Effects of surfactant-based wetting agents on cereal growth and physiology

Vasileios Giannakopoulos

This thesis submitted in partial fulfilment of the requirements for the degree of Doctor of Philosophy at Lancaster University

July, 2021

This project was supported by the Centre for Global Eco-Innovation and is part financed by the European Regional Development Fund.

European Union
European Regional Development Fund

Declaration

Except where references are made to other sources, I declare that the contents in this thesis are my own work and have not been previously submitted, in part or full, for the award of a higher degree elsewhere.

Vasileios Giannakopoulos

Lancaster, U.K.

July 2021

Oral/Poster presentations arising from this work

Oral: **Giannakopoulos V,** Puertolas P, Owen A and Dodd I C. How can surfactants enhance drought resilience and water conservation in agriculture?. Eco-I 2019 - Innovation for Clean and Sustainable Growth, University of Lancaster, Lancaster, U.K. 19th – 20th September 2019.

Poster: **Giannakopoulos V,** Puertolas P, Owen A and Dodd I C. How can surfactants enhance the drought resilience of barley (H. vulgare)?. European Plant Science Retreat 2019, University of Nottingham, Nottingham, U.K. 8th – 10th July 2019.

Acknowledgments

I would like to express my deepest gratitude to my supervisors Dr Jaime Puertolas Simon and Professor Ian C. Dodd. I am tremendously thankful for their help, support, advice, patience, and constructive criticism over the past few years. I would not be the person I am today without their guidance, and I would not have been able to overcome so much throughout my studies without them.

Thank you to Dr Andy Owen, Dr Richard Collins, Lana Farren and Alexander Barr for useful discussions.

I would like to thank members of the Plant Ecophysiology Laboratory and wider community at Lancaster Environment Centre for all the useful discussions, practical help, support and friendship: Dr Annette Ryan, Dr Clare Baskin, Maureen Harrison, Phillip Nott, Dr Vassil Karloukovski, David Lewies, Dr John Crosse, Dr Dawn Worrall, Dr Geoffrey Holroyd, Dr Elizabete Carmo-Silva, Professor Mariana Rufino, Dr Noorliana Mohd Zahn, Dr Estibaliz Leibar-Porcel, Dr Emma Burak, Dr Tom Williams, Javi Sierro, Dr Robert Kempster, Dr Sarah Donaldson, Dr Pedro Castro Valdecantos, Dr Aimee Brett, Hattie Roberts, Brittany Heap, Cristina Mc-Bride Serrano, Katharina Huntenburg, Dr Ryan Edge, Rachel Baxter, Hend Mandour, Patrick Skilleter, Bailey Kretzler, Eric Mensh, Cesar Ponce Ponce De Leon, Hao Zhu, Kellie Smith, Maggie Massam and many more.

I am particularly thankful for having received funding from the Centre for Global Eco-Innovation, European Regional Development Fund, Amega Sciences, ICL Specialty Fertilizers and J. Mallinson Ltd (Local i-68), who provided the financial support which allowed this research to be carried out.

Finally, I am always grateful to my wonderful family for their love, care, continuous support and understanding despite being thousands of miles away. I would not be where I am now without your support.

Abstract

Surfactants (surface-active agents) are amphiphilic molecules, possessing a polar hydrophilic head and a non-polar, hydrophobic, long-chain tail. They reduce the surface tension of water and are widely used by the turfgrass industry to mitigate against soil water repellency and alleviate localised dry spots. More recently, applying surfactants to soil has been considered as an alternative way of enhancing nutrient and water use efficiencies of arable crops. However, the mechanisms by which surfactants affect plant physiological responses to soil water deficit and nutrient status remain largely unknown. Thus, this thesis investigated surfactant effects on soil-plant water relations, water uptake and nutrient acquisition, in drying soil and/or elevated vapour pressure deficit.

Initially, putative effects of surfactants on rhizosheath formation and nutrient uptake were investigated in two barley genotypes that either lacked (*brb*) or had (wild type – WT) root hairs, and thus had less and more rhizosheath respectively. Application of surfactant amplified rhizosheath formation when plants were grown in a sandy soil but did not affect nitrogen and phosphorus uptake. Generally, application of surfactant did not affect plant growth, which was 21% greater in WT than in *brb* plants. Thus, nutrient content (concentration x biomass) was significantly higher in WT than *brb* plants, indicating the importance of root hairs in nutrient acquisition.

Although surfactant application did not affect plant nutrient acquisition, whether they affected plant response to soil water availability was next evaluated. The relationship between soil water potential and soil moisture was determined in surfactant-treated and untreated sandy soils by constructing soil moisture release curves via psychrometry, and by measuring base water potential (leaf water potential of non-transpiring plants) of plants

grown in surfactant-treated and untreated soils. At the same bulk soil water content, surfactant-treated soils had a higher soil water potential and plants grown in these soils had a higher base water potential than plants grown in untreated soils. Since application of surfactant augmented rhizosheath development, WT and *brb* were grown in surfactant-treated and untreated soils and subjected to soil drying and/or elevated evaporative demand, to investigate whether additional rhizosheath development enhanced plant water availability. Surfactant-mediated or genotypic differences in rhizosheath development generally did not affect base water potential or leaf water potential. Surfactant application and genotype did not affect transpiration response to elevated evaporative demand (1-3.5 kPa), thus enhanced rhizosheath formation did not affect water uptake. Overall, applying surfactants enhanced soil water availability independently of rhizosheath formation.

Although surfactant application did not affect plant transpiration during soil drying or under high evaporative demand, surfactant effects on whole plant gas exchange of different species (barley and maize) were determined after rewatering from the top or base of the pot. Surfactant application significantly increased shoot dry biomass by approx. 20% in both species. Although rehydration of upper soil layers was delayed following basal rewatering of surfactant-treated soil, whole plant gas exchange and leaf water potential recovered similarly irrespective of surfactant treatments. Thus, applying surfactant enhanced shoot dry biomass independently of plant gas exchange and leaf water status.

This research showed that surfactant application can increase soil water availability to plants and enhance rhizosheath formation. However, these effects did not improve nutrient or water uptake capacity by the roots. Further research is needed to determine the mechanisms behind plant growth differences observed in some of the experiments.

Contents

List of commonly - used abbreviations

ABA	Abscisic acid
brb	bald root barley
CO_2	Carbon dioxide
D (irrigation treatment)	Drying/Drought (treatment)
DI (water)	Deionised (water)
DW	Dry weight
E	Transpiration rate
FAO	Food and Agriculture Organization
FW	Fresh weight
GHG	Greenhouse gas emissions
g_s	Stomatal conductance
N	Nitrogen
NO_3-N	Nitrate nitrogen
NUE	Nutrient use efficiency
NUpE	Nutrient uptake efficiency
P	Phosphorus
PPFD	Photosynthetic photon flux density

RH	Air relative humidity
RLD	Root length density
RWU	Root water uptake
SWC	Soil water content
SWR	Soil water repellency
VPD	Vapour Pressure Deficit
WT	Wild type
WUE	Water use efficiency
WW (plants)	Well-watered (plants)
θ_v	Volumetric soil moisture
Ψ	Water potential
Ψ_{base}	Base water potential
Ψ_g	Gravitational potential
Ψ_{leaf}	Leaf water potential
Ψ_m	Matric potential
Ψ_p	Pressure potential
$\Psi_{predawn}$	Predawn water potential
Ψ_{root}	Root water potential

Ψ_s	Solute (or osmotic) potential
Ψ_{soil}	Soil water potential

List of Figures

treated with Surfactant 1. Bars are means ± S.E. of 6 replicates and different letters denote significant differences between genotypes and surfactant treatments (P<0.05).

Figure 3.1: Soil moisture release curves of sandy soil without (black circles) and with Surfactants 1 and 2 (hollow circles and triangles, respectively) added. Each point is an individual soil sample, P values from the ANCOVA are reported, and fitted linear regressions lines (medium-dashed line, long-dashed line and solid line for untreated soil and soil treated with Surfactants 1 and 2, respectively) are depicted.

Figure 3.2: (a) Changes in soil water content after withholding water from Day 0. Symbols are means ± SE of 6 replicates (Experiment 1). (b) Base water potential in response to soil drying in soil without (filled circles) and with Surfactants 1 and 2 (hollow circles and filled triangles, respectively) added (Experiment 1). Each point is an individual plant, P values from the ANCOVA are reported, and fitted linear regressions lines (medium-dashed line, long-dashed line and solid line for untreated soil and soil treated with Surfactants 1 and 2, respectively) are depicted.

Figure 3.3: Base water potential of water-stressed plants (harvested at the end of each cycle). Black bars: plants were growing in control (untreated) soil; White bars: plants were growing in soil treated with Surfactant 1 (Experiment 2). Bars are means ± S.E. of 9 replicates and P values reported for genotype, surfactant treatment and their interactions.

Figure 3.4: (a) Total biomass and (b) whole plant leaf area in response to soil drying in Experiment 1 (Day 5). Black bars: plants were growing in untreated (control) soil; White bars: plants were growing in soil treated with Surfactant 1; White/striped bars: plants were growing in soil treated Surfactant 2, respectively. Bars are means ± S.E of 4 replicates. There was no significant (P< 0.05) impact of surfactant treatment on either variable.

Figure 3.5: Leaf elongation (a, b, c; drying cycle 1,2,3 respectively; means ± S.E of 10 replicates) of the youngest leaf (a different leaf was measured in Days 1 and 4; vertical dashed lines) in response to drying soil over 3 drying sequential drying cycles (Experiment 2). Plants were rewatered between cycles. Plants were growing in control (untreated) soil (filled circles) and soil treated with Surfactant 1 (hollow circles), respectively.

Figure 3.6: Whole plant transpiration in response to drying soil (a, b, c; drying cycle 1,2,3 respectively; means ± S.E of 10 replicates) over 3 drying sequential drying cycles (Experiment 2). Plants were rewatered between cycles. Plants were growing in control (untreated) soil (filled circles) and soil treated with Surfactant 1 (hollow circles), respectively.

Figure 3.7: (a) Total rhizosheath weight per plant plotted against its total root length (harvested at the end of each cycle; Experiment 2). WT plants were growing in control (untreated) soil (filled circles) and soil treated with Surfactant 1 (hollow circles), *brb* plants are growing in control (untreated) soil (filled triangles) and soil treated with Surfactant 1 (hollow triangles). Fitted linear regressions are depicted. P values are reported for genotype, surfactant treatment, root length and their interactions. (b) Specific rhizosheath weight (Experiment 3). Black bars: WT plants were growing in control (untreated) soil; Grey bars: WT plants are growing in soil treated with Surfactant 1; Grey/striped bars: *brb* plants were growing in control (untreated) soil; Light grey bars: *brb* plants were growing in soil treated with Surfactant 1. Bars are means ± S.E. of 6 replicates and P values of surfactant treatment, genotype and their interaction are reported.

Figure 3.8: Response of transpiration rate to elevated VPD under well-watered conditions (a), after withholding water for five (b) and nine (c) days (pooling data across both WT and *brb* plants) (Experiment 3). Plants were growing in control (untreated) soil (filled circles) and soil

treated with Surfactant 1 (hollow circles) respectively. Each symbol is mean ± S.E. of 6 replicates and P values of surfactant treatment, genotype and VPD and their interactions are reported.

Figure 3.9: Leaf water potential on different days after ceasing irrigation (0(a), 5(b), 9(c)), at the lowest (black bars) and highest (white bars) VPD level (Experiment 3). Bars are means ± S.E. of 6 replicates (pooling data across both WT and *brb* plants) and P values of surfactant treatment, genotype, VPD and their interactions are reported.

Figure 3.10: Relationship between transpiration rate and leaf water potential for plants growing in soil without (filled circles) and with Surfactant 1 (hollow circles) added (Experiment 3). Each point is an individual plant and fitted linear regression line is depicted.

Figure 4.1: Shoot dry biomass for barley and maize plants. Black bars: plants were growing in untreated (control) soil; White bars: plants were growing in surfactant-treated soil. Bars are means ± SE of 8 replicates. P values are reported for species (Sp), surfactant treatments (S) and their interaction.

Figure 4.2: Soil moisture of upper and lower soil layer over time, for barley (a, b) and maize (c, d). Soil was rehydrated at 0 min (indicated by vertical medium-dashed line), from top (a, c) or from the base (b, d). Symbols are means ± S.E of 4 replicates. Filled and hollow circles denote upper and lower layer of untreated (control) soil, respectively. Filled and hollow triangles denote upper and lower layer of surfactant-treated soil, respectively.

Figure 4.3: Transpiration rate of barley (a, b) and maize (c, d) over time. Plants were rewatered at 0 min (indicated by vertical medium-dashed line), from top (a, c) or from the

base (b, d). Symbols are means ± S.E of 4 replicates. Plants were growing in control (untreated) soil (filled circles) and surfactant-treated soil (hollow circles), respectively.

Figure 4.4: Net photosynthetic rate of barley (a, b) and maize (c, d) over time. Plants were rewatered at 0 min (indicated by vertical medium-dashed line), from top (a, c) or from the base (b, d). Symbols are means ± S.E of 4 replicates. Plants were growing in control (untreated) soil (filled circles) and surfactant-treated soil (hollow circles), respectively.

Figure 4.5: Leaf water potential of barley (a, b) and maize (c, d) before and after rehydration. Plants were rewatered from top (a, c) or from the base (b, d). Black bars: plants were growing in control (untreated) soil; White bars: plants were growing in surfactant-treated soil. Bars are means ± S.E of 4. Different letters denote significant differences between means ($P<0.05$).

Figure 4.6: Foliar ABA concentration of barley (a, b) and maize (c, d) before and after rewatering. Plants were rewatered from top (a, c) or from the base (b, d). Black bars: plants were growing in control (untreated) soil; White bars: plants were growing in surfactant-treated soil. Bars are means ± S.E of 4 plants. Different letters denote significant differences between means ($P<0.05$).

Figure 5.1: The main findings of this thesis are summarised: Plants growing in untreated soil (A) and surfactant treated soil (B).

List of Tables

Table 2.7: Tissue nitrogen [N] concentrations (expressed in mg g^{-1} DW), along with N content (expressed in mg $plant^{-1}$ DW) and total N concentration of WT and *brb* plants grown under high (H) and low (L) nutrient level, in untreated (C) and surfactant-treated (S) soil. Data are means ± SE of 6 replicates with different letters for each row indicating significant differences ($P<0.05$).

Table 2.8: Tissue phosphorus [P] concentrations (expressed in mg g^{-1} DW), along with P content (expressed in mg $plant^{-1}$ DW) and total P concentration and content of WT and *brb* plants grown under high (H) and low (L) nutrient level, in untreated (C) and surfactant-treated (S) soil. Data are means ± SE of 6 replicates with different letters for each row indicating significant differences ($P<0.05$).

Table 3.1. Repeated measures ANOVA (P values reported) examining the effects of time (days after withholding water, D), vapour pressure deficit (VPD), genotype (G) and surfactant treatments (T) on transpiration rate, photosynthesis and leaf water potential. Significant effects ($P<0.05$) are in bold text.

Table 4.1: Soil moisture (expressed in m^3 m^{-3}) of upper and lower soil layer (C and S denote plants grown in untreated and surfactant-treated soil, respectively), 210 min following rewatering from the top. Different letters within a row indicate significant ($P<0.05$) differences.

Table 4.2: Repeated measures ANOVA (P values reported) examining the effects of time (after rewatering, T), soil layer (SL), irrigation location (I) and surfactant treatments (S) and their interactions on soil moisture, in barley and maize. Significant effects ($P<0.05$) are in bold text.

Table 4.3: Repeated measures ANOVA (P values reported) examining the effects of time (after rewatering, T), species (Sp), irrigation (I) and surfactant treatments (S) and their interactions on transpiration rate and photosynthesis. Significant effects (P<0.05) are in bold text.

Table 4.4: Transpiration rate (expressed in g H2O cm^{-2} s^{-1}) and net photosynthetic rate (expressed in µmol CO_2 cm^{-2} s^{-1}) of barley and maize (C and S denote plants grown in untreated and surfactant-treated soil, respectively), 210 min following rewatering from the top or base. There were no significant differences between surfactant treatments (P>0.05).

Table 4.5: Repeated measures ANOVA (P values reported) examining the effects of time (before and following rewatering, R), irrigation (I) and surfactant treatments (S) and their interactions on leaf water potential, in barley and maize. Significant effects (P<0.05) are in bold text.

Table 4.6: Repeated measures ANOVA (P values reported) examining the effects of rewatering time (before and following rewatering, R), irrigation (I) and surfactant treatments (S) and their interactions on foliar ABA concentration, in barley and maize. Significant effects (P<0.05) are in bold text.

Chapter 1: General Introduction

1. Increasing resource-use efficiency in agriculture

1.1. Introduction

The Food and Agriculture Organization (FAO) defines food security as a "*situation that exists when all people, at all times, have physical, social, and economic access to sufficient, safe, and nutritious food that meets their dietary needs and food preferences for an active and healthy life*" (Schmidhuber & Tubiello, 2007). In the middle of 21st century, the global population will reach 9 billion people and the ability of current agricultural systems to maintain its food demands is a substantial issue (Frona *et al.,* 2019). Although 70 to 100% more food will be needed by 2050 (Rosegrant *et al.,* 2003), currently more than one in seven people still lack sufficient protein and energy in their diet, and even more suffer from some form of malnutrition. Remarkable progress has been made in reducing the number of people suffering from hunger, from an estimated 980 million people in 1990–92 to about 690 million people in 2019; however further progress is necessary (Wheeler & von Braun, 2013; FAO, 2020). It is apparent that measures should be taken to counter this dual challenge of increasing global population and eliminating world hunger.

A common concern of the international community is climate change caused by increasing greenhouse gas (GHG) emissions that can negatively affect global food security (Ericksen *et al.,* 2009). An estimated 23% of total anthropogenic GHG (2007-2016) derive from agriculture, forestry and other land use (IPCC, 2019). Furthermore, fertilizer manufacture and application substantially contribute to global GHG emissions, but their liability varies according to different processing technologies and energy sources. The energy required for production,

transport and application of fertilisers is a main source of GHG emissions in agriculture (FAO, 2017). Thus, it is of utmost importance to define ways to reduce excessive use of fertilisers without compromising crop production.

Sufficient nutrient availability to the plant is essential for growth and fertiliser applications boost crop production in most parts of the world (Jin, 2012). Nitrogen (N) and phosphorus (P) are the two most important nutrients limiting biological production and are widely applied in agricultural systems (Hou *et al.,* 2012). However, the environmental damage associated with their use is significant, including GHG emissions and pollution of underground and surface water sources with nutrients. Proper management and efficient applications of fertilisers may maximise crop yield and decrease nutrient leaching below the root zone (Good *et al.,* 2004; Drinkwater *et al.,* 2007).

