roots. *Soil & Tillage Research* **175**: 226–233.

Tang C & Piechota TC. 2009. Spatial and temporal soil moisture and drought variability in the Upper Colorado River Basin. *Journal of Hydrology* **379**: 122–135.

Tate EL & Gustard A. 2000. Drought Definition: A Hydrological Perspective. In *Advances in Natural and Technological Hazards Research* (Springer, Berlin, Germany): 23–48.

Tatewaki Y, Higo M & Isobe K. 2023. Impacts of tillage practices on growth, phosphorus uptake, and yield of maize in controlled and field-based studies in relation to arbuscular mycorrhizal fungi. *Applied Microbiology* **3**: 358–374.

Tawaraya K, Naito M & Wagatsuma T. 2006. Solubilization of insoluble inorganic phosphate by hyphal exudates of arbuscular mycorrhizal fungi. *Journal of Plant Nutrition* **29**: 657–665.

Teklewold H, Kassie M & Shiferaw B. 2013. Adoption of multiple sustainable agricultural practices in rural Ethiopia. *Journal of Agricultural Economics* **64**: 597–623.

Tennant D. 1975. A test of a modified line intersect method of estimating root length. *Journal of Ecology*: 995–1001.

Thornton B & Bausenwein U. 2000. Seasonal protease activity in storage tissue of the deciduous grass *Molinia caerulea*. *The New Phytologist* **146**: 75–81.

Tian H, Wang R, Li M, Dang H & Solaiman ZM. 2019. Molecular signal communication during arbuscular mycorrhizal formation induces significant transcriptional reprogramming of wheat (*Triticum aestivum*) roots. *Annals of Botany* **124**: 1109–1119.

Toberman H, Freeman C, Evans C, Fenner N & Artz RRE. 2008. Summer drought decreases soil fungal diversity and associated phenol oxidase activity in upland Calluna heathland soil. *FEMS Microbiology Ecology* **66**: 426–436.

Trenberth KE, Dai A, van der Schrier G, Jones PD, Barichivich J, Briffa KR & Sheffield J. 2014. Global warming and changes in drought. *Nature Climate Change* **4**: 17–22.

Treseder KK. 2013. The extent of mycorrhizal colonization of roots and its influence on plant growth and phosphorus content. *Plant Soil* **371**: 1–13.

Tsui MTK & Chu LM. 2003. Aquatic toxicity of glyphosate-based formulations: comparison between different organisms and the effects of environmental factors. *Chemosphere* **52**: 1189–1197.

Tuckeldoe RB, Maluleke MK & Adriaanse P. 2023. The effect of coconut coir substrate on the yield and nutritional quality of sweet peppers (*Capsicum annuum*) varieties. *Scientific Reports* **13**: 2742.

Undersander DJ. 2019. Forage Plant Structure, Function, Nutrition, and Growth. In *Horse Pasture Management* (Academic Press, Massachusetts, USA): 1–10.

Urrestarazu M, Guillén C, Mazuela PC & Carrasco G. 2008. Wetting agent effect on physical properties of new and reused rockwool and coconut coir waste. *Scientia Horticulturae* **116**: 104–108.

Vaculík M, Lux A, Luxová M, Tanimoto E & Lichtscheidl I. 2009. Silicon mitigates cadmium inhibitory effects in young maize plants. *Environmental and Experimental Botany* **67**: 52–58.

Van Ha C, Leyva-González MA, Osakabe Y, Tran UT, Nishiyama R, Watanabe Y, Tanaka M, Seki M, Yamaguchi S, Van Dong N *et al*. 2014. Positive regulatory role of strigolactone in plant responses to drought and salt stress. *Proceedings of the National Academy of Sciences of the United States of America* **111**: 851–856.

Ven A, Verlinden MS, Verbruggen E & Vicca S. 2019. Experimental evidence that phosphorus fertilization and arbuscular mycorrhizal symbiosis can reduce the carbon cost of phosphorus uptake. *Functional Ecology* **33**: 2215–2225.

Verma V, Ravindran P & Kumar PP. 2016. Plant hormone-mediated regulation of stress responses. *BMC Plant Biology* **16**: 86.

Verslues PE & Sharma S. 2010. Proline metabolism and its implications for plant-environment interaction. *The Arabidopsis Book* **8***:* e0140.

Vicente-Serrano SM, Beguería S & López-Moreno JI. 2010. A multiscalar drought index sensitive to global warming: The standardized precipitation evapotranspiration index. *Journal of Climate* **23**: 1696–1718.

Voets L, de la Providencia IE & Declerck S. 2006. Glomeraceae and Gigasporaceae differ in their ability to form hyphal networks. *The New Phytologist* **172**: 185–188.

Volgas GC & Lopez H. 2003. Determination of Organosilicone Surfactant Phytotoxicity for Selected Vegetable Species. In *Pesticide Formulations and Application Systems* (ASTM International, Pennsylvania, USA) **23**: 77–84.

Voltr V, Wollnerová J, Fuksa P & Hruška M. 2021. Influence of tillage on the production inputs, outputs, soil compaction and GHG emissions. *Agriculture* **11**: 456.

Wahdan SFM, Asran AGA, Abdellatef M, Atia MAM & Ji L. 2024. Arbuscular Mycorrhizal Fungi in Organic Versus Conventional Farming. In *Arbuscular Mycorrhizal Fungi and Higher Plants* (Springer Nature, London, UK): 259–286.

Wang M, Wang Z, Guo M, Qu L & Biere A. 2023. Effects of arbuscular mycorrhizal fungi on plant growth and herbivore infestation depend on availability of soil water and nutrients. *Frontiers in Plant Science* **14**: 1101932.

Wang R, Cresswell T, Johansen, MP, Harrison JJ, Jiang Y, Keitel C, Cavagnaro TR & Dijkstra FA. 2021. Reallocation of nitrogen and phosphorus from roots drives regrowth of grasses and sedges after defoliation under deficit irrigation and nitrogen enrichment. *The Journal of Ecology* **109**: 4071–4080.

Waraich EA, Ahmad R & Ashraf MY. 2011. Role of Mineral Nutrition in Alleviation of Drought Stress in Plants*. Australian Journal of Crop Science* **5**: 764–777.

Wehner GG, Balko CC, Enders MM, Humbeck KK & Ordon FF. 2015. Identification of genomic regions involved in tolerance to drought stress and drought stress induced leaf senescence in juvenile barley. *BMC Plant Biology* **15**: 125.

Wen Z, Li H, Shen Q, Tang X, Xiong C, Li H, Pang J, Ryan MH, Lambers H & Shen, J. 2019. Tradeoffs among root morphology, exudation and mycorrhizal symbioses for phosphorus-acquisition strategies of 16 crop species. *The New Phytologist* **223**: 882–895.

Wernecke A, Eckert JH, Forster R, Kurlemann N & Odemer R. 2021. Inert agricultural spray adjuvants may increase the adverse effects of selected insecticides on honey bees (*Apis mellifera* L.) under laboratory conditions. *Journal of Plant Diseases and Protection* **129**: 93-105.

Wiebe K, Lotze-Campen H, Sands R, Tabeau A, van der Mensbrugghe D, Biewald A, Bodirsky B, Islam S, Kavallari A, Mason-D'Croz D *et al.* 2015. Climate change impacts on agriculture in 2050 under a range of plausible socioeconomic and emissions scenarios. *Environmental Research Letters* **10**: 085010.

Wilkinson TDJ. 2018. *Interactions between above and below ground symbionts: Implications for food security.* PhD thesis, University of York.

Willett W, Rockström J, Loken B, Springmann M, Lang T, Vermeulen S, Garnett T, Tilman D, DeClerck F, Wood A *et al*. 2019. Food in the Anthropocene: the EAT-Lancet Commission on healthy diets from sustainable food systems. *Lancet* **393**: 447–492.

Wu N, Zhang S, Huang H & Christie P. 2008. Enhanced dissipation of phenanthrene in spiked soil by arbuscular mycorrhizal alfalfa combined with a non-ionic surfactant amendment. *The Science of the Total Environment* **394**: 230–236.

Wu Q-S, Zou Y-N, Fathi A-AE. 2014. Chapter 15 - Mycorrhizal association and ROS in plants. In *Oxidative damage to plants* (Academic Press, Massachusetts, USA): 453–475.

Wu S, Shi Z, Chen X, Gao J & Wang X. 2022. Arbuscular mycorrhizal fungi increase crop yields by improving biomass under rainfed condition: a meta-analysis*. PeerJ* **10**: e12861.

Wu Y, Zhong H, Li J, Xing J, Xu N & Zou H. 2022. Water use efficiency and photosynthesis of *Calamagrostis angustifolia* leaves under drought stress through CO_2 concentration increase. *Journal of Plant Interactions* **17**: 60–74.

Xu J, Liu S, Song S, Guo H, Tang J, Yong JWH, Ma Y & Chen X. 2018. Arbuscular mycorrhizal fungi influence decomposition and the associated soil microbial community under different soil phosphorus availability. *Soil Biology & Biochemistry* **120**: 181–190.

Yang Y-M, Park J-H, An S-I, Yeh S-W, Zhu Z, Liu F, Li J, Lee J-Y & Wang, B. 2022. Increased Indian Ocean-North Atlantic Ocean warming chain under greenhouse warming. *Nature Communications* **13**: 3978.

Zargar SM, Mahajan R, Bhat JA, Nazir M & Deshmukh R. 2019. Role of silicon in plant stress tolerance: opportunities to achieve a sustainable cropping system. *3 Biotech* **9**: 73.

Yılgör I, Yilgör E & Grüning B. 1993. Silicone Surfactants: Part V*: Siloxane copolymers as surface modifying additives for polymers. *Tenside Surfactants Detergents* **30**: 158-164.

Yin C, Casa Vargas JM, Schlatter DC, Hagerty CH, Hulbert SH & Paulitz TC. 2021a. Rhizosphere community selection reveals bacteria associated with reduced root disease. *Microbiome* **9**: 86.

Yin Z, Zhang L & Li R. 2021. Effects of additives on physical, chemical, and microbiological properties during green waste composting. *Bioresource Technology* **340**: 125719.

Ying G-G. 2006. Fate, behavior and effects of surfactants and their degradation products in the environment. *Environment International* **32**: 417–431.

Yoneyama T. 1988. Problems on phosphorus fertility in upland soil; 5. Uptake and metabolism of phosphorus of plant. *Journal of Agriculture and Horticulture* **63**: 16–20.

Zaller J G, Frank T & Drapela T. 2011. Soil sand content can alter effects of different taxa of mycorrhizal fungi on plant biomass production of grassland species*. European Journal of Soil Biology* **47**: 175–181.

van Zelm E, Zhang Y & Testerink C. 2020. Salt tolerance mechanisms of plants. *Annual Review of Plant Biology* **71**: 403–433.

Zhang C & Lu N. 2020a. Soil sorptive potential: Its determination and predicting soil water density. *Journal of Geotechnical and Geoenvironmental Engineering* **146**: 04019118.