Irrigation is also necessary to maintain high yields, specifically in arid and semi-arid regions, where it exceeds 70-80% of global fresh water used (Debaeke *et al.,* 2017). At the same time, continuous global population growth and related developments limit the availability of water, which is considered the main constraint to high crop productivity (Yang *et al.,* 2006). Within the next 40 years, more than 65% of the global population will be estimated to live in areas where water scarcity will be the norm and not the exception (Godfray *et al.,* 2010; Xinchun *et al.,* 2017). Hence, water scarcity is considered as a global systemic risk and the sustainable management of natural resources (i.e., water resources) is essential for maintaining global socio-economic development within an environmentally supportable framework. In conclusion, it is vital to find solutions that will permit crop productivity while using less water, to achieve efficient and effective use of water (Ali *et al.,* 2008; Parry *et al.,* 2010).

Therefore, a multi-faceted global strategy is needed to obtain sustainable and equitable food security. Crop production must be raised to feed a continuously growing population and tackle world hunger, while at the same time enhancing resource use efficiency, by minimising the ecological footprint from excessive use of water sources and application of fertilisers (Godfray *et al.,* 2010; Fan *et al.,* 2011).

1.2. Can surfactant application increase resource-use efficiency?

Currently, turfgrass is the main target market for surfactants, with their application improving greenness, quality and biomass (York et *al*., 1993; Cisar *et al.,* 2000, Kostka *et al*., 2007; Sciavon *et. al,* 2014), by alleviating the occurrence of localised dry spots (Kostka, 2000; Park *et al.,* 2004; Alvarez *et al.,* 2016; Madsen *et al.,* 2016). However, in recent years, using surfactants to improve crop resource use efficiency and yield has attracted increasing attention. Deficit irrigation in combination with surfactants increased total maize (*Zea mays L.*) dry matter, by allowing higher soil water retention (Chaichi *et al.,* 2015). Furthermore, surfactant application enhanced yield of potato (*Solanum tuberosum*) and alfalfa (*Medicago sativa*) grown under limited irrigation treatments (Oostindie *et al.,* 2012; Jafarian *et al.,* 2016). However, not all surfactant treatments have enhanced crop biomass accumulation or yield. Different surfactant application rates had no effect on cotton (*Gossypium hirsutum*) yield (Sullivan *et. al,* 2009). Thus, further investigations of the physiological effects of surfactants in possibly mediating plant water deficits are essential to confront this uncertainty. Surprisingly, the effect of surfactants on plant water relations remains unknown. Plant water status ultimately affects plant growth and yield (Munns *et al.,* 2000; Moradi *et al.,* 2014). Thus, determining plant water availability is essential to understand the physiological mechanisms allowing increased plant growth and yield following surfactant application to drying soils.

Applying surfactant increased leaf N concentration in tomato plants (*Solanum lycopersicum*), likely due to higher water retention and thus enhanced N acquisition (Torres & Santos, 2012). Furthermore, surfactants tended to reduce nitrate nitrogen (NO_3-N) leaching, likely by alleviating preferential flow paths and ensuring more homogeneous soil moisture distribution (McAvoy, 1994; Arriaga *et al.,* 2009; Cooley *et al.,* 2009). Thus, applying surfactants might enhance soil N status by decreasing N losses below the root zone. On the other hand, surfactants had minimal effects or no effect on nutrient availability in several studies, in turfgrass (Wolkowski *et al.,* 1985), maize (Banks *et al.,* 2014) and tomato (Chaichi *et al.,* 2017). Contradictory results between studies suggest that further work is required to better understand whether surfactants enhance nutrient uptake and consequently promote nutrient use efficiency (NUE), either facilitating nutrient mobility or nutrient absorption by the roots.

2. What is a surfactant and how does it affect soil properties?

2.1. Definition, types, and properties of surfactants

Surfactants, a contraction of the term “surface active agents”, are amphiphilic molecules that serve as wetting agents, possessing both hydrophobic and hydrophilic moieties. Surfactants decrease the surface tension of water and increase the wettability of non-hydrophilic organic compounds (Fig. 1.1; Daneshnia *et al.,* 2016). By decreasing surface tension, surfactants reduce the contact angle between soil particles and soil water, facilitating infiltration rate (Arriaga *et. al,* 2009). The polarity of different surfactants varies and based on the nature of the polar head group, are classified into four categories: cationics (positively charged head group), anionics (negatively charged head group), non-ionics (no charge), and amphoterics or zwitterionics (they carry both a positive and a negative charge; Ishiguro & Koopal, 2016).

Anionic surfactants have been used for more than four decades to increase water percolation in hydrophobic substrates. However, both cationic and anionic surfactants have negative effects on soil structure and are phytotoxic (Ruemmele & Amador,1999). Non-ionic surfactants do not have a charge in solution and are chemically less active and consequently less toxic for plants than cationic and anionic (Reinikeinen *et. al,* 1997). Hence, non-ionic surfactants are used most commonly in agriculture as recommended concentrations are harmless to plants (Baratella *et. al,* 2016; Nemmati *et. al,* 2017).

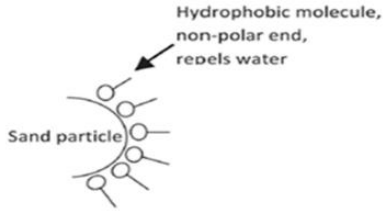

Figure 1.1: The theoretical mode of action of a wetting agent to alleviate water repellent (hydrophobic) soils (redrawn from Gross *et al.,* 2011).

2.2. Soil water movement and effect of surfactants on soil properties

2.2.1. Theoretical background of soil water movement

Water potential (Ψ) is a measure of the free energy that is associated with water and can be defined as the tendency of water to move from one region to another due to matric effects, gravity, osmosis, and pressure mechanisms. Water always moves from the highest to the lowest total Ψ, comprising the following components (Liu *et al.,* 2012).

Matric Potential (Ψ_m)

Matric forces represent the attraction of water to the surface of soil particles where hydrophilic colloids occur (Tyree, 2003).

Gravitational potential (Ψ_g)

Water moves downward due to gravity, with Ψ_g depending on the "*height of the water above the reference-state water, the density of water, and the acceleration due to gravity*" (Taiz & Zeiger, 2002).

Solute (or osmotic) potential (Ψ_s)

Solute accumulation will lower Ψ (Ψ_s=0 in pure water), with water movement across a semi-permeable membrane towards compartments of lower (more negative) solute potential (Hellkvist *et al.,* 1974).

Pressure potential (Ψ_p)

The pressure potential is the component of Ψ due to the hydrostatic pressure that is exerted on water in a cell. In turgid plant cells, Ψ_p has a positive value since the entry of water causes the protoplast to force against the cell wall (turgor). In xylem cells, Ψ_p is negative (tension) due to transpiration. At atmospheric pressure, water has a Ψ_p of zero (Kirkham, 2005).

Water movement in the soil depends on whether it is saturated or unsaturated. Saturated flow occurs when all or almost all soil pores are filled with water, with gravity and pressure potential determining water movement. In saturated soils, Ψ is zero or around zero as all pores are filled with water (Singer & Munns, 2002). In unsaturated conditions, water moves due to differences in Ψ_m, driven by differences in soil water content (SWC). Two forces responsible for this movement are the attraction of soil solids for water (adhesion) and capillarity. In unsaturated soil, water freely drains from macropores due to gravity. As soil becomes drier, soil particles hold water more tightly due to capillary forces and soil hydraulic conductivity (i.e., the ease of flow with which a liquid moves into soil) declines steeply. Thus,

gravitational forces are supplanted by Ψ gradients. Water moves from wetter regions with minimal matric forces (less negative Ψ) to drier regions where water is held more tightly (more negative Ψ). Generally unsaturated soils are more common in agricultural settings than saturated soils (Singer & Munns, 2002; Reinson *et al.,* 2005; Voroney & Heck, 2015).

2.2.2. How do surfactants affect soil properties?

Soil water movement may be disrupted by properties that are generated in the soil due to exogenous factors. During summer dry periods, soils are commonly water-repellent or hydrophobic (Dekker *et al.,* 2004). Soil water repellency (SWR) is mainly observed in sandy substrates (Snyder *et. al,* 2004; Sullivan *et. al,* 2009) and organic coatings of soil particles are widely accepted to enhance SWR. These coatings become hydrophobic after long periods of dry conditions and subsequent rewetting events (Snyder *et. al,* 2004; Kostka *et al.,* 2007). Although the causes of SWR are well known, there are few methods to mitigate this problem. Nevertheless, the turfgrass industry applies surfactants to decrease the surface tension of water, thereby decreasing the contact angle between water molecules and soil particles, ultimately increasing the wettability of organic compounds and hence alleviating SWR (Kostka *et al.,* 2007). Applying these surfactants increase the wettability of hydrophobic substrates, thereby increasing infiltration rate (Feng *et. al,* 2002; Cooley *et. al,* 2009; Lehrsch *et. al,* 2011; Lehrsch, 2013) and enhancing soil water-holding capacity (Elliott 1992; Sullivan *et al.,* 2009). However, when surfactant products were tested on soils that were not water repellent, little or no change in infiltration rate and soil water-holding capacity occurred (Mobbs *et al.,* 2012). Notwithstanding, capillary rise and unsaturated hydraulic conductivity were lower in surfactant-treated sand than untreated controls, but differences were not consistent between four different surfactant treatments. While single applications of either non-ionic or

anionic surfactants might not be sufficient to alter soil water movement and retention, repeated watering and surfactant applications may have different effects (Mobbs *et al.,* 2012). Moreover, initial soil moisture of substrates may affect those responses (Green, 1962), but this information has not been provided in these studies. Thus, surfactants are proposed to increase the wettability and ultimately water-holding capacity of hydrophobic soils (Sullivan *et al.,* 2009; Mehrvarz et al., 2013). Nevertheless, it is not surprising that surfactants had little effect on the wettability of non-repellent soils.

Surfactants have a dual effect on soil water dynamics, as they increase infiltration rate as indicated previously, allowing a more even water distribution within the soil profile (Soldat *et al.,* 2010; Alvarez *et. al,* 2016). This may be explained by rupturing the cohesive forces of water molecules, decreasing surface tension of water and thus increasing infiltration rate within soil profile and consequently into repellent root-zone areas. Moreover, by reducing surface tension of water, surfactants permit the penetration of water into smaller micropores. Specifically, surfactant molecules affect hydraulic properties by altering the size of pores that water can occupy at any given Ψ_m (Lehrsch *et al.,* 2011). Particularly, surfactants increase the number of small pores available to water, thus enhancing lateral water movement (Cid-Ballarin *et al.,* 1998). Ultimately, application of surfactant enhances soil moisture distribution across all soil pores in both hydrophobic and hydrophilic root zones (Schiavon *et al.,* 2014; Alvarez *et al.,* 2016).

However, improving soil moisture homogeneity is not necessarily correlated with higher SWC. Studies in diverse species have shown that surfactants can increase (Oostindie *et al.,* 2008; Barton & Colmer, 2011; Oostindie *et al.,* 2012), decrease (Aamlid *et al.,* 2009) or have no effect (Chaichi *et al.,* 2017) on SWC. Although surfactants increase soil moisture homogeneity,

putative effects of surfactants on bulk soil water relations have not been studied yet. Soil water potential (Ψ_{soil}) and SWC are inseparably linked, with Ψ_{soil} becoming less negative as SWC increases. The shape of this relationship depends on soil characteristics (soil texture, organic matter content; Singer & Munns, 2002). Whether surfactants alter the relationship between Ψ_{soil} and SWC by changing water and/or soil physical properties is not clear.

3. Water absorption by plants

3.1. Root water uptake and limitations

Root water uptake (RWU) depends on the Ψ gradient between root (Ψ_{root}) and soil (with uptake occurring when $\Psi_{root} < \Psi_{soil}$), and on the hydraulic conductivity of all parts of the water movement pathway (Nobel & Cui, 1992). Root water uptake is greater in moist than dry soil. In moist substrates, RWU is mainly limited by root hydraulic conductivity, whereas soil hydraulic conductivity plays a key role under dry conditions and decreases sharply as soil dries (North & Nobel, 1997; Zarebanadkouki & Carminati, 2013). In soils of intermediate SWC, water movement is determined by the hydraulic conductivity of the root-soil interface (Liu *et al.,* 2015). As soil dries, air gaps are generated between roots and soil mainly due to root shrinkage, reducing the hydraulic conductivity of soil-root interface (Ahmed *et al.,* 2015). Previous studies found that air gaps are generated after transpiration has been substantially reduced, indicating that air gaps are a consequence rather than a cause of inadequate water availability (Carminati *et al.,* 2013). Roots may shrink radially up to 40%, when transpirational demands are substantial (Liu *et al.,* 2015). However, rewetting the soil allows root swelling to partially close air-filled gaps (Carminati *et al.,* 2013).

3.2. Rhizosphere: the dynamic processes that affect water absorption

The rhizosphere is defined as the layer of soil in the vicinity of plant roots. It is actively modified by root growth, exudation and microbial activity and comprises a radius of a few millimetres up to a maximum of a few centimetres (Benard *et al.* 2016). Studies indicate that physical, chemical, and biological properties of rhizosphere differ from that of bulk soil (Moradi *et al*., 2012; Ahmed *et al.,* 2015) while mucilage modifies the physical properties of the rhizosphere (Moradi *et al.,* 2011). Mucilage, which is exuded by most roots, is a polymeric gel that substantially affects hydraulic properties of rhizosphere (Ahmed *et al.,* 2017). Mucilage acts to a) maintain contact between soil particles and roots by facilitating rhizosheath formation, b) facilitate root penetration of the soil by reducing friction, c) promote RWU by increasing rhizosphere water content and d) prevent dehydration of root tissues (Carminati *et al.,* 2010; Ahmed *et al.,* 2015). Mucilage affects rhizosphere physical properties by effectively absorbing large volumes of water as the rest of the soil dries (Young, 1995), which may explain higher water content in the rhizosphere than in the bulk soil during soil drying (Ahmed *et al.,* 2014; Zarebanadkouki *et al.,* 2018). Mucilage retention of water content in the rhizosphere may enhance hydraulic conductivity of the root-soil interface. In this case, mucilage exudation would partly favour water uptake as soil dries.

However, mucilage also contains amphiphilic components such as small phospholipid fractions that become water repellent upon drying and rewetting events (Zarebanadkouki *et al.,* 2018). Indeed, it has been argued that roots secrete organic compounds that cause SWR (Kostka *et. al*, 2000; Oostindie *et. al,* 2012). Moreover, the rhizosphere of lupin (*Lupinus albus*) grown in sandy soil became water repellent after drying and subsequent rewetting (Carminati *et al,* 2010). Upon rewetting, the rhizosphere remained temporarily dry and recovered a few

hours later, in contrast to bulk soil that rehydrated immediately. This response was attributed mainly to mucilage properties.

Taken together, it is proposed that water movement in the rhizosphere is dynamic and differs from that in the bulk soil, due to the different actions of mucilage with drying and rewetting. When water is adequate, mucilage absorbs a substantial volume of water thereby facilitating rhizosphere wettability under gradually drying soil and before any rewetting event occurs. On the other hand, phospholipids (and other amphiphilic components) in the mucilage make the rhizosphere water repellent after a period of drying and subsequent rewetting. Therefore, rhizosphere wettability is lower after rewetting and recovers after a certain period. However, 60 h after rewatering the water content of the rhizosphere exceeded that of the bulk soil (Carminati *et al,* 2010).

3.3. The putative role of rhizosheath in water uptake and drought resilience

Sufficient contact between soil particles and roots is essential for water and nutrient uptake. Loose soil structure and/or root or soil shrinkage diminishes root-soil contact, restricting absorption of water and nutrients (North & Nobel, 1997). However, efficient contact between soil particles and roots is difficult to accomplish, particularly in sandy, shallow, or shifting substrates. In addition to loose soil structure that could create air gaps around the roots, prompt drainage of these substrates causes rapid drying (Pang *et al.,* 2017). The tendency of roots of certain species to form rhizosheaths, composed of soil particles bound together with root hairs (i.e., tubular projections from root epidermal cells that function in nutrient and water uptake as well as in anchoring the root in the soil) and root exudates, can be advantageous in such habitats. Particularly as the soil dries, mucilage partly dehydrates and is maintained close to the roots. Root hairs also assist in adhering soil particles to the root

surface (Datta *et al.,* 2011; Ahmadi *et al.,* 2017; Benard *et al.,* 2018; Burak *et al.,* 2021). Formation of these rhizosheaths has been suggested to facilitate water and nutrient uptake (Liu *et al.,* 2018) since they occur in many plants, including agriculturally important crops such as maize and other mesophytic and desert grasses (Hartnett *et al.,* 2013; Brown *et al.,* 2017). Moreover, soil particles adhere more firmly to root surfaces under dry conditions, where rhizosheaths are denser rather than under moist conditions (Watt *et al.,* 1994; Bailey & Scholes, 1997). Additionally, wheat cultivars with greater rhizosheath development maintained higher transpiration rates in drying soil, compared to cultivars that formed thinner rhizosheaths. Genotypic differences in transpiration might be attributed to greater rhizosheath size facilitating root access to water but further work is needed to confirm it (Basirat *et al.,* 2019). The most convincing evidence that rhizosheaths may mitigate drought stress *in planta* was provided by observations that the rhizosheath of wheat (*Triticum aestivum*) plants was significantly wetter than bulk soil (Young 1995). Mucilage was suggested to increased rhizosheath soil moisture, since it can absorb large amounts of water. Thus, rhizosheath formation may enhance water uptake in dry soil, but further work is needed to confirm it.

Hydrophobic parts of surfactant molecules associate with hydrophobic groups of mucilage, decreasing mucilage swelling and consequently increasing its viscosity (cross-linking; Ahmadi et al., 2017). Ultimately, more mucilage is retained in the vicinity of the roots and the bonds between soil particles and root surface are strengthened, forming more extensive rhizosheath layers (Ahmadi *et al.,* 2017). The proposed ability of surfactants to enhance rhizosheath formation may facilitate further water uptake, particularly at low soil moisture. However, the plant physiological implications of soil modifications following surfactant application (enhanced rhizosheath formation) have not been explored. Specifically, surfactant application

could enhance plant water status by increasing soil-root contact via greater rhizosheath formation. Denser rhizosheath layers following surfactant applications could enhance plant water availability (at the same bulk soil water content), thereby improving water status. Investigating these hypotheses is essential to better understand the mechanisms by which surfactants might affect soil - plant water relations.

4. Soil moisture, regulation of plant water use and effect of surfactants

4.1. Plant water use and transpiration

Plant water use plays a key role in soil - plant water relations. Transpiration, which is defined as *"a process of water movement from soil solution to the atmosphere through the plant vascular system"* occurs through the stomata that are specialized pores in the leaf epidermis bordered by two guard cells (Liu *et al.,* 2012; Torres-Ruiz *et al.,* 2015; Jezek & Blatt, 2017). Regulating transpiration is crucial to maintaining plant functions. Transpiration rate is partially regulated by stomatal conductance (g_s), which is determined by stomatal density and stomatal aperture. Theoretically, high g_s may emanate from many small open stomata, or fewer but larger open stomata per unit leaf area (Bueckert, 2013). Transpiration is essential for maintaining carbon dioxide (CO_2) acquisition. While stomata are open, CO_2 enters through stomata and is converted into oxygen and energy-rich organic compounds through photosynthesis. Stomatal opening incurs continuous water losses while the process of photosynthesis happens. Plant tissues comprise at least 90% water and their structure depend on cell turgidity. Water must be taken up continuously to replenish transpirational losses and drive turgor-mediated cell expansion. Under drought conditions, plants close their stomata to prevent water losses, but this reduces photosynthesis as less CO_2 enters the leaves. Thus, it becomes clear that transpiration is a vital process for plants since it is

inseparably linked to photosynthesis and plant water status (Bois *et al.,* 1985; Mishra, 2004; Buckley, 2005; Galmés *et al.,* 2007).