Zhang F, Zou Y-N, Wu Q-S & Kuča K. 2020b. Arbuscular mycorrhizas modulate root polyamine metabolism to enhance drought tolerance of trifoliate orange. *Environmental and Experimental Botany* **171**: 103926.

Zhang H, Zhao Y & Zhu J-K. 2020c. Thriving under stress: How plants balance growth and the stress response. *Developmental Cell* **55**: 529–543.

Zhang M, Wang L-F, Zhang K, Liu F-Z & Wan Y-S. 2017. Drought-induced responses of organic osmolytes and proline metabolism during pre-flowering stage in leaves of peanut (*Arachis hypogaea* L.). *Journal of Integrative Agriculture* **16**: 2197–2205.

Zhang P, Wang K, He L, Fan R, Liu Z, Yang J, Guo R & Gao Y. 2023. Surfactants improving the wetting behavior and adhesion mechanism of pesticide dilution droplets on jujube leaf surfaces. *ACS Omega* **8**: 22121–22131.

Zhao S, Zhang Q, Liu M, Zhou H, Ma C & Wang P. 2021. Regulation of plant responses to salt stress. *International Journal of Molecular Sciences* **22**: 4609.

Zidack NK, Backman PA & Shaw JJ. 1992. Promotion of bacterial infection of leaves by an organosilicone surfactant: Implications for biological weed control. *Biological Control: Theory and Applications in Pest Management* **2**: 111–117.

Zipfel C & Oldroyd GE. 2017. Plant signalling in symbiosis and immunity. *Nature* **543**: 328–336.

Zocco D, Fontaine J, Lozanova E, Renard L, Bivort C, Durand R, Grandmougin-Ferjani A & Declerck S. 2008. Effects of two sterol biosynthesis inhibitor fungicides (fenpropimorph and fenhexamid) on the development of an arbuscular mycorrhizal fungus. *Mycological Research* **112**: 592–601.

6 Appendices

6.1 Appendix 1 for general introduction

Table S1. Some commercially available soil adjuvants and soil conditioners, with details about their particular chemical composition and how their action in soil can benefit plant growth and development. Unique features are indicated in bold.

Manufacturer / product name	Product and formulation type	Main composition	Key benefits
Silwet by Momentive® / Silwet™ Power Super Wetter	Powder activator adjuvant: non-ionic organosilicone surfactant	Siloxane Polyalkyleneoxide Copolymer	Improves soil-air exchange capacity for soil microbiome health, increases water holding capacity of soil for improved nutrient uptake and soil-applied agrochemical application, reduced water use, reduced agrochemical application needed, **promotes feeder root development, powder formulation enables dry broadcast application**.
Silwet by Momentive® / Silwet™ 408 Super-Spreader	Liquid spray adjuvant: non-ionic organosilicone surfactant	Trisiloxane ethoxylate	Soluble liquid and emulsifiable in concentrate formulations, reduced agrochemical application needed, increased uptake of agrochemicals, **meets EPA 40 CFR §180.910 requirements, OMRI listed for organic use**
Plant Health Technologies /	Liquid activator adjuvant: non-ionic surfactant	Poloxalene, alkyl polyglucoside,	Increases water holding capacity of soil, improves water infiltration and distribution. **Tailored for use**

AD-SORB RST®		vegetable oil ethoxylate	**in specific adjuvant formulations.**
ORO-AGRI / ORO®-RZ	Liquid activator adjuvant: Soil Surfactant Penetrant	Alcohol Ethoxylate, Orange Oil (Cold Pressed)	Improves soil-air exchange capacity for soil microbiome health, improves water infiltration and distribution, improving soil-applied agrochemical application. **Designed for edaphic application with pesticides to improve their efficacy.**
Precision Laboratories, LLC / Cascade Plus™	Liquid activator adjuvant: Soil Surfactant Penetrant	Alkyl Phenol Ethoxylate, Polyethylene – polypropylene Glycol – Block Copolymer, Cellulose Complex	Reduced water use, increases water infiltration and water holding capacity of soil for improved nutrient uptake, **relieves localised dry spots on turfgrass.**
Precision Laboratories, LLC / Stretta	Liquid activator adjuvant: Irrigation surfactant	Cellulose, carboxymethyl ether, potassium salt	Plant derived formula, increases water holding capacity of soil, improves soil-applied agrochemical application and availability.
Harrell's® / Fleet 100	Liquid activator adjuvant: Soil Surfactant Penetrant	Polyoxyalkylene polymers	Non-phytotoxic formulation, improves water infiltration and distribution, protects from salt sensitivity in crops, neutralises soil bicarbonates, improves soil oxygen levels for favourable microbiome conditions.
Wilbur Ellis / AQUATE® MAX	Liquid activator adjuvant: Soil Surfactant Penetrant	Ethylene oxide-propylene oxide copolymer	Increases water infiltration and water holding capacity of soil for improved nutrient uptake, reduced agrochemical application needed.