4.2. Control of plant water use under drought stress

Regulating stomatal aperture is the major physiological control of plant water use in drying soil. Stomatal aperture can be altered directly by changes in plant water relations (hydraulic signalling), but also through chemical signals within the plant (Gollan *et al.,* 1986; Giorio *et al.,* 1999; Dodd, 2005). Both hydraulic and chemical signals may play a key role in root to shoot communication and stomatal regulation under drought stress (Čereković *et al.,* 2014).

Since water is characterized by cohesion and tension properties, changes in Ψ in any plant tissue can be rapidly distributed within the plant via the xylem. Thus, soil drying decreases Ψ_{soil} and consequently Ψ_{root}, thereby generating hydraulic signals that decrease Ψ_{leaf}. While hydraulic signalling plays a significant role in stomatal regulation in response to drying soil, it is not always the case (Schmidhalter, *et al.,* 1998). Soil water status can also modify g_s without affecting shoot water relations and hence Ψ_{leaf} (Bates & Hall, 1981; Gollan *et al.,* 1986; Zhang & Davies, 1989). Plants respond to water scarcity by transporting chemical signals through the xylem that regulate stomatal aperture and also leaf growth (Wilkinson, 1999). Particularly, root-sourced abscisic acid (ABA) is a plant hormone that triggers physiological responses as soil dries, regulating stomatal aperture (Tuteja & Sopory, 2008; Claeys & Inze, 2013; de Ollas & Dodd, 2016). Abscisic acid synthesis in root tips increases as Ψ_{root} declines, in response to gradually drying soil (Simonneau *et al.,* 1998). Some of this ABA is exported into the transpiration stream, thereby increasing xylem sap ABA concentration. Ultimately, it reaches the leaves inducing stomatal closure to avoid dehydration (Comstock, 2002; Lee & Mudge,

2013). Commonly, both hydraulic and chemical signals are involved in regulating plant water use (Pou *et al.,* 2008; Lee *et al.,* 2013).

4.3. Effect of surfactants on plant water use

Very little research has explored whether surfactants alter plant water use, even though interactions between mucilage and surfactant molecules following rewetting (Section 3.3) provide a possible mechanism. By decreasing the contact angle of solid-liquid interfaces, surfactant molecules may enhance rhizosphere rewetting. With time, mucilage rehydrates, and water flow depends on the mucilage swelling. Surfactant molecules decrease mucilage swelling and therefore decrease the saturated conductivity of the soil–mucilage mixture, potentially lowering transpiration rate (Ahmed *et al*. 2017). However, in this conceptual model it is not clear how decreased rhizosphere hydraulic conductivity after a rewetting event could benefit RWU. Moreover, it is not known whether this conceptual model can be generalized to species with different mucilage properties (Naveed *et al.,* 2019). Thus, further work is needed to further investigate if and how application of surfactants affects plant transpiration, especially following rewatering.

Alternatively, it has been hypothesised that surfactant molecules enter the xylem, thereby decreasing transpiration rates and g_s (Yang, 2008; Sibley *et al.* 2018). While surfactant application decreased irrigation needs by decreasing g_s but not limiting photosynthesis, further studies are needed to determine the mechanisms behind this response, since only instantaneous measurements of leaf gas exchange are provided in the abovementioned works and surfactant concentrations in xylem sap were not measured. In conclusion, several authors have suggested that surfactants alter plant water use either directly (anti-transpirant

activity of surfactants) or indirectly (lowering rhizosphere hydraulic conductivity), but evidence is scant.

5. Water distribution within the root -zone and surfactant effects on root architecture

5.1. Soil moisture distribution in the root-zone

Soil water accessibility is strongly dependent on the spatial geometry of the root system. Thus, roots are able to acquire water within a limited region of soil profile, delimiting an upper threshold of water volume that roots can exploit (Ahmed *et al.,* 2015). However, water is rarely distributed evenly, generating soil moisture heterogeneities. Furthermore, water is distributed unevenly within the root system due to the differences in axial and radial conductivities within root segments as well as the conductance of the shortest path that connects them with the shoot base (Lobet *et al.,* 2014). This concept of root hydraulic architecture greatly affects the hydraulic conductance of the root system. Eventually, these differences result in certain parts of the root system taking up more water than others, creating soil moisture heterogeneity (Doussan *et al*., 2006). Taken together, RWU may be limited by root hydraulic architecture and soil hydraulic conductivity, which may be altered as the soil dries.

5.2. Do surfactants affect root length?

Although surfactants significantly increased root length of turfgrass and maize compared to untreated plants in field trials (Brumbaugh *et al.,* 2001; Karnok & Tucker, 2001), other studies showed no differences in maximal root length of turfgrass (Aamlid *et al.,* 2009). Surfactants may positively affect root traits by ensuring more homogeneous soil moisture distribution and improved soil wettability. However, these studies do not clarify whether increased root

length depends on plant size or is due to altered biomass allocation. Additionally, measurements were conducted either visually or using gridded templates, with more accurate quantification of specific root traits needed. Digital root analysis (eg. WinRhizo; Gu *et al.,* 2017) has scarcely been used in previous studies. Thus, a recent field study determined thinner roots and higher root length density (RLD) of turfgrass growing in surfactant-treated soil (Serena *et al.,* 2020). These differences were attributed to enhanced soil wettability due to application of surfactants. Taken together, applying surfactants may affect root traits, however further work is needed to confirm it.

6. Root nutrient uptake

6.1. Uptake of major elements and related problems

Nitrogen and phosphorus are two major elements required for plant growth and development that differ in terms of mobility in the soil, with deficiencies occurring due to different reasons (Razaq *et al.,* 2017). Plants require N in the highest quantity, and although nitrate is highly soluble in the soil solution (moving towards the roots via both mass flow and diffusion), it can be easily lost by leaching (Lambers *et al.,* 2006; Sorgonà *et al.,* 2007; Trubat *et al.,* 2012). On the other hand, P (the second most important element in terms of plant development), is highly immobile and moves mainly via diffusion. Moreover, inorganic phosphate ions which are bio-available to plants, are strongly adsorbed to surfaces dominated by Al^{3+}, Fe^{3+} and Ca^{2+}, forming insoluble complexes. Thus, mobility of phosphate ions is substantially restricted (Hodge, 2004).

6.2. Nutrient uptake and soil moisture distribution under drought conditions: The putative role of surfactants

Soil drying decreases nutrient uptake by limiting nutrient transport towards the roots, which may also inhibit plant growth (Mouat *et al.,* 1985; Htoon *et al.,* 2014). Since mass flow is the main mechanism for N absorption, low soil water availability decreases transpiration rate thereby substantially restricting N movement towards the roots (Junjittakarn *et al.,* 2013). Moreover, drought stress restricts P movement and availability in soil, as well as P acquisition due to lower diffusion rate of P from the soil matrix to the absorbing root surface (Garg, 2003; Singh *et al.,* 2004; Fan *et al.,* 2015). It is apparent that limited soil water availability can restrict nutrient uptake, which may limit growth. Homogenisation of soil moisture distribution by surfactant addition might facilitate nutrient uptake by enhancing mass flow of nitrate, particularly in drying soil. Moreover, soil moisture homogeneity may enhance root surface area and root distribution, allowing more exposed sites to be available to take up diffusible ions (i.e., phosphate). However, as discussed above, putative effects of surfactants on root traits have received little attention and further investigation is needed to confirm this assumption.

6.3. Effect of rhizosheath on nutrient uptake: The putative role of surfactants

Another root-related trait that may enhance nutrient uptake is rhizosheath formation. As with drought resilience (Section 3.3), the rhizosheath may play a key role under nutrient deficient conditions, even if the mechanisms may differ. Root hairs are known to be important for rhizosheath development and might confer benefits associated with the rhizosheath, including nutrient acquisition (Haling *et al.,* 2013). A few studies have investigated rhizosheath effects on nutrient uptake and biomass accumulation. Wild-type plants with root

hairs (that formed a substantial rhizosheath) had greater shoot P accumulation than root hairless mutants (with less rhizosheath formation) in barley (*Hordeum vulgare*) (George *et al.,* 2014). Furthermore, barley plants grown in P-deficient soils developed 18% more rhizosheath than plants grown in P-sufficient soils (Brown *et al.,* 2012). Overall, the presence of root hairs, which ultimately contribute to rhizosheath formation, seems to play a vital role in nutrient acquisition, especially for P. However, it is not clear whether rhizosheath formation contributes to nutrient acquisition beyond the effect of root hairs (Pang *et al.,* 2017).

In addition to root hairs, mucilage plays an important role in rhizosheath formation (Liu *et al.,* 2018). Species with higher root exudation formed more rhizosheath and were better able to acquire immobile elements (zinc) than species with lower root exudation, in drying soil (Nambiar, 1976). Based on the concept described in Section 3.3, surfactant-induced rhizosheath formation is hypothesised to facilitate nutrient uptake and biomass accumulation. However, experimental evidence is needed to determine whether surfactant-engineered rhizosheath formation affects nutrient uptake.

Statement of Research Objectives

This research aims to determine the impact of surfactant application on nutrient uptake, soil-plant water relations and plant water use in drying soil and/or under elevated evaporative demand. Initial experiments (Chapter 2) focused on effects of applying surfactants on nutrient acquisition and biomass accumulation in drying soil. Nutrient uptake was determined in two genotypes of barley, with contrasting rhizosheath traits, growing in surfactant-treated soil or untreated controls and irrigated with two different strengths of Hoagland's Solution (100% and 10%). This study aimed to determine whether putative surfactant effects on nutrient acquisition depend on rhizosheath development and unravel effects of rhizosheath and

presence of root hairs on nutrient uptake. Thus, it was hypothesised that surfactant-induced rhizosheath formation will have an additional positive effect on nutrient uptake and plant growth, beyond that of root hairs.

Then a series of experiments (Chapter 3) assessed whether surfactants enhance soil - plant water relations in drying soil and under elevated evaporative demand. These experiments aimed to determine whether any differences in soil-plant water relations were attributed to changes in soil physics (soil moisture release curves) or had a physiological explanation (effects of rhizosheath on root access to water). Thus, putative differences between surfactant treatments were suggested to result from a) alterations in the relationship between Ψ_{soil} and SWC, with surfactants enhancing the water status of drying soil b) surfactant-mediated rhizosheath formation increasing plant water availability under drought conditions.

Finally, in Chapter 4, surfactant effects on whole plant gas exchange following rewatering were investigated. This study investigated whether surfactant effects on plant gas exchange, following rewatering, depend on contrasting mucilage properties of varied species (barley/maize) and/or rehydration techniques. Based on previous studies, maize root exudates were hypothesised to act as surfactants (drying the rhizosphere at smaller suctions) whereas barley exudates act as hydrogels (holding more water in the rhizosphere, but with slower rewetting). It was hypothesised that (a) applying surfactant will inhibit soil rehydration when plants are rewatered from the base of the pot, thereby limiting whole plant gas exchange, and (b) species will differ in their responses to surfactant application following rehydration; with surfactants decreasing maize gas exchange but not affecting barley gas exchange following rewatering.

The major objectives of this study are:

1) Investigate whether surfactants enhance nutrient uptake and plant growth and determine the putative mechanism(s) involved.

2) Evaluate whether surfactants alter soil-plant water relations in drying soil and/or at higher evaporative demand, and to understand the underlying physiological processes associated with those effects.

3) Determine surfactant effects on whole plant gas exchange during recovery from drought and understand the physiological mechanism(s) involved.

Chapter 2: Root hairs and not surfactant-induced rhizosheath formation facilitate nutrient acquisition of barley (*Hordeum vulgare*) in drying soil.

2.1. Introduction

High demand and frequent use of nutrients in modern agriculture requires alternative management solutions to improve NUE (Baligar *et al.,* 2001; Baligar & Fageria, 2005). Agronomically, NUE is the total biomass/yield per unit nutrient in the soil or applied nutrients (Baligar *et al.,* 2001). Applying surfactants to soils has been proposed as an alternative way to improve NUE. Applying surfactants tended to decrease NO_3-N leaching, likely by alleviating preferential flow paths and homogenising soil moisture distribution (McAvoy, 1994; Arriaga *et al.,* 2009; Cooley *et al.,* 2009). Thus, applying surfactants may enhance soil N availability by decreasing N losses below the root zone. Since most studies have explored putative effect of surfactants on nutrient leaching, whether surfactants affect nutrient uptake remains largely unknown.

A conceptual model proposed that surfactants affect nutrient distribution in the soil by allowing water to occupy smaller soil micropores (Baratella & Trinchera, 2018). Also, surfactants were hypothesised to enhance root nutrient uptake by facilitating solute movement across the plasma membrane (Baratella & Trinchera, 2018), but supporting experimental evidence has not been forthcoming. Empirical studies show that surfactants have inconsistent effects on plant nutrient accumulation and growth (Banks *et al.,* 2014; Dadresan *et al.,* 2015; Chaichi *et al.,* 2017; Trinchera & Baratella, 2018). Interestingly, applying surfactants tended to decrease P accumulation in plants (Table A-1), but the putative

mechanism remains obscure. Further research is needed to reconcile discrepancies between studies and determine mechanisms that might explain surfactant effects on nutrient uptake and plant growth.

Surfactants may enhance nutrient acquisition by promoting rhizosheath formation. The rhizosheath is defined as soil particles that adhere to the root system by a combination of root hairs which penetrate the soil and mucilage secreted from the roots (Pang *et al.,* 2017). Surfactant molecules are hypothesised to interact with root mucilage, leading to decreased swelling and increased viscosity of mucilage (Ahmadi *et al*., 2017). Therefore, mucilage is retained more abundantly in the vicinity of the root surface, adhering more soil particles, and thus enhancing rhizosheath formation (Ahmadi *et al*., 2017). However, whether this facilitates nutrient uptake is less clear.

Secretion of water-absorbing mucilage by roots may enhance rhizosheath moisture content compared to the bulk soil (Young, 1995). Thus, rhizosheath soil is considered a more favourable habitat for microbial growth and development than bulk soil (Marasco *et al.,* 2018; Moreno-Espíndola *et al.,* 2018). Such microorganisms contribute substantially to availability and accessibility of essential elements to the plants by producing enzymes (such as phosphatase) that solubilize nutrients during mineralization (Ortíz-Castro *et al.,* 2009). Additionally, the rhizosheath is considered to act as a niche for nitrogen-fixing bacteria since nitrogen fixation has been associated with the rhizosheath of several species of grasses (Wullstein *et al.,* 1979; Wullstein, 1980; Bergmann *et al.,* 2009). Thus, by enhancing microbial activity and including bacteria that fix nitrogen, the rhizosheath may increase plant nutrient acquisition.

Furthermore, rhizosheaths are thought to enhance water and nutrient uptake by preserving soil-to-root contact (Hartman, 2020). Mass flow accounts for nutrient uptake of mobile elements that are easily soluble in the soil solution, such as N (Mengel, 1982). By facilitating water uptake, rhizosheath formation could promote nutrient movement to the root system, facilitating N acquisition. Surfactant-induced rhizosheath augmentation might allow higher N uptake, and ultimately enhance plant growth, but this hypothesis has not been verified yet.

Previous studies have indirectly investigated the effects of rhizosheath formation on P acquisition and biomass accumulation, by comparing these variables in genotypes differing in root hair traits. Genotypes with larger rhizosheath mass (due to longer root hairs) accumulated more P and biomass than those with smaller rhizosheath mass (due to shorter or no root hairs), due to an extended P depletion zone since P is immobile and absorbed mainly via diffusion (Brown *et al.,* 2012; George *et al.,* 2014; James *et al*., 2016). Rhizosheath formation strongly correlates with root hair length and density, as root hairs contribute significantly to soil binding (Haling et al., 2014; Delhaize *et al.,* 2015). While greater P acquisition and biomass accumulation were attributed to favourable root hair traits, their impact on nutrient acquisition could not be readily differentiated from their effect on rhizosheath formation. Hence, it remains ambiguous whether the presence of root hairs increases nutrient uptake only by significantly increasing root surface area, or whether they have an additional effect by augmenting rhizosheath formation (Pang *et al.,* 2017).

Applying surfactants to genotypes that either have or lack root hairs would determine whether putative surfactant effects on nutrient uptake depend on rhizosheath development, potentially disentangling effects of rhizosheath and presence of root hairs on nutrient acquisition. Consequently, a factorial experiment applied surfactants to soils within which

two barley genotypes with contrasting root hair phenotypes were grown under two fertilisation regimes (high and low nutrient application). Nitrogen and phosphorus concentrations and contents, plant biomass, rhizosheath formation and rhizosheath and bulk soil moisture content were determined. It was hypothesised that: a) surfactant application will facilitate nutrient uptake and plant growth by increasing rhizosheath size and b) rhizosheath formation will have an additional effect of enhancing nutrient acquisition beyond that of root hairs.

2.2. Materials and Methods

2.2.1. Plant material, growth conditions, application of surfactant and irrigation treatments

Two barley genotypes, the root hairless bald root barley (*brb*) mutant and its wild-type (*cv. Pallas* - WT), were cultivated in a sandy soil, in a controlled environment (CE) Room. The substrate, hereafter described as a sandy soil, was a 3:1 (v:v) mixture of silica sand (0.4 mm-0.8 mm; Bathgate Silica Sand Ltd, Sandbach, UK) and topsoil (Bailey's of Norfolk Ltd, Norwich, UK), which contained 90% w/w sand, 3% w/w clay and 7% w/w silt. After germination, seeds were sown 2.5 cm deep in rectangular 2 L pots (10×8×20 cm height; water holding capacity of substrate was 200 g ± 2.8) filled with sandy soil. An Ektron II sensor (HortiMax, Pijnacker, Netherlands) monitored environmental conditions in the centre of the CE Room. Maximum daily air temperature was 23.6^{o}C, minimum daily air temperature was 21.8^{o}C and average relative humidity was 65.3% ± 0.1. Two strengths of Hoagland's Solution (2.1% v/v and 0.21% v/v) were applied to plants every two days (100 mL per plant on each occasion) by surface watering. Hoagland's Solution was prepared by adding deionised (DI) water to the appropriate volume of three different stock solutions to avoid precipitation of incompatible solutes (Table 2.1). One surfactant product was used, a dipropylene glycol methyl ether

(H2Pro AquaSmart, Amega Sciences, Daventry, UK). Aquasmart is referred to as “Surfactant 1”. Surfactant (5 mL) made up to 1000 mL of DI water and stored in clear glass laboratory bottles wrapped with aluminium foil under dark conditions. This concentration corresponds to commercial application of surfactants in the field, according to manufacturer recommendations. Solution was evenly applied to surface of each pot (25 mL), seven and fourteen days after germination. In control plants, an identical volume of DI water was added. All plants were irrigated with approx. 175 mL of tap water after each surfactant application to ensure its even distribution in the root-zone. Saucers were placed underneath each pot to capture any drainage water, which was re-incorporated to the pots. Plants were kept well-watered (WW) for three weeks.