EVONIK / BREAK-GARD® IR 100	Liquid activator adjuvant: trisiloxane surfactant/ wetting agent	Oxirane, 2-methyl-, polymer with oxirane, mono [3-[1,3,3,3-tetramethyl-1-[(trimethylsilyl)oxy]-1-disiloxanyl] propyl] ether	Non-phytotoxic formulation, increases water infiltration and distribution, protects from salt sensitivity in crops, **active at high temperatures**, increases water holding capacity of soil.
Exacto® Inc/ AquiMax®	Liquid soil surfactant and moisture holding polymer	Ethylene oxide, propylene oxide block polymer, Polyacrylamide	Increases water infiltration and distribution, increases water holding capacity of soil, thus reducing water use, **improved nutrient retention, plant photosynthesis, transpiration and CO_2 uptake**, overall increasing crop yield and quality.
CRODA / Hydravance™ 200	Liquid activator adjuvant: Non-ionic surfactant blend	Not disclosed by the manufacturer	Increases water infiltration and distribution, improving agrochemical absorption into soil, reduced water use, reduced agrochemical application needed, **reduced evaporation rate from the soil.**
Aquatrols® / Revolution®	Liquid activator adjuvant: surfactant/ wetting agent	Oxirane, methyl-, polymer with oxirane, dimethyl ether	Increases water infiltration and distribution, improves soil-air exchange capacity for soil microbiome health, **in turf - antioxidant levels and self-cooling ability through evapotranspiration are increased.**
Tradecorp / Adjufirst Pre	Liquid super spreading adjuvant	Not disclosed by the manufacturer	Increases water infiltration and distribution, for improved soil-

			applied agrochemical application, reduced water use.
Heritage PPG / Aquisync®	Liquid surfactant: soil wetting agent	Ethylene oxide-propylene oxide copolymer	Increases water infiltration, distribution, increases water holding capacity of soil, reducing localized dry spots for more consistent playing surface (golf course specific), reduces water use, reduces agrochemical application needed, **most effective on non-sandy soils.**
ICL Specialty Fertilisers / H2Pro AquaSmart	Liquid surfactant penetrant/water retention	Dipropylene Glycol Methyl Ether	Increases water infiltration and distribution, increases water holding capacity of soil, reduces localised dry patch development, formulated for all soil types, **flexible application rates.**
ICL Specialty Fertilisers / H2OPro FlowSmart	Liquid penetrant	heptamethyl glycidyl oxypropyl trisiloxane polymer with ethoxylated cocoamine and acetic 57 acid	Increases water infiltration and distribution, reduced water use, reduced agrochemical application needed, **provides a drier surface in wet conditions, reduces carbonate and salt build up in rootzones.**
UPL / Zeba® SP	Liquid soil conditioner/ amendment	Starch-g-Poly (2-propenamide-co-2-propenoic acid), potassium salt	Increases water infiltration, distribution and retention, reduced water use, increased soil oxygenation, **starch-based** and biodegradable.
TerraCottem / Universal	Granular soil conditioner/ amendment	Mixture containing volcanic lava, water-absorbing polymers, NPK fertiliser with	Absorb moisture to reduce water use in degraded soils, **stimulating root development, faster plant growth.**

		trace elements and biostimulants.	
TerraCottem / Turf	Granular solid soil conditioner/ amendment	Mixture containing zeolite, water absorbent polymers, NPK fertilisers containing magnesium and biostimulants	Increases soil water holding capacity, reduced water use, **increases soil cation exchange capacity**, less nutrient leaching, **stronger and deeper root development**, increased microbiological activity.
JFM Horticulture / Aqualatus®	Liquid soil surfactant	64% Tri Block Co-Polymer and 19% Gluco-ethers	Increases soil water holding capacity, distribution, retention and lateral movement in soil, can be applied to all soil types, **long term re-wetting in root zones for any crop type.**
JFM Horticulture / Aqualatus Ca™	Liquid surfactant and nutrient regulator for coir substrate	18.5% CaO and 30% surfactants/ hydrating polymers	**Increases hydration, water penetration, expansion and salt leaching in coir, improves moisture and nutrient distribution.**
Bharat Certis / Ignition	Liquid silicone adjuvant	Not disclosed by the manufacturer	Enhances the efficacy of agrochemicals, improves soil moisture content, **reduces the occurrence of blockages in drip irrigation systems.**

Silwet BY MOMENTIVE

Technical Data Sheet

Silwet™ Power Super-Wetter

Description

Silwet Power super-wetter is an organosilicone based powder adjuvant that can enhance the water absorption efficiency of hard-to-wet soils by increasing their water infiltration, drainage, water retention and hydrophilicity. Silwet Power super-wetter was designed to overcome the challenges an application in the powder form can face, including low uptake, poor absorption, leaching, and run-off of the broadcasted fertilizers, while improving their performance and efficiency.

Key Features and Typical Benefits

- Improves soil-air exchange capacity resulting in favorable aerobic conditions for soil microbes, helping to hasten their metabolic processes
- Brings better uniformity to soil wetting, increasing the availability and uptake of plant nutrients and the efficacy of soil-applied agrochemicals
- Helps to reduce hydrophobicity of soils, boosting water infiltration and promoting less water use
- Enables low dose rates and reduced number of applications
- Promotes the development of feeder (white & hairy) roots

Typical Physical Properties

Physical Property	Standard Value
Appearance	White free-flowing homogenous powder
Spread Diameter, mm (0.2% wt%)	43
Aqueous pH, 1%	7
Surface Tension, mN/m (0.1% wt%)	21
Bulk Density, g/mL (0 untapped[a]	0.24
Bulk Density, g/mL (20 tapped)[b]	0.30

Typical properties are average data and are not to be used as or to develop specifications.
(a) Bulk density without any compaction or tapping.
(b) Bulk density after tapping 20 times.

Potential Applications and Use Rates

Application (Broadcasting)	Typical Use Rate (Kg/Acre)
Fertilizers and Micronutrients	0.5 to 2.25
Fungicide	0.5 to 1
Herbicide	0.5 to 1
Insecticide	0.5 to 1
Plant Growth Regulators	0.5 to 1

General Conditions for Use

In Agrochemical In-Can Formulations
Silwet Power super-wetter may be used as a component in agrochemical formulations as a wetting agent, to help provide improved fertilizer, pesticide, and water distribution in the soil profile. It is recommended that for typical applications, Silwet Power super-wetter be used at a concentration of at least 5%, based on the total formulation.