Prior to starting soil drying, plants were watered at 1600 h until water drained from the base of the pot, and next morning were weighed on a precision balance to 0.1 g (Scout Pro Portable balance, Ohaus, Switzerland) to establish pot capacity. The surface of each pot was covered with black duct tape to limit evaporation losses (less than 5% of plant evapotranspiration; determined by weighing pots without plants that were similarly covered with duct tape). Water was withheld for eight days and plants (D) were harvested at the end of drying cycle.

Table 2.1: Constituent chemicals of Hoagland's stock solution (g L-1). Stock solution was made in 1 L preparations for each Solution A, B, C. 100 mL of A and B and 10 mL of C or 10 mL of A and B and 1 mL were added to 10 L of water to make a working solution of 2.1% v/v or 0.21% v/v Hoagland's, respectively.

Source	Stock solution (g L^{-1})	Element	Final concentration for full-strength solution (ppm)	Final concentration for 10% solution (ppm)
A				
NH_4NO_3	8	N	195.8	19.58
$Ca(NO_3)_2{\cdot}4H_2O$	82.6	Ca	161.2	16.12
KNO_3	40.7	K	250.6	25.06
B				
KH_2PO_4	27.4	P	45.4	4.54
$MgSO_4{\cdot}7H_2O$	24.6	S	45.4	4.54
$MnSO_4{\cdot}5H_2O$	0.053	Mg	34.4	3.44
H_3BO_3	0.14	Mn	0.10	0.01
$CuSO_4{\cdot}5H_2O$	0.015	B	0.24	0.024
$(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$	0.008	Cu	0.04	0.004
ZnSO4·7H2O	0.06	Mo	0.01	0.001
ZnSO4·7H2O	0.06	Zn	0.34	0.034
C				
NaFe EDTA	36.71	Fe	0.50	0.05

2.2.2. Transpiration, leaf elongation and shoot biomass

Transpiration was monitored gravimetrically daily, using a precision balance to 0.1 g (Scout Pro Portable balance, Ohaus, Switzerland), simultaneously to measuring the length of the youngest expanding leaf on the main tiller using a flexible ruler. The newest leaf was measured as soon as it appeared on the main tiller and for one day both leaves were measured. Leaf elongation rate was calculated as the difference in leaf length between successive days. At the end of the experiment, shoot tissues were dried at 80°C for at least 72 h to obtain dry biomass weight.

2.2.3. Rhizosheath formation, root length, root biomass and soil moisture

De-topped roots were extracted from the soil, whilst minimising soil disturbance to retain root-soil contact (Young, 1995; Pang *et al.,* 2017). Rhizosheath formation was quantified using the method of Haling *et al*. (2010), with slight modifications. Fresh weight (FW) of roots with adhered soil particles ($FW_{root+rhizosheath}$) was recorded. Afterwards, the entire root system was placed in a pre-weighed metal tray filled with DI water and gently agitated until the rhizosheath separated from the roots. Larger aggregates were fragmented using a small paintbrush whilst caution was taken not to damage the roots. Immediately roots were blotted with absorbent tissue, root fresh weight (FW_{root}) was recorded and roots were placed in tubes filled with 50% v/v ethanol and stored at 4^{o}C for further analysis (Haling *et al.,* 2010). The metal tray containing rhizosheath soil was oven-dried at 105^{o}C to constant mass, with rhizosheath dry weight $DW_{rhizosheath}$ determined by subtracting the weight of the metal tray. Fresh weight of rhizosheath soil was determined as:

$$FW_{rhizosheath} = (FW_{root+rhizosheath}) - FW_{root}$$

Immediately after extracting the rhizosheath, the rest of the soil not adhered to the root was weighed to obtain bulk soil fresh weight (FW_{bulk}) and oven dried as described above to measure dry weight (DW_{bulk})

Soil water content of bulk soil and the rhizosheath (SWC_{bulk} and $SWC_{rhizosheath}$, respectively) was calculated as:

$$SWC_{bulk} = (FW_{bulk} – DW_{bulk})/ DW_{bulk}$$

$$SWC_{rhizosheath} = (FW_{rhizosheath} – DW_{rhizosheath})/ DW_{rhizosheath}$$

Roots were placed in a clear acrylic tray with a thin film of DI water. To avoid as much overlap as possible, they were splayed using plastic pipette tips. A single root system was sometimes separated into multiple scans. Images (.jpg; 8-bit grayscale; 400 dpi) were created using a scanner (Epson Expression 11000XL Pro, Seiko Epson, Nagano, Japan) with transparency unit. Root length was analysed using WinRHIZO (2013e, Regent Instruments Inc., Sainte-Foy, QC, Canada). Root tissues were dried at 80°C for at least 72 h to obtain dry biomass weight.

2.2.4. Nutrient analyses

Total N and carbon (C) of shoot tissues, root tissues, bulk soil and rhizosheath soil were measured by an Elemental Analyser (Vario MICRO Cube, Elementar UK Ltd., Cheadle, UK). Prior to measurements, samples were ground to a fine powder using a ball mill. Powder (10 ± 1 mg and 20 ± 1 mg, for plant tissues and soil samples, respectively) was weighed into tin cups and folded. Samples were combusted at 800^{o}C to determine total N and C.

Total P concentration of shoot tissues, root tissues, bulk soil and rhizosheath soil was determined by an Autoanalyzer after acid digestion (Allen *et al.,* 1974 – modified Kjeldahl). Briefly, 0.2 ± 0.05 g of powdered tissue were digested using 4.4 mL of digest reagent (0.42 g

selenium, 14 g lithium, 350 mL hydrogen peroxide and 420 mL sulphuric acid) at 350^{o}C for 2.5 h. Digested samples were diluted to 50 mL with milliQ water, filtered (Whatman no 44, Fisher Scientific UK Ltd, Loughborough, UK), diluted a further 5x with milliQ water and stored at 4^{o}C for further analysis. Blanks were digested in the same way. Total P was measured spectrophotometrically at 880 nm (AutoAnalyzer 3 HR, Seal Analytical Ltd, Southampton, UK). Nutrient content (uptake) of each element was calculated as nutrient concentration multiplied by dry biomass.

2.2.5. Statistical analysis

Data were tested for normality using Shapiro-Wilk test. Statistical differences ($P < 0.05$) in dry biomass and N, P concentrations/contents between genotypes, nutrient levels and surfactant treatments were determined by three-way ANOVA. Three-way ANCOVA assessed whether nutrient levels, genotypes and surfactant treatments differed in rhizosheath production, with root length as the covariate. Differences in plant transpiration and leaf elongation rate at different times during drying cycle were analysed using repeated measures ANOVA, with time as within-subjects factor and genotypes, nutrient levels, and surfactant treatments as between-subject factors. Differences between SWC_{bulk} and $SWC_{rhizosheath}$, N, P concentration between bulk soil and rhizosheath soil were determined using repeated measures ANOVA, with soil type as within-subjects factor and genotypes, nutrient levels, and surfactant treatments as between-subject factors. All statistical analyses were performed using SPSS 25 (SPSS Statistics 25, IBM, Armonk, New York, USA).

2.3. Results

2.3.1. Total biomass

Application of surfactant had no significant effect on plant growth while total biomass of WT plants was ca. 22% higher (across surfactant and nutrient treatments) than *brb* plants (Table 2.2). In plants growing under high nutrient conditions, total biomass was ca. 51% higher than in plants growing under low nutrient conditions (across two genotypes and surfactant treatments; Table 2.2). A significant Treatment x Genotype x Nutrient Level interaction (P=0.04; Table 2.3) revealed that under high nutrient conditions, total biomass of *brb* plants growing in surfactant - treated soil was ca. 45% greater than in untreated soil (Table 2.2) while under low nutrient conditions, total biomass was ca. 13% higher in plants growing in surfactant-treated soil than untreated controls (across two genotypes; Table 2.2). Overall, applying surfactant under high nutrient conditions increased total biomass of *brb* plants.

Table 2.2: Total biomass for WT and *brb* plants grown under high and low nutrient level, in untreated and surfactant-treated soil. Data are means ± SE of 6 replicates with different letters indicating significant differences (P<0.05).

Treatment	Genotype	Nutrient Level	Total Biomass (g)
Control	WT	High	0.77 ± 0.01 b
Control	WT	Low	0.56 ± 0.03 c
Control	*brb*	High	0.64 ± 0.03 c
Control	*brb*	Low	0.34 ± 0.03 d
Surfactant	WT	High	0.70 ± 0.05 b
Surfactant	WT	Low	0.58 ± 0.08 c
Surfactant	*brb*	High	0.94 ± 0.03 a
Surfactant	*brb*	Low	0.40 ± 0.06 d

Table 2.3: Three-way ANOVA (P values are reported) examining the effects of surfactant treatment (T), genotype (G) and nutrient level (NL) on total biomass. Significant effects (P<0.05) are in bold text.

	P value
Treatment (T)	0.58
Genotype (G)	**0.04**
Nutrient Level (NL)	**<0.001**
Treatment x Genotype	0.06
Treatment x Nutrient level	0.69
Genotype x Nutrient level	0.19
TxGxNL	**0.04**

2.3.2. Bulk and rhizosheath soil moisture

Bulk SWC did not significantly differ between surfactant (P=0.44) or nutrient treatments (P=0.06). However, soil moisture was significantly higher in *brb* than in WT plants (P=0.03), which may be attributed to lower absolute transpiration of *brb* than WT plants during Days 1-4. No significant differences were found between bulk and rhizosheath SWC (Table 2.4; Table A-2).

Table 2.4: Rhizosheath and bulk soil water contents for WT and *brb* plants grown under high and low nutrient level, in untreated and surfactant-treated soil. Measurements were taken at the end of drying cycle, on Day 8. There were no significant (P<0.05) differences between rhizosheath and bulk soil. Data are means ± SE of 6 replicates.

Treatment	Genotype	Nutrient Level	$SWC_{bulk\ soil}$ ($g\ g^{-1}$)	$SWC_{rhizosheath}$ ($g\ g^{-1}$)
Control	WT	High	0.033 ± 0.008	0.032 ± 0.010
Control	WT	Low	0.035 ± 0.005	0.036 ± 0.003
Control	*brb*	High	0.054 ± 0.005	0.056 ± 0.003
Control	*brb*	Low	0.065 ± 0.009	0.063 ± 0.003
Surfactant	WT	High	0.036 ± 0.010	0.035 ± 0.001
Surfactant	WT	Low	0.035 ± 0.005	0.036 ± 0.006
Surfactant	*brb*	High	0.051 ± 0.002	0.050 ± 0.006
Surfactant	*brb*	Low	0.062 ± 0.006	0.063 ± 0.006

2.3.3. Transpiration and leaf elongation

Soil drying decreased transpiration after Day 6 (Fig. 2.1) similarly in the two surfactant treatments (Fig. 2.1 a). Transpiration was ca. 25% higher in WT plants than in *brb* plants from Days 1 to 4 (Fig. 2.1 b; Table A-3), possibly because WT plants had a greater transpiring (leaf) area, as determined at the end of the experiment. Plants that were growing under high nutrient conditions had ca. 24% higher transpiration than plants growing under low nutrient conditions, for Days 6-8 (Fig. 2.1 c; Table A-3). Furthermore, normalising Day 8 transpiration by leaf area also demonstrated that low nutrient plants had 40% lower transpiration rate. Thus, WT and well-fertilised plants had higher transpiration, but there was no surfactant effect.

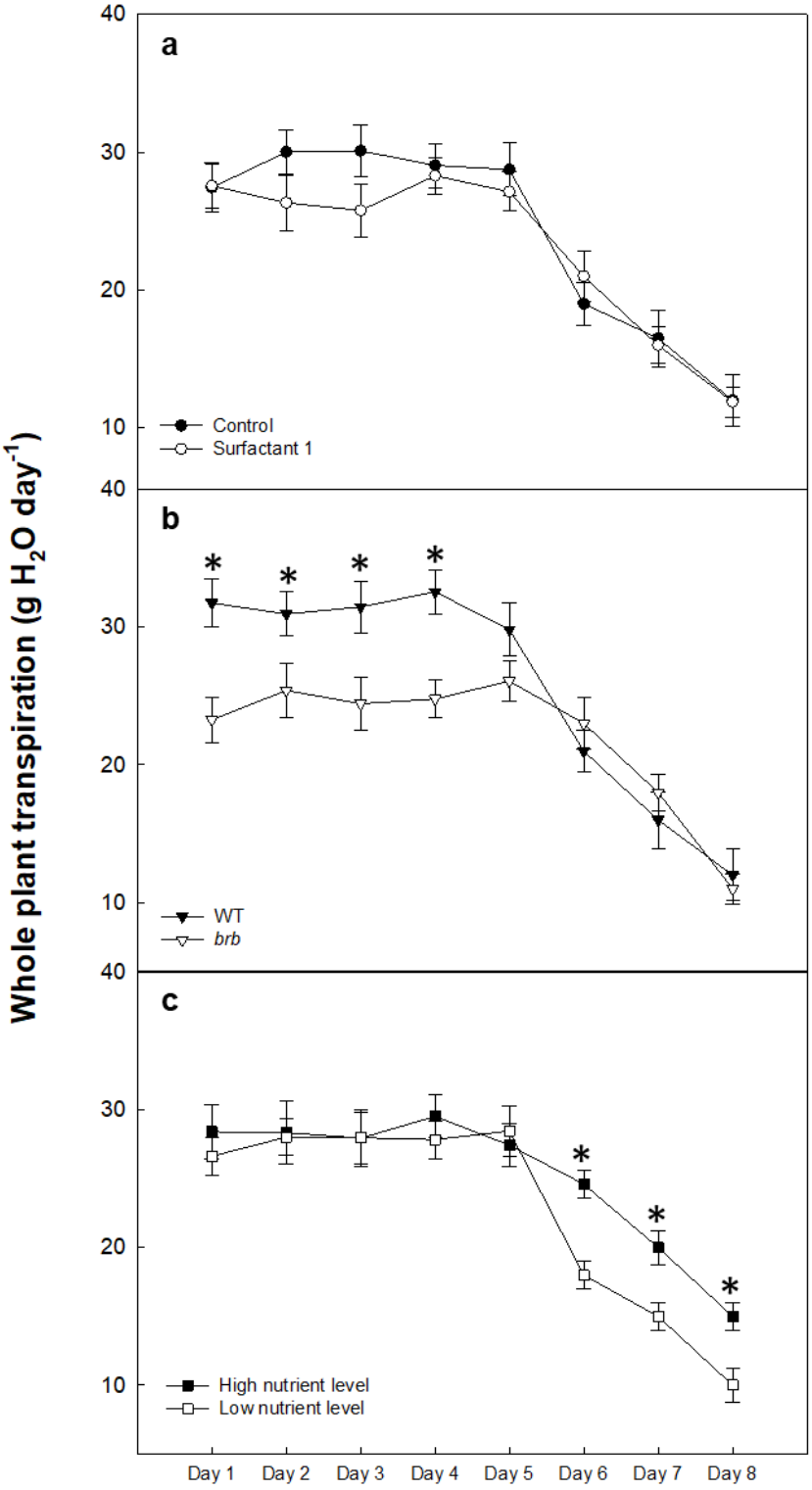

Figure 2.1: Whole plant transpiration in response to drying soil (means ± S.E of 6 replicates), in control (untreated) soil (filled circles) and soil treated with Surfactant 1 (hollow circles) (a), in WT (filled triangles) and *brb* (hollow triangles) plants (b) and under high (filled squares) and low (hollow squares) nutrient conditions (c). Single asterisks indicate statistically significant differences between surfactants, genotypes, and nutrient levels.

Soil drying decreased leaf elongation after Day 2 (Fig. 2.2), similarly in the two surfactant treatments (Fig. 2.2 a; Table A-3). Leaf elongation was higher in WT than in *brb* plants in Days 2 and 6 (significant Day x Treatment interaction; p=0.02) (Fig. 2.2 b; Table A-3). Nutrient conditions did not alter leaf elongation at any measurement occasion (Table A-3). Overall, only WT leaf elongation was greater than *brb* leaf elongation on a few measurement occasions.

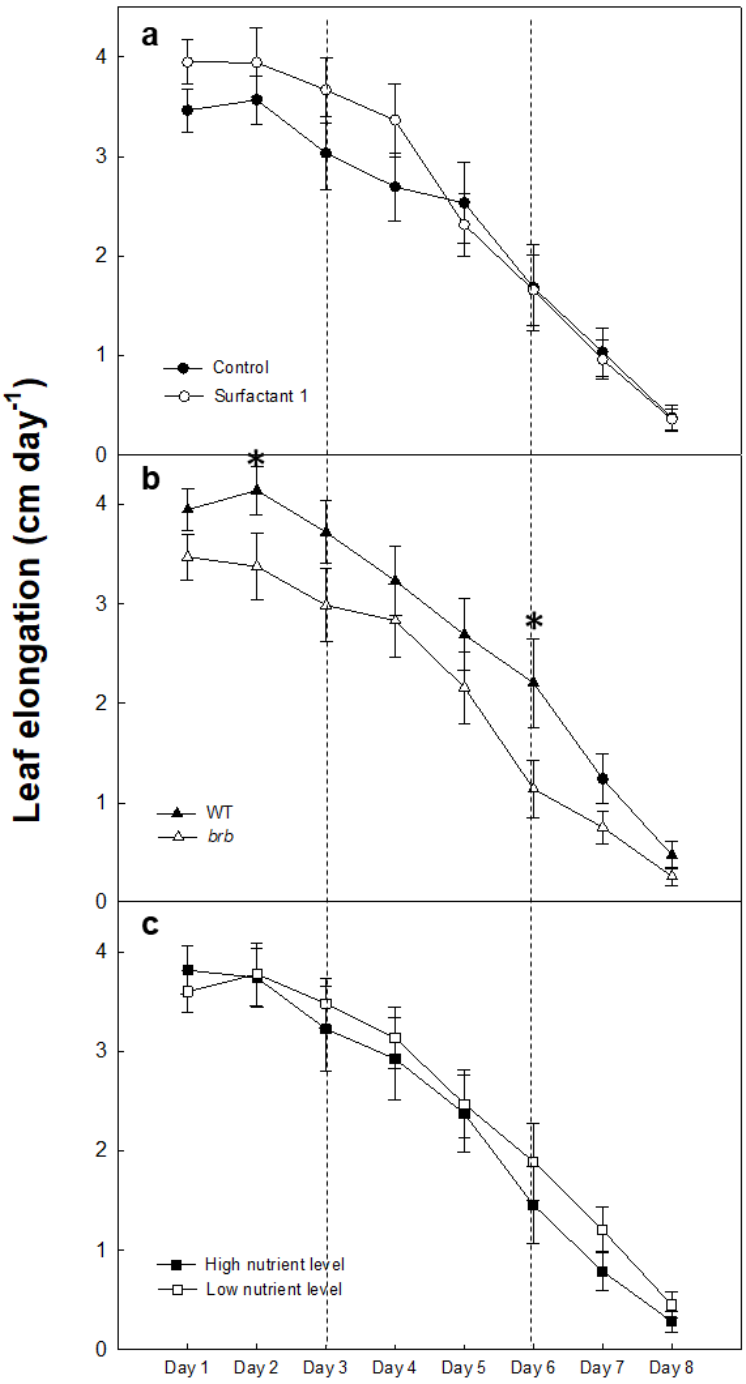

Figure 2.2: Leaf elongation of the youngest leaf (a different leaf was measured in Days 1, 4 and 7; vertical dashed lines) in response to drying soil (means ± S.E of 6 replicates), in control (untreated) soil (filled circles) and soil treated with Surfactant 1 (hollow circles) (a), in WT (filled triangles) and *brb* (hollow triangles) plants (b) and under high (filled squares) and low (hollow squares) nutrient conditions (c). Single asterisks indicate statistically significant differences between surfactants, genotypes, and nutrient levels.