As a Tank-Mix Adjuvant
Silwet Power super-wetter is also readily dispersible in water and can be applied as a tank-mix adjuvant through spray, drip irrigation, and drenching, for example.

As a Dry Adjuvant for Broadcast Applications
Silwet Power super-wetter is a dry, white free-flowing homogenous powder which can be applied while broadcasting fertilizers. When using this method, following label instructions, use the recommended dose (kg of fertilizer/ha) and add Silwet Power super-wetter to the mixture at a rate of 5-15 g/kg (or 0.5 to 2.25 kg/acre) mixing thoroughly by hand (wearing appropriate personal protective equipment). The mixture can be directly broadcasted in the field, especially near the root zone. Proper irrigation and agronomic practices specific to each crop should be observed as usual after the broadcasting.

Figure S1. The technical data sheet (TDS) for Silwet™ Power (SP) super-wetter, a powder organosilicone surfactant developed by Momentive that can be used for soil application to improve soil moisture content. This can have benefits for crops cultivated in drought-affected soils.

Silwet BY MOMENTIVE

Technical Data Sheet

Silwet™ 408 Super Spreader

Description

Silwet 408 super spreader is a trisiloxane ethoxylate based adjuvant that lowers the surface tension of spray solutions beyond that which is achievable with conventional nonionic surfactants.

Key Features and Typical Benefits

- Super spreader for soluble liquid and emulsifiable concentrate formulations
- Promotes spray volume reduction
- Promotes rapid uptake of agrochemicals (rainfastness)
- Can significantly improve spray coverage
- Nonionic
- Meets EPA 40 CFR §180.910 requirements(1)
- OMRI Listed for organic use

products available OMRI LISTED For Organic Use

Typical Physical Properties

Property	Value
Surface Tension mN/m (0.1% wt%)(a)	21.5
Cloud Point (0.1 wt%), °C	<10
Viscosity (cSt at 25 °C)	35
CMC (Wt%)(b)	0.007
Pour Point, °C	-8
Specific Gravity at 25 °C	1.020
Flash Point(c), °C (°F)	118 (244)

Typical properties are average data and are not to be used as or to develop specifications.

Potential Applications and Use Rates

Application	Typical Use Rate
Fertilizers and Micronutrients	0.015% to 0.1%
Fungicide	0.015% to 0.1%
Herbicide	0.025% to 0.15%
Insecticide	0.025% to 0.1%
Plant Growth Regulators	0.025% to 0.1%

Use rates are dependent on crop, agrochemical and spray volume requirements.

(1) The components meet the requirements of U.S. regulation EPA 40 CFR §180.910, and therefore are exempt from tolerances when used as an inert ingredient in agricultural applications in accordance with the other conditions of that regulation

(a) Surface tension by Wilhelmy Plate Method
(b) Critical Micelle Concentration
(c) Pensky-Martens Closed Cup, ASTM Method D93

General Conditions for Use

In Agrochemical In-Can Formulations

Silwet 408 super spreader may be used as a component in agrochemical formulations. Although organosilicone surfactants are subject to hydrolysis under acidic or basic conditions, optimum performance is achieved by buffering the formulation to pH 6.5 to 7.5. Additionally, it is recommended that Silwet 408 super spreader be used at a concentration of at least 5%, based on the total formulation.

As a Tank-Mix Adjuvant

Silwet 408 super spreader, when used as a tank-side adjuvant may be used to improve spray coverage, improve uptake or to allow for a reduction in spray volume. Silwet 408 super spreader is most effective as a tank-side adjuvant when spray mixtures are within a pH range of 5 to 8 and used within 24 hours of preparation.

High spray volumes, coupled with high surfactant rates, are not required to achieve sufficient coverage with Silwet 408 super spreader. In fact, it has the potential to provide adequate coverage in many low volume spray applications at rates between 0.025% and 0.1%.

Figure S2. The technical data sheet for Silwet™ 408 (S408) super spreader, a liquid organosilicone surfactant developed by Momentive primarily for foliar applications with agricultural formulations. Due to similarities in chemical composition and properties to Silwet™ Power (SP), it was utilised in experiments reported in the present thesis for soil application, to improve soil moisture content of droughted soils.

Figure S3. Silwet™ Power super-wetter, a white, powder organosilicone surfactant.

6.2 Appendix 2 for chapter 2

Table S2. Contents of Modified Strullu and Romand (MSR) medium, lacking vitamins and solidified with Bacto Agar (Le Pioufle & Declerck, 2018) and concentrations of each component.

Nutrient component	**Concentration (mg l^{-1})**
$MgSO_4.7H_2O$	739
KNO_3	76
KH_2PO_4	4
$Ca(NO_3)_24H_2O$	359
NaFeEDTA	8
KCl	65
$MnSO_4.4H_2O$	2
$ZnSO_4.7H_2O$	0.3
H_3BO_3	1.9
$CuSO_4.5H_20$	0.2
$(NH_4)_6Mo_7O_{24}.4H_2O$	0.04
$Na_2MoO_4.2H_2O$	2×10^{-3}
Sucrose	1×10^4
Bacto Agar (Becton Dickson, New Jersey, USA)	8×10^3

Table S3. The results of an unbalanced 3-way ANOVA comparing the effects of the type (Silwet™ Power [SP], Silwet™ 408 [S408]), concentration (SP 0.05 g l-1, SP 0.1 g l-1, S408 0.025 g l-1, S408 0.05 g l-1, 0 g l-1 [untreated control]) and exposure time (0.25, 3, 24 h) to an adjuvant on percentage of germinated *Rhizophagus irregularis* spores (%). Significant *P* values are indicated in bold (*P* ≤ 0.05).

Source of variation	% Germinated Spores				
	Type III Sum of Squares	***df***	**Mean Square**	***F***	***P***
Adjuvant type (SP, S408)	0.01	1,335	0.01	0.27	0.613
Adjuvant concentration (l, m, h)	0.13	2,335	0.07	1.56	0.246
Exposure time (0.25, 3, 24 h)	0.049	2,335	0.025	0.598	0.571
Adjuvant type * Adjuvant concentration	0.17	2,335	0.08	1.98	0.175
Adjuvant type * Exposure time	0.18	4,335	0.04	0.82	0.537
Exposure time * Adjuvant concentration	0.34	6,335	0.06	1.36	0.325

6.3 Appendix 3 for chapter 3

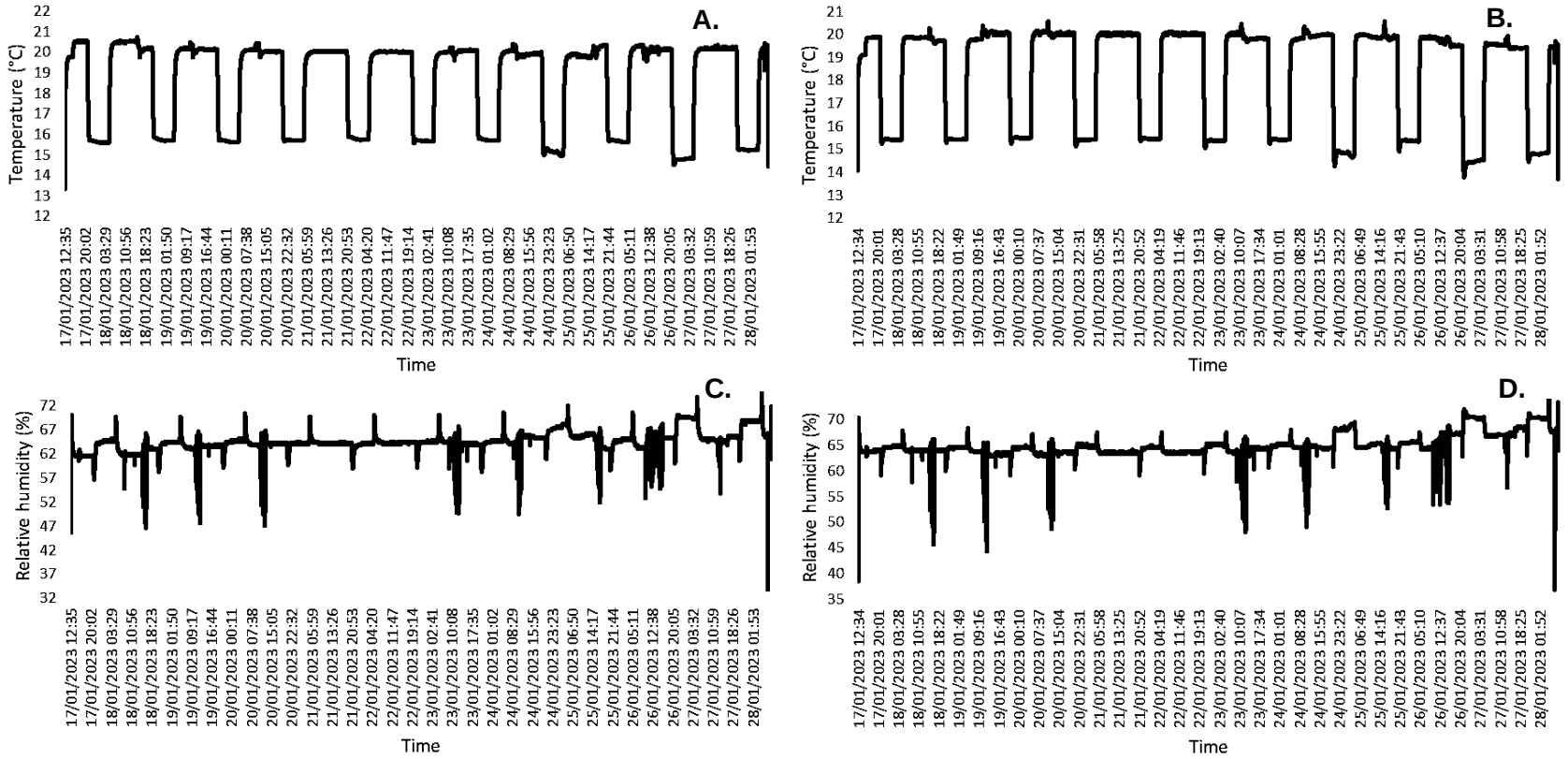

Figure S4. Information retrieved from the data loggers (View 2 TV-4500, TinyTag, Sussex, UK) kept in the growth cabinet throughout the duration of the experiment which tested the effect of Silwet™ Power (SP) and Silwet™ 408 (S408) on the growth substrate water retention. Temperature (°C; bottom shelf: Figure S4A, top shelf: Figure S4B) and RH (%; bottom shelf: Figure S4C, top shelf: Figure S4D) in the growth cabinet did not show any notable unexpected changes; dips in RH recorded during day periods can be accounted for when the growth cabinet was opened to take % SMC measurements.

Table S4. Instrument conditions of the Flash 1112 NC analyser (Thermo Scientific, Massachusetts, USA) used to analyse carbon and nitrogen content of *Zea mays* L. and *Vicia faba* L. plants.