2.3.4. Rhizosheath formation

Nutrient level had no significant effect on rhizosheath formation (Table 2.5); thus, data were pooled and re-analysed in order to determine any genotypic and surfactant treatment differences. Surfactant treatment had no significant effect on total root length (Table 2.5) Rhizosheath weight significantly increased with root length and was higher in WT than *brb* plants (Fig. 2.3 a). Applying surfactant significantly increased rhizosheath formation of both WT and *brb* plants (Fig. 2.3) and WT plants growing in untreated soil had the same rhizosheath size as *brb* plants growing in surfactant-treated soil (Fig. 2.3 b). No significant Genotype x Surfactant interaction was found (Table 2.5), indicating additive effects of roots hairs and surfactants on rhizosheath development.

Table 2.5: Three-way ANCOVA (P values are reported) testing the effects of surfactant treatments (T), genotype (G) and nutrient level (NL) on the relationship between total rhizosheath and root length (RL). Significant effects (P<0.05) are in bold text.

	P value
Treatment (T)	**0.02**
Genotype (G)	**0.04**
Nutrient level (NL)	0.36
Root Length (RL)	**0.01**
TxNL	0.43
GxNL	0.31
TxG	0.15
NLxTxG	0.85
NLxRL	0.49
TxRL	0.88
GxRL	0.85
TxGxRL	0.44
NLxTxRL	0.65
NLxGxRL	0.71
NLxGxTxRL	0.76

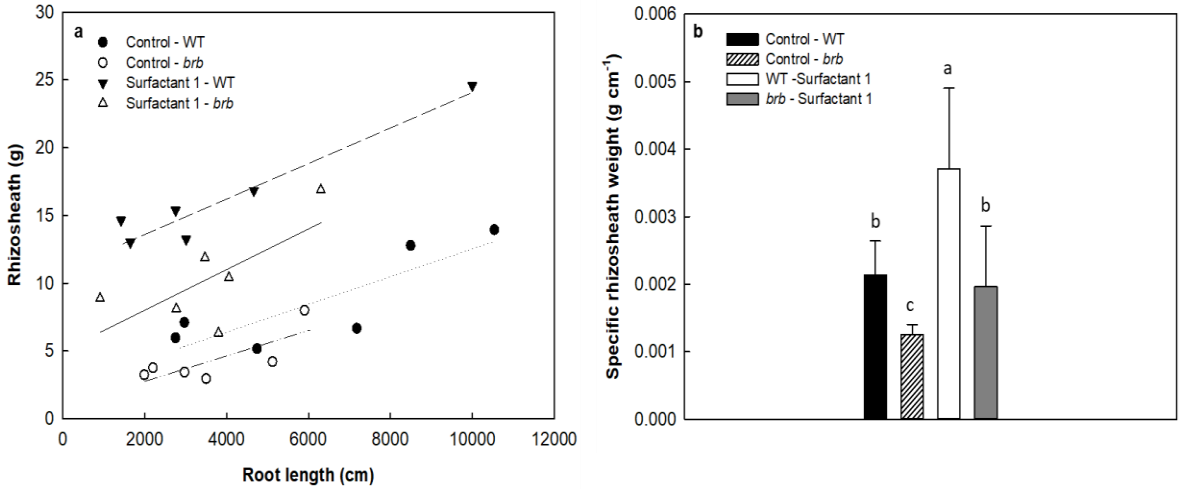

Figure 2.3: (a) Total rhizosheath weight per plant plotted against its total root length (data of two nutrient levels have been pooled). WT plants were growing in control (untreated) soil (filled circles) and soil treated with Surfactant 1 (filled triangles), *brb* plants were growing in control (untreated) soil (hollow circles) and soil treated with Surfactant 1 (hollow triangles). Fitted linear regressions are depicted. (b) Specific rhizosheath weight (data of two nutrient levels have been pooled). Black bars: WT plants were growing in control (untreated) soil; White/striped bars: *brb* plants were growing in control (untreated) soil; White bars: WT plants were growing in soil treated with Surfactant 1; Grey bars: *brb* plants were growing in soil treated with Surfactant 1. Bars are means ± S.E. of 6 replicates and different letters denote significant differences between genotypes and surfactant treatments ($P<0.05$).

2.3.5. Nitrogen and phosphorus analyses

Nitrogen and phosphorus contents were not statistically different between bulk soil and rhizosheath soil (Table 2.6). No differences were found in N and P contents between surfactant treatments and fertilisation regimes. Soil C content was significantly higher (ca. 27% across both surfactant treatments and fertilisation regimes) in rhizosheath than in bulk soil (Table 2.6). Overall, N and P contents were similar between bulk soil and rhizosheath soil.

Table 2.6: Total nitrogen (N) and phosphorus (P) content (expressed in mg g^{-1}), along with % carbon (C), in bulk soil and rhizosheath soil. Data are means ± SE of 3 replicates with different letters for each column indicating significant differences (P<0.05).

Soil type	Treatment	Genotype	Nutrient Level	N	C	P
Bulk	Control	WT	High	0.25 ± 0.02 a	0.27 ± 0.02b	0.12 ± 0.00 a
	Control	WT	Low	0.22 ± 0.03 a	0.26 ± 0.03 b	0.11 ± 0.00 a
	Surfactant	WT	High	0.26 ± 0.03 a	0.28 ± 0.03 b	0.11 ± 0.00 a
	Surfactant	WT	Low	0.23 ± 0.03 a	0.26 ± 0.01 b	0.11 ± 0.00 a
Rhizosheath	Control	WT	High	0.24 ± 0.01 a	0.40 ± 0.06 a	0.13 ± 0.00 a
	Control	WT	Low	0.21 ± 0.04 a	0.35 ± 0.02 a	0.12 ± 0.01 a
	Surfactant	WT	High	0.23 ± 0.01 a	0.40 ± 0.03 a	0.12 ± 0.00 a
	Surfactant	WT	Low	0.21 ± 0.01 a	0.36 ± 0.01 a	0.11 ± 0.00 a

Surfactant treatment had no significant effect on shoot, root, or total N concentration/content. High nutrient level increased shoot, root, and total N concentration by approximately 48%, 14% and 62% (across both genotypes and surfactant treatments, respectively (Table 2.7). Genotype did not affect root N concentration (Table A-4); but shoot and total N concentration/content were significantly higher in WT than in *brb* plants, under low nutrient conditions (Table 2.7). Overall, WT plants accumulated more N than *brb* plants.

Table 2.7: Tissue nitrogen [N] concentrations (expressed in mg g^{-1} DW), along with N content (expressed in mg $plant^{-1}$ DW) and total N concentration of WT and *brb* plants grown under high (H) and low (L) nutrient level, in untreated (C) and surfactant-treated (S) soil. Data are means ± SE of 6 replicates with different letters for each row indicating significant differences (P<0.05).

	H-WT-C	**H-WT-S**	**H-*brb*-C**	**H-*brb*-S**	**L-WT-C**	**L-WT-S**	**L-*brb*-C**	**L-*brb*-S**
Shoot [N]	58.29 ± 2.50 a	55.05 ± 2.32 a	58.30 ± 1.31 a	50.35 ± 1.80 a	28.92 ± 0.74 b	26.55 ± 1.88 b	18.35 ± 1.09 c	18.10 ± 0.80 c
Root [N]	20.19 ± 0.48 a	17.70 ± 0.60 a	20.89 ± 0.58 a	17.65 ± 1.00 a	16.21 ± 0.22 a	14.31 ± 1.30 b	14.65 ± 0.50 b	12.19 ± 0.41 b
Total [N]	50.30 ± 1.31 a	52.60 ± 2.58 a	55.78 ± 1.81 a	51.68 ± 2.31 a	26.18 ± 0.93 b	25.40 ± 1.89 b	18.10 ± 0.78 c	18.51 ± 2.31 c
Shoot N Content	47.45 ± 0.62 a	48.29 ± 0.89 a	36.28 ± 2.35 a	39.38 ± 1.40 a	8.69 ± 0.11 b	8.12 ± 0.59 b	6.11 ± 1.00 c	6.36 ± 1.10 c
Root N content	3.40 ± 0.49 a	3.60 ± 0.56 a	1.48 ± 0.89 b	1.94 ± 0.71 b	2.30 ± 0.81 b	2.08 ± 0.60 b	1.70 ± 0.30 b	1.80 ± 0.60 b
Total N content	50.81 ± 1.00 a	51.88 a ± 1.74 a	37.48 ± 2.10 b	41.24 ± 1.28 b	10.91 ± 1.09 c	10.10 ± 1.70 c	7.89 ± 1.50 d	8.12 ± 1.40 d

Surfactant treatment had no significant effect on shoot, root, or total P concentration/content (Table 2.8; Table A-5). No genotypic or nutrient level differences were found in root P concentration, but plants grown under high nutrient level conditions had higher shoot and total P concentration. Shoot and total P concentrations of WT plants were around 38% and 36% higher (across treatments and nutrient level) than in *brb* plants (Table 2.8; Table A-5). Plants grown under high nutrient level had higher P content than those grown under low nutrient level conditions (Table A-5). Taken together, WT plants accumulated more P than *brb* plants, under both low and high nutrient conditions.

Table 2.8: Tissue phosphorus [P] concentrations (expressed in mg g^{-1} DW), along with P content (expressed in mg $plant^{-1}$ DW) and total P concentration and content of WT and *brb* plants grown under high (H) and low (L) nutrient level, in untreated (C) and surfactant-treated (S) soil. Data are means ± SE of 6 replicates with different letters for each row indicating significant differences (P<0.05).

	H-WT-C	**H-WT-S**	**H-*brb*-C**	**H-*brb*-S**	**L-WT-C**	**L-WT-S**	**L-*brb*-C**	**L-*brb*-S**
Shoot [P]	1.24 ± 0.09 a	1.14 ± 0.09 a	0.87 ± 0.11 b	0.82 ± 0.10 b	0.81 ± 0.14 b	0.91 ± 0.13 b	0.60 ± 0.05 c	0.49 ± 0.03 c
Root [P]	0.17 ± 0.02 a	0.18 ± 0.01 a	0.16 ± 0.01 a	0.15 ± 0.01 a	0.17 ± 0.01 a	0.17 ± 0.01 a	0.16 ± 0.01 a	0.16 ± 0.06 a
Total [P]	5.22 ± 0.16 a	4.70 ± 0.50 a	3.14 ± 0.40 b	3.33 ± 0.35 b	3.21 ± 0.46 b	3.56 ± 0.38 b	1.95 ± 0.16 c	1.78 ± 0.12 c
Shoot P content	5.00 ± 0.58 a	4.10 ± 0.82 a	2.13 ± 0.36 b	2.00 ± 0.67 b	1.94 ± 0.20 b	2.16 ± 0.46 b	0.75 ± 0.10 c	0.74 ± 0.15 c
Root P content	0.12 ± 0.01 a	0.10 ± 0.01 a	0.14 ± 0.08 a	0.11 ± 0.01 a	0.12 ± 0.01 a	0.13 ± 0.03 a	0.12 ± 0.01 a	0.11 ± 0.02 a
Total P content	5.12 ± 0.59 a	4.20 ± 0.83 a	2.27 ± 0.56 b	2.11 ± 0.68 b	2.06 ± 0.14 b	2.29 ± 0.48 b	0.87 ± 0.10 c	0.85 ± 0.17 c

2.4. Discussion

2.4.1. Surfactant application amplified rhizosheath formation but did not affect nutrient acquisition

Surfactant application enhanced rhizosheath formation in WT and *brb* plants (Fig. 2.3), with similar rhizosheath development between *brb* plants growing in surfactant-treated soil and WT plants growing in untreated soil (Fig. 2.3 b). Similarly, applying surfactant augmented rhizosheath development, presumably by cross-linking root mucilage and surfactant molecules (Ahmadi *et al.,* 2017). Presence of root hairs also promoted rhizosheath development (George *et al.,* 2014) by entangling soil particles (Delhaize *et al.,* 2012), but this effect did not interact with that caused by application of surfactant (Table 2.5). Greater root hair development better facilitated rhizosheath formation than root exudate adhesiveness in WT barley plants, but root exudate adhesiveness was important in root hairless mutants (Burak *et al.,* 2021). Regardless of root hair traits, surfactant application presumably increased root mucilage adhesiveness, similarly in WT and *brb*, facilitating rhizosheath formation. Overall, surfactant application allowed root hair effects to be separated from rhizosheath effects as WT barley in the absence of surfactant had the same rhizosheath development as *brb* plants growing in surfactant-treated soil.

Application of surfactant to soil had no cumulative effect on nutrient uptake (Tables A-4; A-5). Previous studies provide empirical evidence of surfactant effects on nutrient uptake; however, results are frequently contrasting (Table A-1), and mechanisms on how surfactants affect nutrient acquisition remain unclear. In this study, N and P uptake did not differ between surfactant treatments (Tables 2.7; 2.8), and thus augmented rhizosheath formation did not promote nutrient acquisition or biomass accumulation.

That crop nutrient uptake was independent of surfactant application (Tables 2.7; 2.8) was consistent with similar rhizosheath nutrient content in surfactant-treated and untreated soils, and the similar N and P content in bulk soil and rhizosheath (Table 2.6). Growing plants in a sandy soil and irrigating them uniformly with Hoagland's Solution (prior to withholding water) might have masked any diffusional limitations to nutrient transfer. Nevertheless, rhizosheath phosphatase activity has been correlated with P utilization efficiency in lupin growing in loamy soil (Wu *et al.,* 2021), thus further studies need to determine whether surfactant-enhanced rhizosheath formation might augment nutrient acquisition in certain soil types.

2.4.2. Rhizosheath and root hairs effects on nutrient acquisition and plant growth

The higher rhizosheath moisture content than the bulk soil ensures a favourable habitat for greater microbial activity (Othman *et al.,* 2004). However, rhizosheath and bulk soil water content did not differ (Table 2.4), in contrast to Young's (1995) pioneering study of a sandy loam. Both studies used similar methodologies for rhizosheath collection, but there were quantitative differences in soil water contents (SWC~0.046 and 0.044 g g^{-1} in rhizosheath and bulk soil at the end of drying cycle in Table 2.4 versus ca. 0.24 g g^{-1} and 0.18 g g^{-1} in Young 1995) related to the timing of sample collection. In the current study, soil drying had likely already depleted moisture from mucilage, leading to similar water contents between rhizosheath and bulk soils. Thus, higher water retention in rhizosheath compared to bulk soil might be feasible up to a certain degree of soil water depletion.

Root hairs can considerably enhance nutrient uptake by increasing the surface area of the root system (Leitner *et al.,* 2009). Root hair-mediated P uptake has been extensively studied in barley, *Arabidopsis thaliana* and maize, with the presence of root hairs enhancing P content and shoot growth, especially under P deficient conditions (Bates & Lynch, 2000; Brown *et al.,*

2012; Klamer *et al.,* 2019). By comparing rhizosheath formation in WT plants growing in untreated soil and *brb* plants growing in surfactant-treated soil (Fig. 2.3) this study confirms previous results that root hairs enhance P accumulation (Table 2.8). In contrast, *brb* and WT plants accumulated similar amounts of iron, which is mainly supplied by diffusion similar to P (Zuchi *et al.,* 2011). Inconsistencies between studies make apparent that contribution of root hairs to nutrient acquisition strongly depends on nutrient element. Particularly, differences in element distribution in the soil may affect root hairs role on nutrient uptake, as well as plant species and environmental conditions (i.e., soil nutrient availability; Jungk, 2001). Taken together, these results confirm that root hairs facilitate P uptake and biomass accumulation in barley but suggests that this facilitation is not due to enhanced rhizosheath formation.

Similar N content independent of surfactant treatment suggests that surfactant-induced rhizosheath formation did not have a role in N uptake (Table 2.7). Nonetheless, WT plants accumulated more N than *brb* (Table 2.7), in agreement with previous work in wheat, where N accumulation was significantly positively correlated with root hair length and density (Wang *et al.,* 2016). Nitrogen deficiency stimulated root hair length and density in various species (Foehse & Jungk, 1983; Robinson & Rorison, 1987; Vatter *et al.,* 2015), implying that root hairs facilitate N accumulation under low N conditions. However, to our best knowledge, such differences in N uptake have not been previously reported in barley genotypes that either lack or have root hairs. In conclusion, WT plants accumulated more N and P than root hairless mutant *brb*, suggesting that root hairs but not enhanced rhizosheath formation significantly facilitate nutrient uptake, particularly under nutrient deficient conditions.

2.4.3. Plant transpiration and growth

Towards the end of the soil drying cycle, transpiration decreased more acutely in plants growing under low than high nutrient conditions (Fig. 2.1 c). Nutrient starvation likely decreased root hydraulic conductance, affecting water-transport capacity (Trubat *et al.,* 2006; Zhang *et al.,* 2014), in combination with soil drying that further decreased hydraulic conductance leading to a more pronounced restriction of transpiration. Transpiration was higher in WT than in *brb* plants in Days 1- 4 (Fig. 2.1 b), which may be attributed to smaller leaf area of *brb* than WT (Table A-3). Lack of root hairs might compromise nutrient uptake impairing daily leaf growth, hence decreasing transpiration. However, in Days 6-8 combination of nutrient and water stresses eliminated genotypic differences and transpiration declined at the same degree in WT and *brb* (Fig. 2.1 b). On the contrary, nutrient regimes did not affect leaf elongation (Fig. 2.2 c) and started to decline earlier during the drying cycle than transpiration since leaf elongation is more sensitive to tissue water status than transpiration (Hsiao, 1973). Thus, daily leaf growth and plant transpiration were affected differently by combined effects of water stress and nutrient deficiency.

Since nutrient transfer to the shoots is determined by transpiration rate (Junjittakarn *et al.,* 2013), the absence of surfactant effects on transpiration (Fig. 2.1 a) is consistent with the similar nutrient status of plants growing in surfactant-treated and untreated soils (Tables 2.7; 2.8). Surfactant application had no significant effect on daily transpiration (Fig. 2.1 a) consistent with results showing that surfactants do not change the rate of soil drying (Table 2.4). On the contrary, surfactants decreased transpiration of lupin when dried soil was rehydrated. This effect was attributed to reduced soil-to-root hydraulic conductivity caused by surfactants altering root mucilage properties (Ahmed *et al.,* 2017). Several factors might

account for the differences between two studies: watering method (from the top or base of the pot) or plant species. Basal rewatering by capillarity (Ahmed *et al.,* 2017), might hinder rehydration of upper soil layers, due to decreased capillary rise caused by applying surfactants (Mobbs *et al.,* 2012). Moreover, contrasting mucilage properties of lupin (properties similar to maize; behaves as hydrogel that holds more water in the rhizosphere, but with slower rewetting) and barley (acts as surfactants, drying the rhizosphere at smaller suctions; Naveed *et al.,* 2019) may account for transpirational differences. Future work (Chapter 4) will focus on reconciling those discrepancies and determining whether surfactant effects on transpiration depend on irrigation methods or species.

Similar to transpiration, application of surfactant to soil had no cumulative effect on plant growth (Tables 2.2; 2.3). However, *brb* plants growing in surfactant-treated soil and fed with full strength of Hoagland's solution accumulated ca. 25% more biomass than untreated plants. These differences cannot be attributed to enhanced nutrient status since N and P contents did not significantly differ between surfactant treatments (Tables 2.7; 2.8). Other plant physiological mechanisms might be involved, and thus further work is needed to determine how surfactants might promote plant growth.

2.5. Conclusions

Overall, surfactant application to soil amplified rhizosheath development, but this had no cumulative effect on nutrient acquisition. In contrast, the presence of root hairs allowed N and P uptake. Further work will focus on understanding putative effects of surfactant-induced rhizosheath formation on soil – plant water relations (Chapter 3).

Chapter 3: Soil-applied surfactants increase soil water potential and rhizosheath formation in drying soil but have limited impacts on plant water relations of barley (*Hordeum vulgare*).

3.1. Introduction

Surfactant application to soil has been considered as an alternative way of enhancing drought tolerance and irrigation efficiency (Kostka *et al*., 2007; Baratella and Trinchera, 2018). Several studies have reported agronomic benefits of surfactants (especially in drying soils), recording higher biomass accumulation and/or yield. Laboratory and field experiments have reported positive effects of surfactants in diverse crops, such as maize, potato, tomato and alfalfa and soil types (for instance sand and silt loam), under varying soil moistures (Cooley *et al.,* 2009; Chaichi *et al.,* 2015; Jafarian *et al*., 2016; Table B-1). Those beneficial effects are mainly attributed to their ability to facilitate water movement within the soil profile (Daneshnia *et al.,* 2016). However, few studies have investigated the physiological mechanisms regulating surfactant effects on plant growth under water deficit conditions.

Surfactants may affect plant physiological responses by altering soil water retention. At field capacity, water molecules are held against gravity due to matric forces (in meso-pores and micro-pores). As soil dries, water is held increasingly more firmly to soil particle surfaces due to capillary forces, decreasing Ψ_{soil}, which makes soil water less available to plants (O'Geen, 2013). As surfactant molecules reduce the surface tension of water and thereby decrease the contact angle between water molecules and soil particles, applying surfactants may decrease capillary rise by reducing surface tension (Gross *et al.,* 2011; Mobbs *et al*., 2012). Hence, as surfactant-treated soil dries, water might be held less firmly to the surface of the soil particles.

Decreased matric forces following surfactant application should increase soil and plant water potential for a given soil volumetric water content. However, there has been little attempt to determine whether these hypothesised changes in soil moisture release characteristics enhance soil water availability to crops.

Surfactants may also enhance plant drought tolerance by promoting rhizosheath formation, i.e., soil particles that adhere to the root surface due to enmeshment by root hairs and/or mucilage action (Haling *et al.,* 2013). Drought-tolerant grasses growing in sandy desert environments generally have a thicker rhizosheath than drought-sensitive grasses (Hartnett *et al*., 2013). Applying surfactants augmented rhizosheath formation in lupin plants grown in sandy soil (Ahmadi *et al.,* 2017). As discussed in Chapter 2, surfactant molecules were proposed to interact with root mucilage, leading to decreased swelling and increased viscosity of mucilage (cross-linking). When root surfaces retain more mucilage in their vicinity, more soil particles adhere thus enhancing rhizosheath production (Ahmadi *et al.,* 2017). However, this response cannot be generalized as species differences in root exudation and mucilage properties have different effects on soil aggregation (Naveed *et al.,* 2017).

Rhizosheath formation may be effective in providing and maintaining efficient contact at the root-soil interface (Liu *et al.,* 2015; Brown *et al.,* 2017). As transpiration dries the soil, root tissues tend to shrink (Nye, 1994). Root shrinkage leads to root–soil air gaps that sharply increase hydraulic resistance, compromising RWU (Carminati and Vetterlein, 2012; Koebernick *et al.,* 2018). Rhizosheath formation may alleviate high hydraulic resistance of the root-soil interface by minimising air gaps, allowing continued RWU (North and Nobel, 1997; Pang *et al.,* 2017; Basirat *et al.,* 2019). Rhizosheath formation strongly correlates with root hair length and density, as they contribute substantially to soil binding (Haling *et al.,* 2014;

Delhaize *et al.,* 2015). Root hairs improve soil-to-plant hydraulic conductivity, with xylem water potential of the root hairless barley mutant *brb* (with minimal rhizosheath development - George *et al.* 2014) sharply decreasing as transpiration increased, in comparison to its WT (Carminati *et al.* 2017). While this greater hydraulic conductivity was attributed to the presence of root hairs, it could also be due to enhanced rhizosheath formation as described above, as rhizosheath *versus* root hair effects could not be separated in that study. Applying surfactants to genotypes that either have or lack root hairs (and thus differ in rhizosheath formation) would determine whether putative surfactant effects depend on rhizosheath development.

Although surfactant application may potentially enhance plant water relations in drying soil, to our knowledge no comprehensive and integrated evaluation of surfactant effects on soil physical and plant physiological mechanisms facilitating plant water relations has been undertaken. Hence, three experiments determined surfactant effects under soil water deficit and increased evaporative demand conditions. It was hypothesized that (a) Applying surfactants will enhance plant water status and ultimately plant growth, due to increased rhizosheath formation and/or increased Ψ_{soil} in drying soil. (b) Surfactant-enhanced rhizosheath formation would support higher transpiration under elevated evaporative demand.

3.2. Materials and Methods

3.2.1 Soil moisture release properties

A sandy soil was used, as detailed in Section 2.2.1. Two surfactant products were used, a dipropylene glycol methyl ether (H_2Pro AquaSmart, Amega Sciences, Daventry, UK) and a heptamethyl glycidyl oxypropyl trisiloxane polymer with ethox-ylated cocoamine and acetic

acid (H_2Pro FlowSmart, Amega Sciences, Daventry, UK). AquaSmart and FlowSmart are referred to as “Surfactant 1” and “Surfactant 2”, respectively. Sandy soil (4.5 kg) was air-dried and sieved to 10 mm, divided into nine aliquots, and spread in pre-weighed metal trays (500 g each aliquot). Trays were assigned randomly to three different treatments: control (no surfactant), Surfactant 1, and Surfactant 2. Each surfactant (5 mL) made up to 1000 mL of DI water and stored in clear glass laboratory bottles wrapped with aluminium foil under dark conditions. This concentration corresponds to commercial application of surfactants in the field, according to manufacturer recommendations. Each surfactant solution (50 mL) was applied to the soil surface of three trays using a plastic spray bottle to ensure the solution covered the entire area. In control trays, 50 mL of DI water was applied using same method. Soil in the trays was mixed carefully to ensure solutions were homogeneously distributed. Afterwards, sufficient tap water was applied to all trays, based on previous calculations of WHC. Trays were kept at room temperature (approximately 20^{o}C) during the experiment. Trays were weighed and sampled every 30 min. Thirteen consecutive samples were taken during soil drying, aiming to measure Ψ at SWC ranging from 0.22 g g^{-1} to 0.03 g g^{-1}. For each sampling, soil was mixed to homogenise moisture, immediately mounted on clean sample holders, and wrapped in aluminium foil to prevent evaporative losses. Samples were unwrapped and loaded into C52 chambers (Wescor Inc., Logan, UT, USA) and voltages recorded by a microvolt meter (Model HR-33T, Wescor Inc., Logan, UT, USA). Voltage data were converted into Ψ_m based on calibration curves with sodium chloride solutions of known Ψ_s. Samples were oven dried and DW recorded to estimate SWC.

3.2.2. Plant material, growth conditions and application of surfactants

Barley seeds (cv. *Irina*) were germinated and cultivated as detailed in Section 2.2.1. All plants were kept WW for three weeks and maintained in a naturally lit glasshouse compartment with an average daytime temperature of 27 ± 2°C, with a RH of 30–40% and supplementary lighting providing a PPFD at bench height of 250–400 µmol m^{-2} s^{1} for a 12 h day photoperiod (0800 h-2000 h). In Experiments 2 and 3, two barley genotypes, *brb* and its WT, were cultivated as detailed in Section 2.2.1. An Ektron II sensor (HortiMax, Pijnacker, The Netherlands) monitored environmental conditions in the centre of the glasshouse/CE Room. Surfactant solutions (25 mL of each) were applied as detailed in Section 2.2.1. Saucers were placed underneath each pot to capture any drainage water, which was re-incorporated to the pots.

3.2.3. Irrigation treatments

Prior to starting treatments, plants were watered at 1600 h until water drained from the base of the pot, and next morning were weighed on a precision balance to 0.1 g (Scout Pro Portable balance, Ohaus, Switzerland) to establish pot capacity. The surface of each pot was covered with black duct tape to limit evaporation losses. Pots were allocated randomly to two irrigation treatments: well-watered (WW) or drying (D). Well-watered plants were watered daily, by replacing transpirational losses (determined gravimetrically) or water was withheld for five days (D plants). All pots were weighed daily to calculate plant water uptake and three plants of each treatment were harvested daily during a drying cycle that lasted five days (Experiment 1). In Experiment 2, treatments were similar, but water was withheld from D plants for six days before rewatering, and plants were exposed to three drying/rewetting cycles. Stressed plants (D) were harvested at the end of each cycle (three plants of each

genotype and treatment), while WW plants were harvested at the end of the second cycle. In Experiment 3, irrigation treatments were similar to Experiment 1, but a single drying cycle lasted nine days and plants were harvested on Days 0, 5 and 9 (three plants of each genotype and treatment per day).

3.2.4. Transpiration, plant water status, biomass, and soil moisture

Transpiration was monitored gravimetrically, as described in Section 2.2.2, daily. Elongation of the youngest leaf on the main tiller was monitored daily using a flexible ruler. Leaf elongation rate was calculated as difference in leaf length between successive days, as detailed in Section 2.2.2. These measurements were made between 1000 h-1300 h. At harvest, the whole plant was sealed into a black plastic bag to prevent transpiration (Wang *et al.,* 2008) and placed in the dark for at least five hours. Preliminary experiments established that five hours were needed for Ψ to stabilise following plant enclosure (Fig. B-2). After equilibration, a fully expanded leaf of the main tiller was excised, placed in a plastic bag and moved to the laboratory to measure base water potential (Ψ_{base}) with a Scholander-style pressure chamber (Soil Moisture Equipment Corp., Santa Barbara, CA, USA). Total leaf area was measured using a leaf area meter (Li-3100 Leaf Area Meter, Li-Cor Inc., Lincoln, Nebraska, USA). Roots were separated from the soil and cleaned carefully. All tissues were dried at 80°C for at least 72 h to obtain dry biomass weight. Bulk soil from each pot was weighed and oven dried at 105°C to constant mass. Soil water content was calculated as: SWC = (FW – DW)/DW.

3.2.5. Rhizosheath formation and root length measurements

Rhizosheath formation was determined only in D plants, as roots in wet soil produce weaker and less coherent rhizosheaths (Watt *et al.,* 1994), as detailed in Section 2.2.3. Root length was analysed and measured as detailed in Section 2.2.3.

3.2.6. Transpiration responses to evaporative demand

Transpiration rate (E) responses to elevated vapour pressure deficit (VPD) were measured using a whole plant gas exchange system. Details about construction and function of this system were previously published (Jauregui *et al*., 2018). Prior to each measurement sequence, each plant was acclimated for approximately 30 min to the light conditions of the chamber. Two high-pressure sodium lamps (Son-T, Philips, Amsterdam, Netherlands) providing 450 μmol m^{-2} s^{-1} photosynthetically active radiation (PAR) were directly above the plants. The aboveground part of plant was placed in the chamber and the shoot base sealed using a polymeric sealant (Qubitac, Qubit Systems, Kingston, Ontario, Canada). Air relative humidity (i.e., the ratio of the current absolute humidity to the highest possible absolute humidity) inside the chamber was initially set to ∼70% by passing dry air (Relative Humidity, RH=11%; [CO_2] =420 ppm) through a humidifier compartment. Differences in [CO_2] and [H_2O] between air entering and exiting the chamber were measured and recorded using an infra-red gas analyser (Model LI-6400XT, Li-Cor Inc., Lincoln, Nebraska, USA). Once [CO_2] and [H_2O] differences were steady for more than 10 min (typically after 50-60 min), values were logged every 15 s for 5-10 min, and averaged. Then the chamber was opened (for less than 30 s) and a fully expanded leaf excised to measure Ψ_{leaf}. After closing the chamber again, gas exchange was allowed to stabilise again (typically 10 min), and then RH inside the system was reduced to 50% by introducing a mixture of dry and humidified air to the chamber. After the following

RH level was achieved (typically 3 min), plant gas exchange was allowed to stabilise (typically 20-30 min) and [CO_2] and [H_2O] values logged again. Each plant was exposed to four sequentially decreasing humidity levels achieved by increasing the ratio of dry to humid air (70%, 50%, 25%, 11%), approximately corresponding to VPD values of 1 KPa, 1.5 KPa, 2 KPa and 3 KPa (measurements taken at ambient temperature of ~26^{o}C). The leaf removed to measure Ψ_{leaf} accounts for approx. 4%-6% of total leaf area. Leaf water potential was determined again at the highest VPD level. Total leaf area was measured using a leaf area meter (Model Li-3100, Li-Cor Inc., Lincoln, Nebraska, USA).

3.2.7 Statistical analysis

Data were tested for normality using Shapiro-Wilk test. Statistical differences ($P<0.05$) in leaf area, dry biomass, Ψ_{base}, rhizosheath, root length and SWC between genotypes, irrigation and surfactant effects were determined by multi-factorial ANOVA. Whenever a factor was significant, means were differentiated using Tukey's multiple comparison test ($P<0.05$). Differences in transpiration and leaf elongation rate at different time points during the drying cycle in Experiments 1 and 2 were analysed using repeated measures ANOVA, with time as within-subjects factor and genotype (in Experiment 2), treatment and irrigation as between-subject factors. Surfactant effects on the relationship between both Ψ_{soil} and Ψ_{base} and SWC were tested using ANCOVA, using SWC as the covariate. Two-way ANCOVA assessed whether the genotype and surfactant treatments differed in rhizosheath production, with root length as the covariate. Repeated measures ANOVA determined effects of time (days after withholding water), VPD, genotype, surfactant treatment, using VPD as within-subjects factor and time, genotype and surfactant treatment as between-subjects factors (Experiment 3). All

statistical analyses were performed using SPSS 25 (SPSS Statistics 25, IBM, Armonk, New York, USA).

3.3. Results

3.3.1. Soil and plant water status

Well-watered soils had a similar Ψ_{soil} irrespective of surfactant treatment, but Ψ_{soil} of untreated soils declined more sharply as the soil dried. At any given SWC, Ψ_{soil} of surfactant-treated soils was significantly higher than untreated soil (Fig. 3.1) as indicated by a significant Treatment x SWC interaction (P=0.003). Both surfactants had similar effects on Ψ_{soil}.

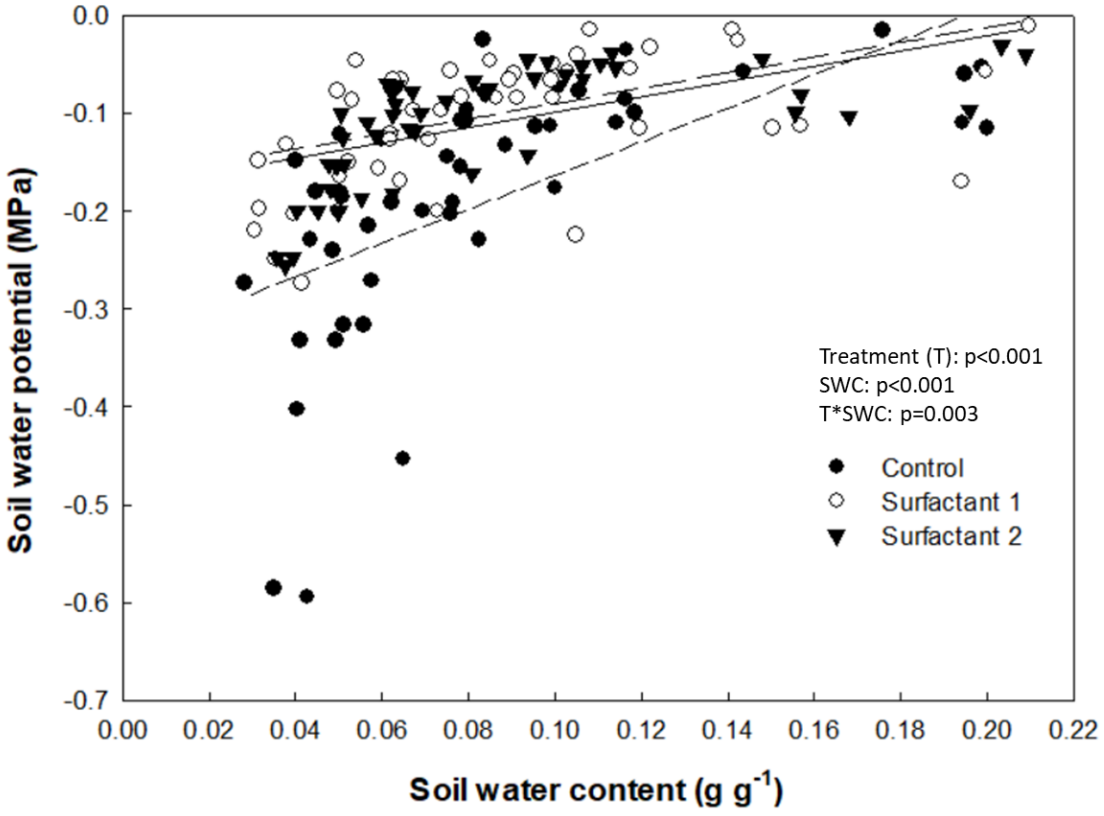

Figure 3.1: Soil moisture release curves of sandy soil without (black circles) and with Surfactants 1 and 2 (hollow circles and triangles, respectively) added. Each point is an individual soil sample, P values from the ANCOVA are reported, and fitted linear regressions lines (medium-dashed line, long-dashed line and solid line for untreated soil and soil treated with Surfactants 1 and 2, respectively) are depicted.