Variable	**Parameter**	**Set value**
Temperature	Left furnace	On / 900°C
	Right furnace	On / 840°C
	Oven	On / 50°C
	Set instrument to stand-by	Off
Flow / Timing	Carrier	On / 140 ml min^{-1}
	Oxygen	On / 250 ml min^{-1}
	Reference	On / 100 ml min^{-1}
	Cycle time	300s
	Sampling delay	12s
	Oxygen injection end	10s
Detector	Filament	On
	Gain	1
	Autozero	On

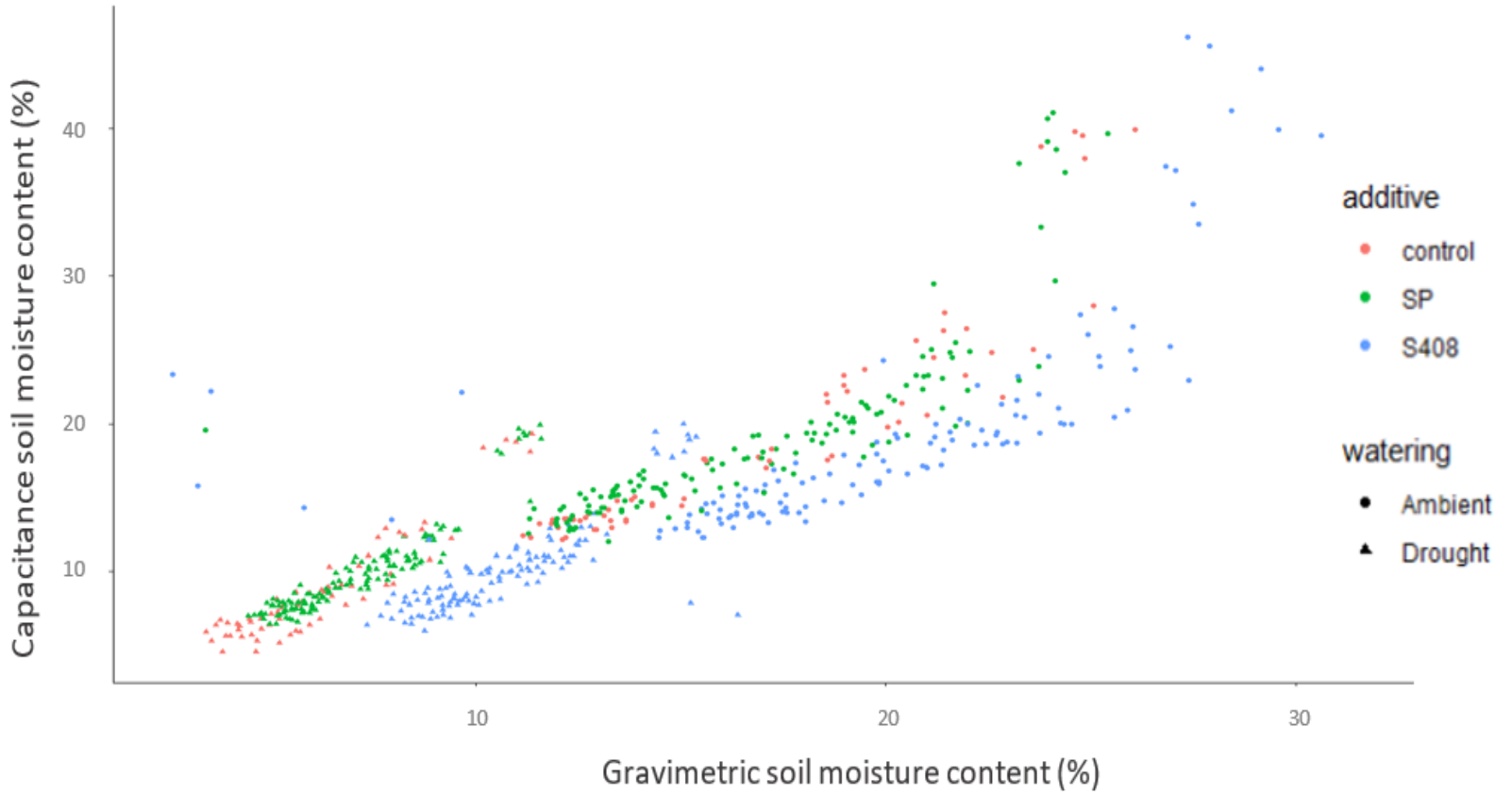

Figure S5. Soil moisture content (%) that was collected gravimetrically *vs*. by capacitance probe were plotted against each other to examine which data set was more accurate and reliable. Gravimetric measurements show a positive shift away from control values and groups of anomalous data points. Therefore, gravimetric measurements were omitted from proceeding analysis of treatment effects. The growth substrate was treated with Silwet™ Power (SP), Silwet™ 408 (S408) or untreated (control) and watered to ambient (50% of the soil water holding capacity [SWHC]) or drought (20% of the SWHC) level.