Surfactant application did not change the rate of soil drying in Experiment 1 (Fig. 3.2 a). Plants grown in surfactant-treated soils maintained a higher Ψ_{base} than plants grown in untreated soils as the soil dried (Fig. 3.2 b). For the driest soil (0.05 g g^{-1}), Ψ_{base} of plants grown in surfactant-treated soil was ca. 0.1 MPa higher than plants grown in untreated soil, while Ψ_{base} did not significantly differ between treatments in wet soil (0.15 g g^{-1}). While surfactant application did not alter the rate of soil drying, it allowed plants to maintain a higher Ψ_{base} in dry soil.

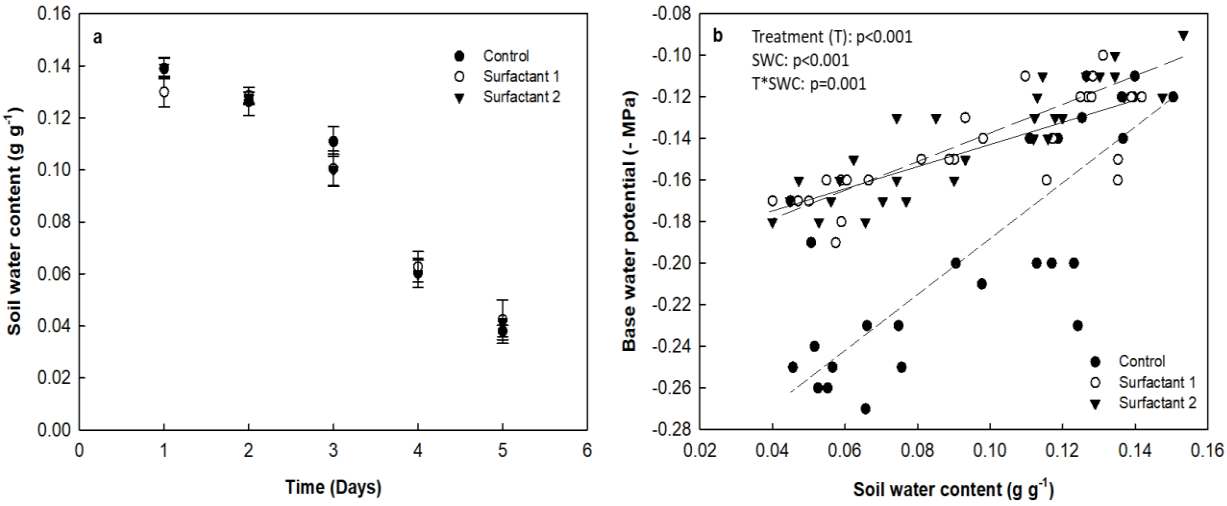

Figure 3.2: (a) Changes in soil water content after withholding water from Day 0. Symbols are means ± SE of 6 replicates (Experiment 1). (b) Base water potential in response to soil drying in soil without (filled circles) and with Surfactants 1 and 2 (hollow circles and filled triangles, respectively) added (Experiment 1). Each point is an individual plant, P values from the ANCOVA are reported, and fitted linear regressions lines (medium-dashed line, long-dashed line and solid line for untreated soil and soil treated with Surfactants 1 and 2, respectively) are depicted.

In Experiment 2 using both *brb* and WT plants, Ψ_{base} was significantly higher (by 0.06 MPa averaged across both genotypes) at the end of each drying cycle, when plants were grown in surfactant-treated than untreated soils (Fig. 3.3). However, no significant genotypic differences or Genotype x Treatment interaction were observed for Ψ_{base} (Fig. 3.3). Thus, surfactant application improved plant water relations when both genotypes were in drying soil.

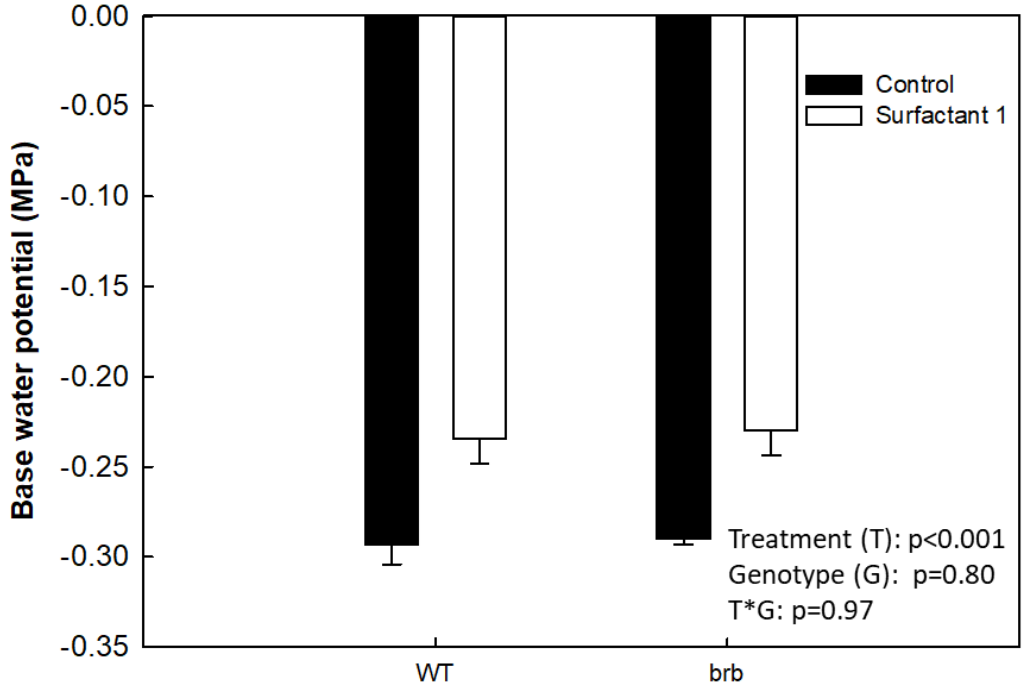

Figure 3.3: Base water potential of water-stressed plants (harvested at the end of each cycle). Black bars: plants were growing in control (untreated) soil; White bars: plants were growing in soil treated with Surfactant 1 (Experiment 2). Bars are means ± S.E. of 9 replicates and P values reported for genotype, surfactant treatment and their interactions.

3.3.2. Transpiration and plant growth and rhizosheath formation

In Experiment 1, biomass accumulation and leaf area did not significantly differ between surfactant treatments (Fig. 3.4 a, b). Soil drying substantially inhibited leaf elongation rate on Day 6, with fluctuations between drying cycles, in Experiment 2 (Fig. 3.5 a, b, c). No significant differences were found in leaf elongation between surfactant treatments (no significant Day x Treatment interaction; P=0.29, P=0.29, P=0.20, for 1st, 2nd and 3rd drying cycles, respectively), in Experiment 2. Soil drying decreased transpiration after Day 4, with similar results for three consecutive drying cycles (Experiment 2; Fig. 3. 6 a, b, c). Surfactant application had no significant effect on transpiration in any of the three drying cycles (no significant day x treatment interaction; P=0.18, P=0.38, P=0.15, for 1st, 2nd and 3rd drying cycles, respectively). There was no significant effect of genotype on transpiration and leaf elongation at any time in Experiment 2. Rewatering restored transpiration and leaf elongation within 24 hours.

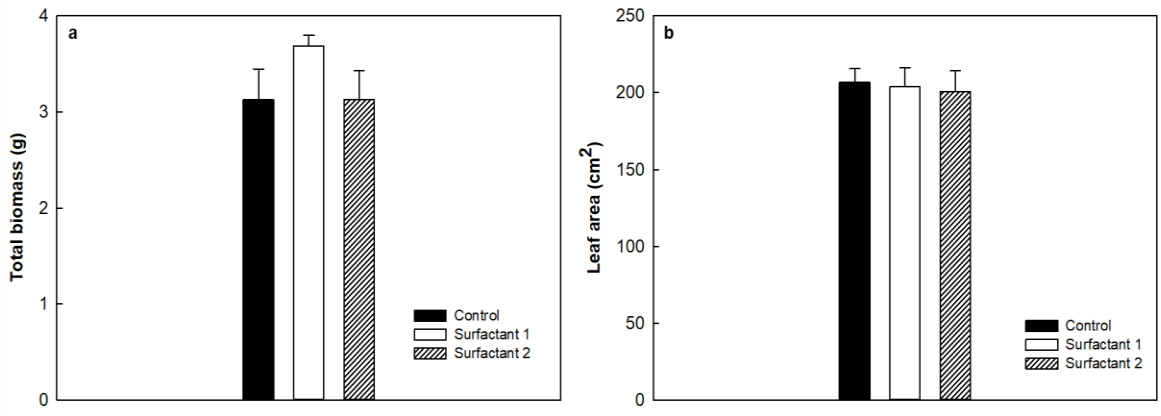

Figure 3.4: (a) Total biomass and (b) whole plant leaf area in response to soil drying in Experiment 1 (Day 5). Black bars: plants were growing in untreated (control) soil; White bars: plants were growing in soil treated with Surfactant 1; White/striped bars: plants were growing in soil treated Surfactant 2, respectively. Bars are means ± S.E of 4 replicates. There was no significant (P< 0.05) impact of surfactant treatment on either variable.

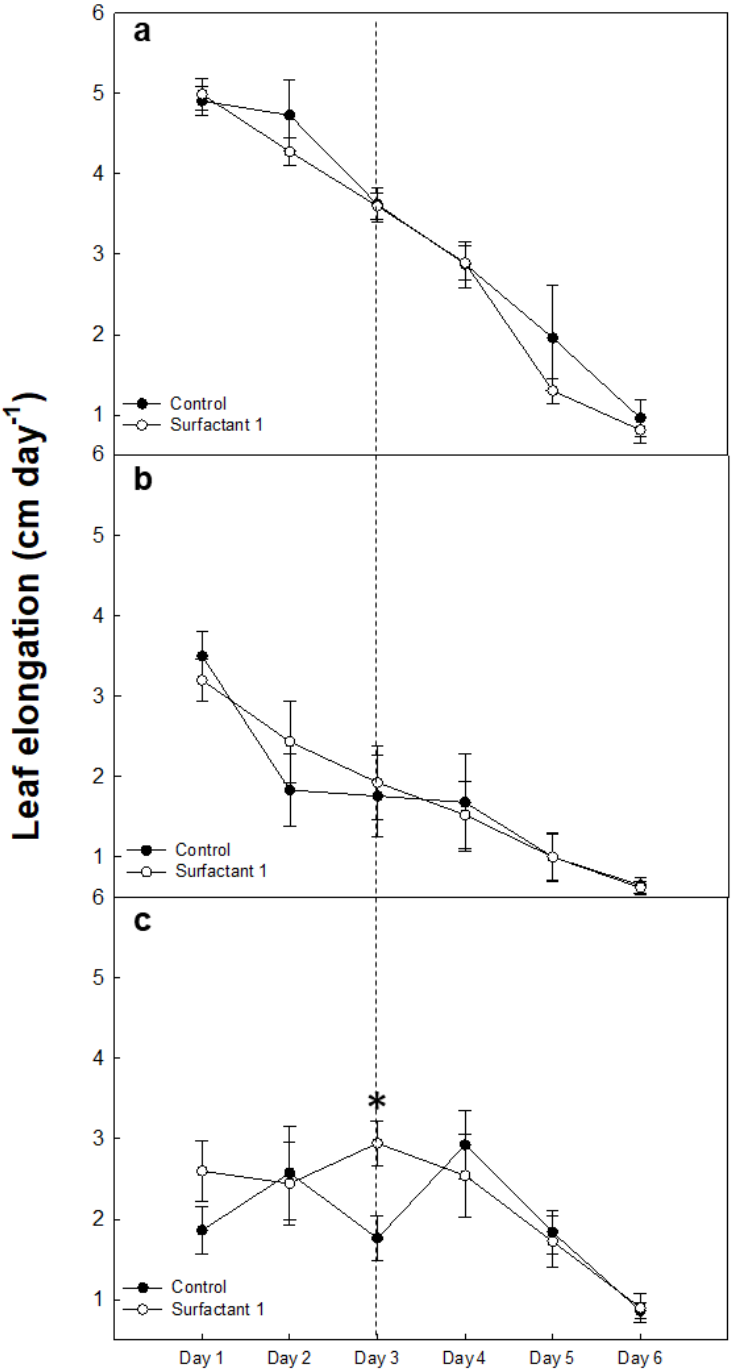

Figure 3.5: Leaf elongation (a, b, c; drying cycle 1,2,3 respectively; means ± S.E of 10 replicates) of the youngest leaf (a different leaf was measured in Days 1 and 4; vertical dashed lines) in response to drying soil over 3 drying sequential drying cycles (Experiment 2). Plants were rewatered between cycles. Plants were growing in control (untreated) soil (filled circles) and soil treated with Surfactant 1 (hollow circles), respectively.

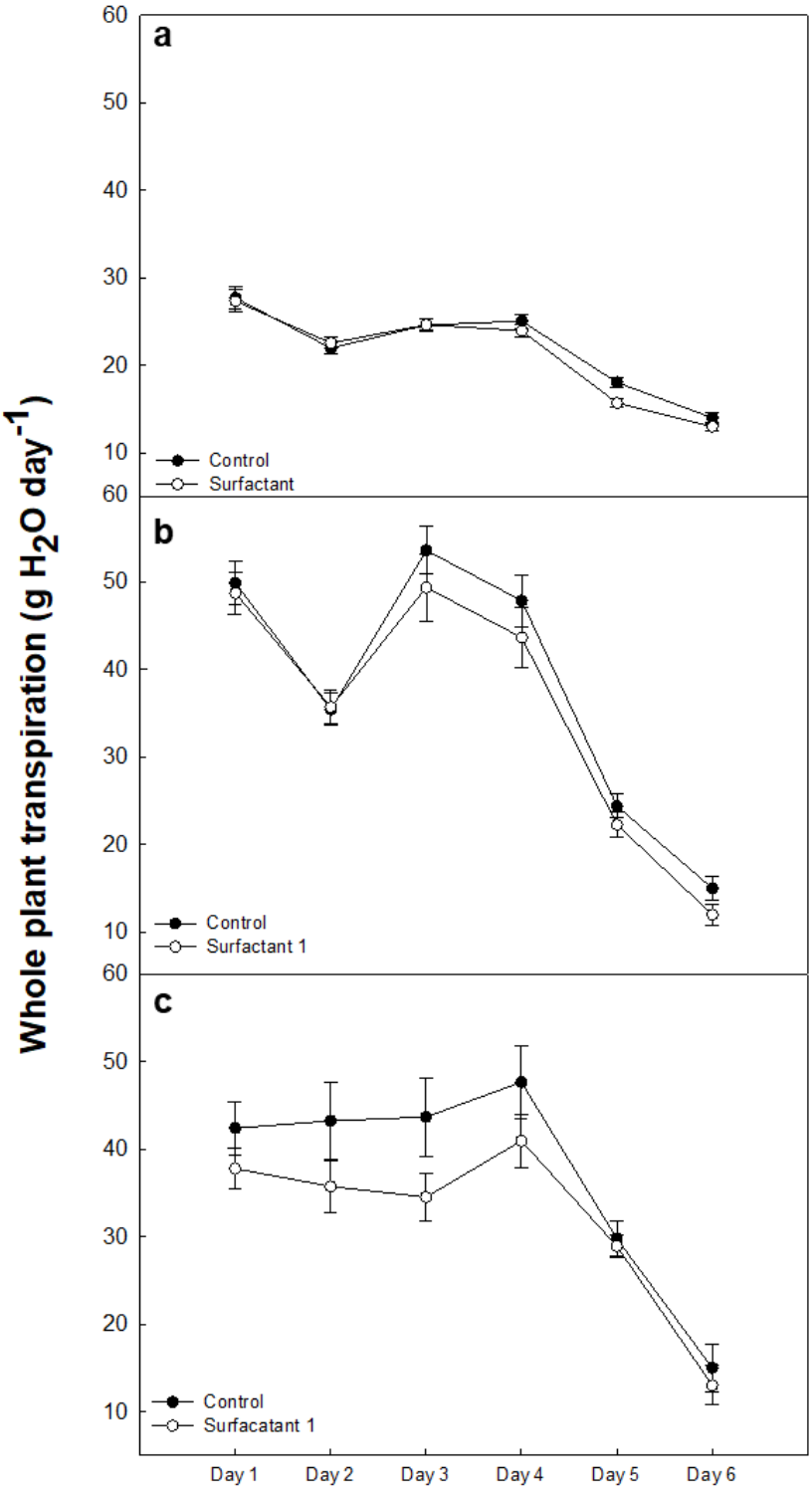

Figure 3.6: Whole plant transpiration in response to drying soil (a, b, c; drying cycle 1,2,3 respectively; means ± S.E of 10 replicates) over 3 drying sequential drying cycles (Experiment 2). Plants were rewatered between cycles. Plants were growing in control (untreated) soil (filled circles) and soil treated with Surfactant 1 (hollow circles), respectively.

In both Experiments 2 and 3, rhizosheath weight significantly increased with root length and was higher in WT than *brb* plants (Fig. 3.7 a, b). In both experiments, applying Surfactant 1 significantly increased rhizosheath formation of both WT and *brb* plants (Fig. 3.7 a, b), by an average of 60% and 50% across genotypes, respectively. No significant Genotype x Treatment interaction was found, suggesting additive effects of roots hairs and surfactants on rhizosheath formation. Applying Surfactant 1 to *brb* plants restored rhizosheath weight to the levels of untreated WT plants.

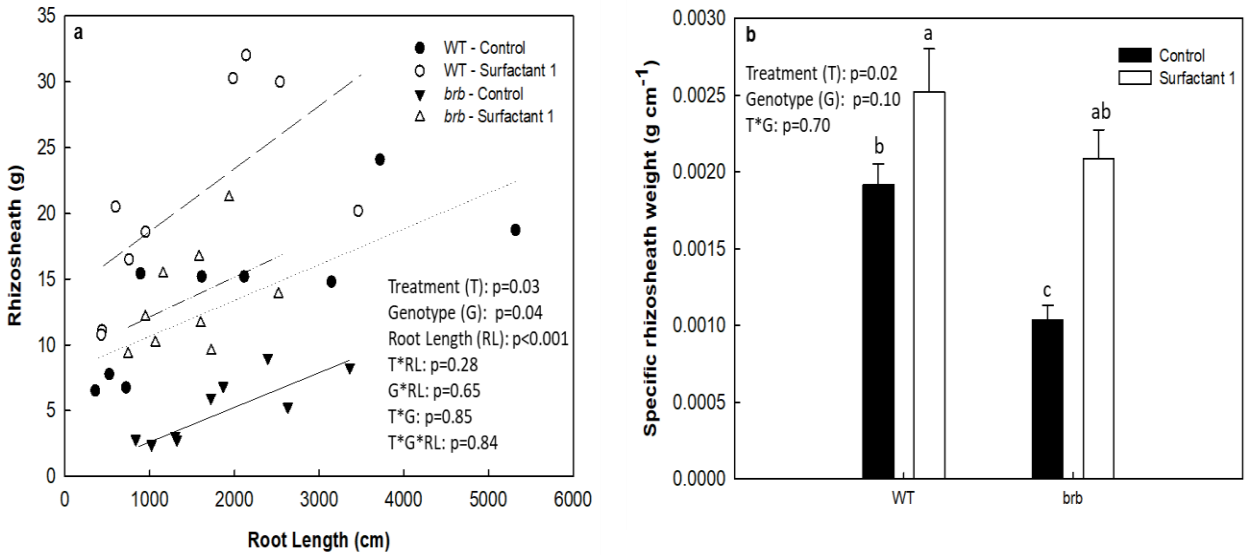

Figure 3.7: (a) Total rhizosheath weight per plant plotted against its total root length (harvested at the end of each cycle; Experiment 2). WT plants were growing in control (untreated) soil (filled circles) and soil treated with Surfactant 1 (hollow circles), *brb* plants are growing in control (untreated) soil (filled triangles) and soil treated with Surfactant 1 (hollow triangles). Fitted linear regressions are depicted. P values are reported for genotype, surfactant treatment, root length and their interactions. (b) Specific rhizosheath weight (Experiment 3). Black bars: WT plants were growing in control (untreated) soil; Grey bars: WT plants are growing in soil treated with Surfactant 1; Grey/striped bars: *brb* plants were growing in control (untreated) soil; Light grey bars: *brb* plants were growing in soil treated with Surfactant 1. Bars are means ± S.E. of 6 replicates and P values of surfactant treatment, genotype and their interaction are reported.