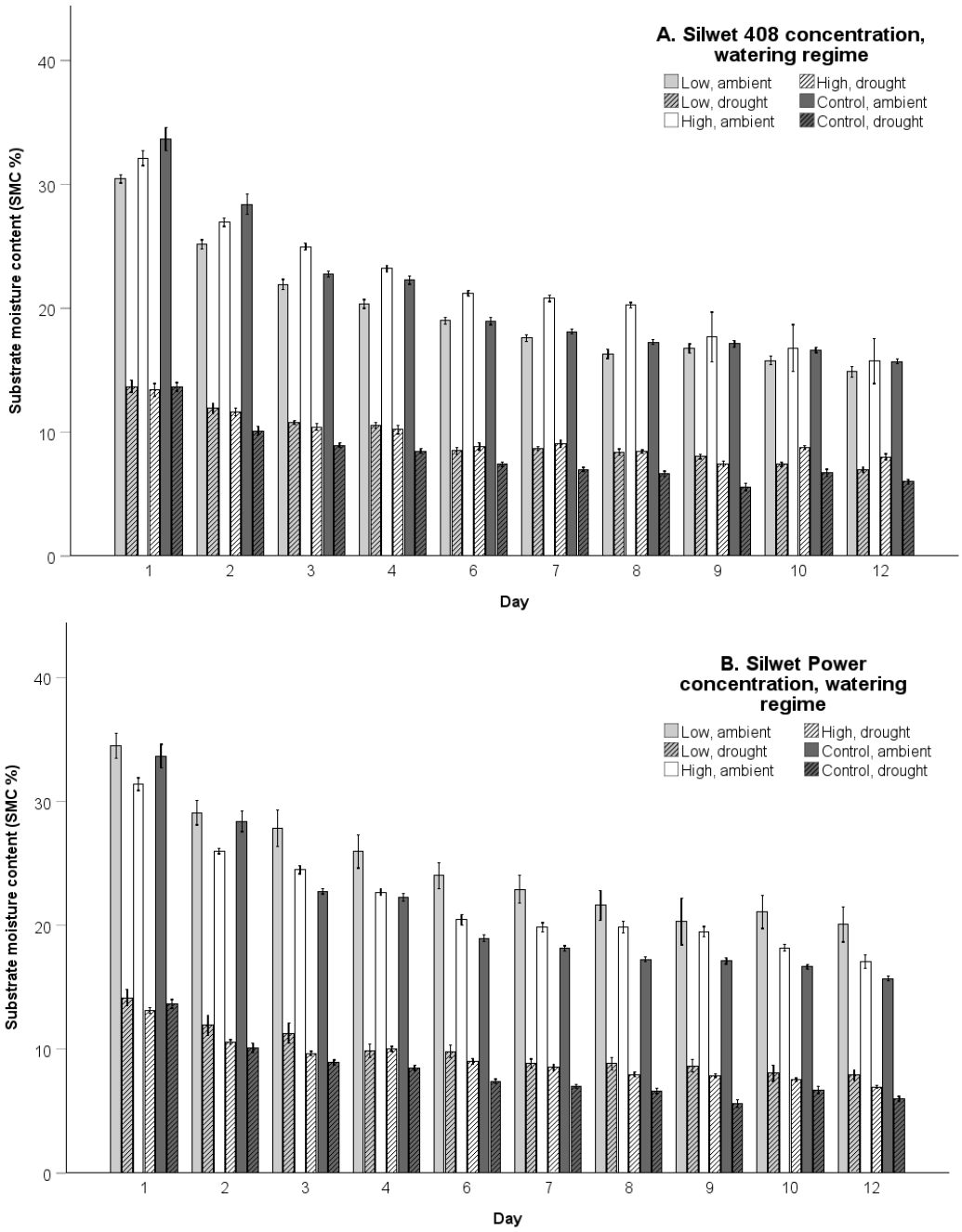

Figure S6. Graphs showing the trend of data collected from the experiment which tested the effect of two Silwet™ adjuvants (Silwet™ Power and Silwet™ 408) on the moisture content of a sandy growth substrate, prior to statistical analysis. Graphs show the moisture content of the growth substrate over 14 d when watered at ambient and drought watering levels and treated with Silwet™ 408 (A) or Silwet™ Power (B). Ambient and drought watering were 50% and 20% of the soil water holding capacity, respectively. Control pots received no adjuvant treatment. Error bars are ± 1 standard error of the mean (*n* = 5).

Table S5. The results of a generalised linear model (GLM) with Gamma probability distribution and log link function comparing the effects of watering regime (ambient: 50%, drought: 20% of the soil water holding capacity), adjuvant type (Silwet™ Power [SP], Silwet™ 408 [S408], control with no adjuvant) and adjuvant concentration (SP 0.05 g l^{-1}, SP 0.1 g l^{-1}, S408 0.025 g l^{-1}, S408 0.05 g l^{-1}, 0 g l^{-1} [untreated control]) on growth substrate moisture content (% SMC) from d 14 (when the growth substrate was rewatered to return to 50 or 20% SWHC) to d 27.

Source of variation	**Wald Chi-Square**	***df***	***P***
(Intercept)	70984.4	1	**<.001**
Watering (50% SWHC, 20% SWHC)	1897.5	1	**<.001**
Adjuvant type (SP, S408)	0.1	1	0.745
Adjuvant concentration (low, high)	3.4	1	0.065
Watering * adjuvant type	0.6	1	0.442
Watering * adjuvant concentration	0.8	1	0.377
Adjuvant type * adjuvant concentration	2.2	1	0.135
Watering * adjuvant type * adjuvant concentration	0	1	0.999

df = degrees of freedom *n* = 5, significant differences are indicated in bold ($P \leq 0.05$).

Figure S7. The experimental setup testing the effect of arbuscular mycorrhizal fungi (AMF) and Silwet™ Power (SP) on drought tolerance of *Vicia faba* L. and *Zea mays* L. Germinated seeds were sown in growth substrate treated with AMF only, SP only, AMF+SP or untreated for controls. Plants were then watered to either ambient (50% of the total soil water holding capacity [SWHC]) or drought (30% of the total SWHC) level every 3 d for 56 d until harvest. Biomass, specific leaf area, root system size, water content, macro and micronutrient content, root colonisation and hyphal length density were assessed. There were 5 replicates for each treatment in a randomised block design, with 5 blocks for each species (a single block is indicated by white brackets). *V. faba* plants were sown a week after *Z. mays* plants.