3.3.3. Genotype and surfactant effects on transpiration rate (E) response to elevated VPD

Since surfactant application had minimal effects on transpiration when plants slowly dried the soil, the effects of abrupt changes in transpiration rate were explored. Transpiration rate (E) increased with VPD, similarly when plants were grown in untreated and surfactant-treated soils, respectively (Fig. 3.8 a). No genotypic differences in the E vs VPD relationship were observed ($P>0.05$). Surfactant did not significantly alter the response of E to elevated VPD (Table 3.1), even though WW plants growing in surfactant-treated soil tended to transpire slightly more (by an average of 14% across all four VPDs) than plants growing in untreated soil (Fig. 3.8 a). Thus, genotype and surfactant had no impact on plant transpiration under well-watered conditions.

Plants in drying soil showed similar patterns (Fig. 3.8 b, c), where E increased with VPD, but absolute transpiration rates were lower than WW plants (by 25% and 51% after five and nine days of soil drying respectively, averaged across genotypes and treatments). Surfactant application did not alter the E vs VPD response after either five or nine days of withholding water (Fig. 3.8 b, c). Generally, surfactant application had minimal effects on transpiration responses to evaporative demand.

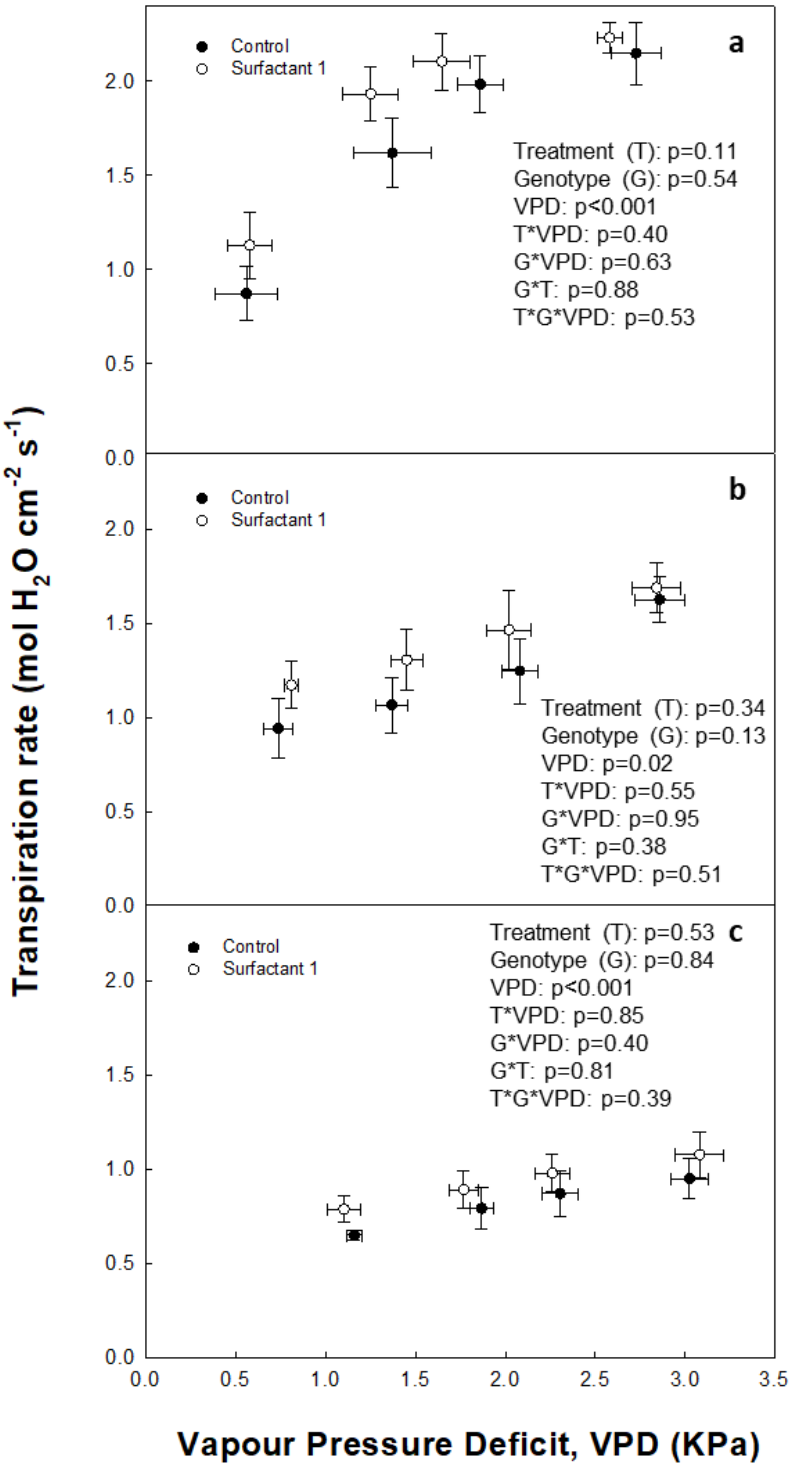

Figure 3.8: Response of transpiration rate to elevated VPD under well-watered conditions (a), after withholding water for five (b) and nine (c) days (pooling data across both WT and *brb* plants) (Experiment 3). Plants were growing in control (untreated) soil (filled circles) and soil treated with Surfactant 1 (hollow circles) respectively. Each symbol is mean ± S.E. of 6 replicates and P values of surfactant treatment, genotype and VPD and their interactions are reported.

Leaf water potential decreased as the plants dried the soil, from -0.15 MPa (averaged across both genotypes and VPDs) on Day 0 when plants were in well-watered soil to -0.51 MPa on Day 9 when plants were in dry soil. Elevated VPD decreased Ψ_{leaf} on all sampling days ($P=0.028$, $P<0.001$, $P=0.001$, for Days 0, 5 and 9, respectively) by 0.03 MPa, 0.12 MPa and 0.11 MPa, respectively (Fig. 3.9). Genotype did not alter the Ψ_{leaf} response to elevated VPD on any measurement occasion (Fig. 3.9). Surfactant application had no impact on Ψ_{leaf} at either VPD when plants were in well-watered or very dry soil (Fig. 3.9 a, c). However, after 5 days of soil drying (SWC in both treatments was ca. 0.08 g g^{-1}), surfactant-treated plants had a significantly higher Ψ_{leaf} (ca. 0.1 MPa) than control plants (Fig. 3.9 b). Thus, surfactants increased Ψ_{leaf} when plants were growing in moderately dry soil and exposed to low VPD, but generally surfactant application did not affect Ψ_{leaf}.

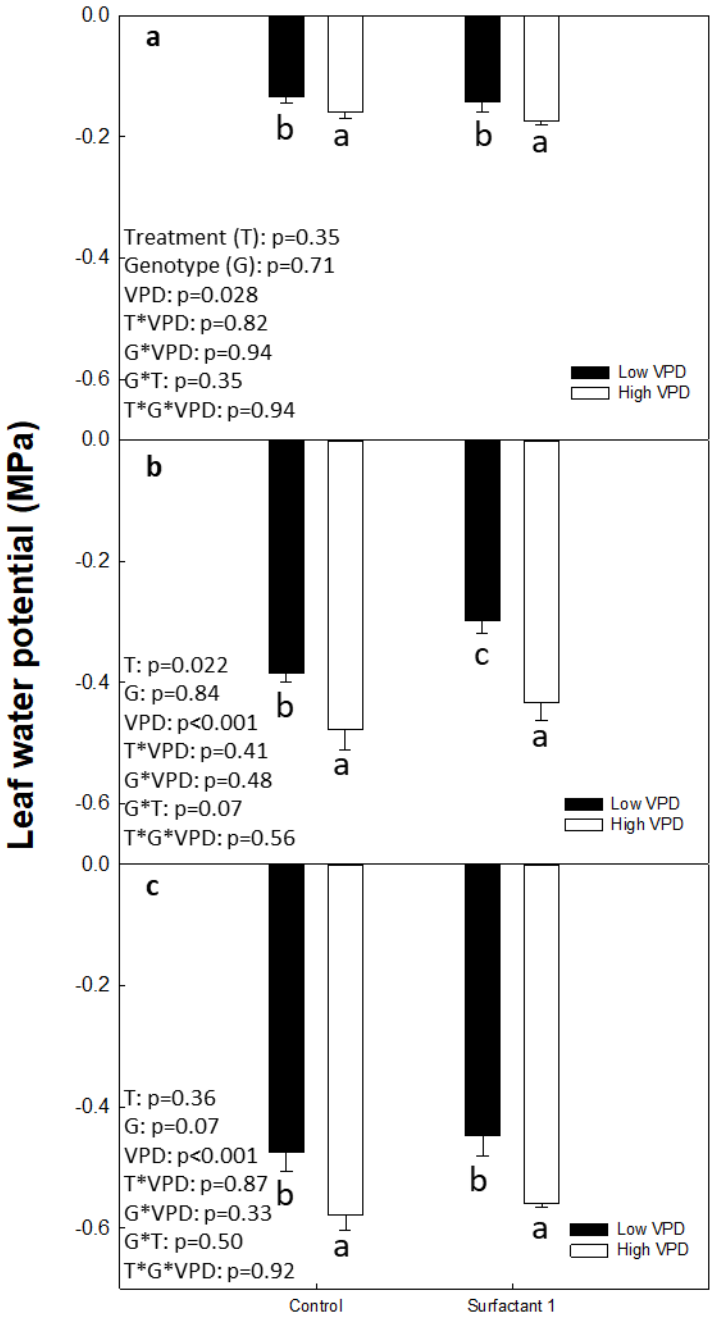

Figure 3.9: Leaf water potential on different days after ceasing irrigation (0(a), 5(b), 9(c)), at the lowest (black bars) and highest (white bars) VPD level (Experiment 3). Bars are means ± S.E. of 6 replicates (pooling data across both WT and *brb* plants) and P values of surfactant treatment, genotype, VPD and their interactions are reported.

Table 3.1. Repeated measures ANOVA (P values reported) examining the effects of time (days after withholding water, D), vapour pressure deficit (VPD), genotype (G) and surfactant treatments (T) on transpiration rate, photosynthesis and leaf water potential. Significant effects (P<0.05) are in bold text.

	Transpiration	**Photosynthesis**	**Leaf water potential**
Day (D)	**<0.001**	**0.02**	**<0.001**
VPD	**<0.001**	**<0.001**	**<0.001**
Treatment (T)	0.14	0.18	0.10
Genotype (G)	0.72	0.71	0.39
VPD*D	**<0.001**	**0.04**	**<0.001**
VPD*T	0.85	0.53	0.31
VPD*G	0.37	0.8	0.13
VPD*D*T	0.87	0.45	0.64
VPD*D*G	0.91	0.5	0.48
VPD*T*G	0.56	0.23	0.67
VPD*D*T*G	0.99	0.62	0.69
D*T	0.95	0.86	0.15
D*G	0.40	0.36	0.27
T*G	0.37	0.69	0.37
D*T*G	0.47	0.92	0.23

Transpiration rate was poorly linearly related to Ψ_{leaf} for plants growing in untreated soil (R^2=0.11) and surfactant-treated soil (R^2=0.20) respectively, without differences between the two slopes (Fig. 3.10). Since the slope of the relationship between transpiration rate and Ψ_{leaf} is interpreted as plant resistance (Dube *et al.,*1975; Hayat *et al.,* 2020), applying surfactant did not alter plant resistance (Fig. 3.10).

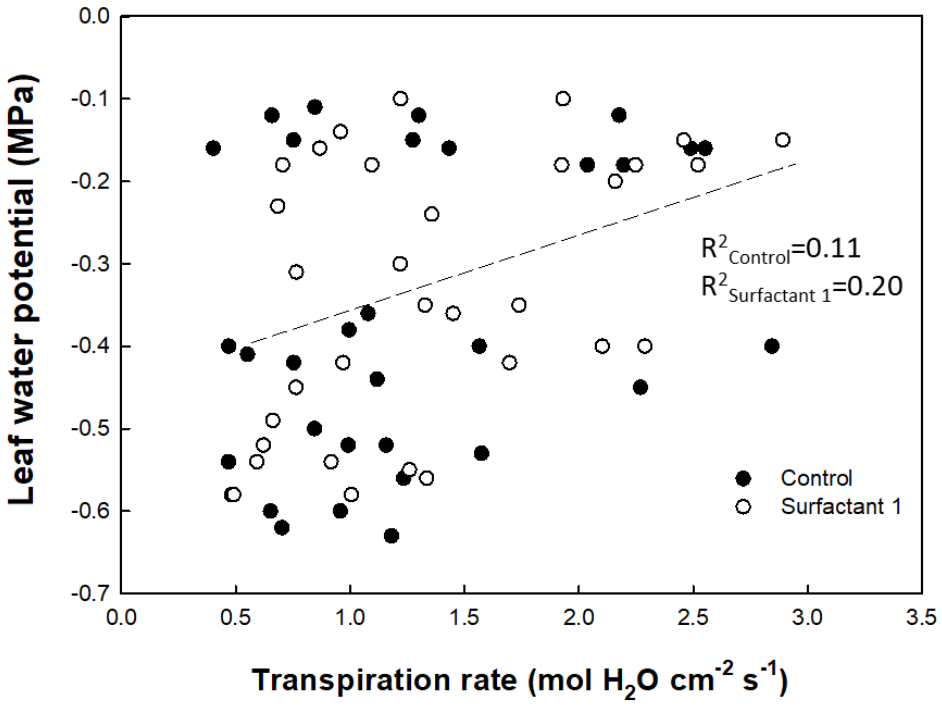

Figure 3.10: Relationship between transpiration rate and leaf water potential for plants growing in soil without (filled circles) and with Surfactant 1 (hollow circles) added (Experiment 3). Each point is an individual plant and fitted linear regression line is depicted.

3.4. Discussion

Surfactants enhanced rhizosheath formation as reported previously (Ahmadi *et al.,* 2017) and increased Ψ_{soil} for the same SWC in dry soil. By assessing surfactant effects on plant water relations in genotypes with different rhizosheath formation, this study demonstrated that surfactants increased soil water potential as the soil dried (Fig. 3.1; 3.2 b) but had limited impact on water relations of plants grown in surfactant-treated soil (Fig. 3.8; 3.9). Under high evaporative demand, neither surfactant nor genotype effects on rhizosheath formation (Fig. 3.7) altered transpiration responses to elevated VPD (Table 3.1), consistent with longer-term experiments demonstrating that surfactant application did not change the rate of soil drying (Fig. 3.2 a here; Lyons *et al*. 2009). Moreover, surfactant application had no cumulative effect on leaf area and biomass accumulation (Fig. 3.4; Table B-3), even though plants growing in surfactant-treated soils maintained higher Ψ_{base} in drying soil compared those grown in untreated soil (Fig. 3.2 b). Previous reports of surfactants improving plant growth in drying soil (Chaichi *et al.,* 2015; Daneshnia *et al*., 2016) are consistent with hydraulic mediation of leaf expansion (Tardieu *et al.* 2010), but these putative effects were not detected in our experiments. Surfactant application had no long-term effect on daily leaf growth in drying soil (Fig. 3.5 a, b, c), consistent with observations that soil drying decreased leaf expansion independent of leaf water status (Passioura, 1988). Surfactant effects on plant water relations (albeit statistically significant on one occasion) were likely restricted to only part of the drying cycle (Fig. 3.9 b) and had no cumulative effect on plant growth. Nevertheless, the mechanisms by which surfactant application enhance soil-plant water relations are discussed below.

3.4.1. Surfactant effects on soil water relations and rhizosheath formation

Applying surfactants to soil likely decreased the surface tension and thus capillary forces (Wiel-Shafran *et al.,* 2006; Gross *et al.,* 2011). Although these studies did not determine soil moisture release curves, lower capillary forces would imply that water molecules are retained less firmly to soil particles, thereby making soil water more available to plants in drying soil. Whether these changes affected plant water availability was assessed using two independent methods (thermocouple psychrometry of soil samples, measuring Ψ_{base} of plants grown in such soils). Since Ψ_{base} measures plant water potential when it is in equilibrium along the soil-plant-continuum in the absence of water fluxes, it can be considered as a proxy of Ψ_{soil} in the root-zone (Sellin, 1999). Both soil moisture release curves (Fig. 3.1) and measuring Ψ_{base} (Fig. 3.2 b, 3.3) indicated that surfactants increase Ψ_{soil} in drying soils.

Whether surfactants improve plant water relations by enhancing rhizosheath formation (Fig. 3.7) was evaluated by measuring Ψ_{base} in genotypes with (WT) and without (*brb*) root hairs, that differed in rhizosheath development. Previous work proposed that cross-linking between root mucilage and surfactant molecules enhance rhizosheath formation (Ahmadi *et al.,* 2017). Root hairs also promoted rhizosheath formation (Brown *et al.,* 2017) in WT plants, presumably by enmeshing soil particles (Koebernick *et al.,* 2018), but this effect did not interact with that induced by surfactant application (Fig. 3.7 a). Both genotypes had the same Ψ_{base} (Fig. 3.3) despite greater rhizosheath development in WT plants (Fig. 3.7), indicating that plant water relations were not enhanced by greater rhizosheath formation in non-transpiring plants. Nevertheless, it was important to evaluate plant water relations when plants were exposed to drying soil and/or increased evaporative demand, and thus transpiring.

3.4.2. Surfactant effects on plant water relations

Surfactant application had no consistent effect on daily transpiration during drying and re-wetting cycles (Fig. 3.6 a, b, c), suggesting that rhizosheath formation did not affect water uptake. In contrast, applying surfactant decreased transpiration (compared to untreated controls) after lupin plants grown in sandy soil were rewatered, which was attributed to reduced soil-to-root hydraulic conductivity caused by surfactants changing mucilage properties (Ahmed *et al.,* 2017). Possibly changes in rhizosheath size or chemical composition (mucilage properties) of lupin might magnify effects of surfactant-rhizosheath interactions on plant water uptake. However, these effects cannot be generalised to other species or other surfactants, as surfactants did not change transpirational responses to rewatering in barley (Fig. 3.6 a, b, c). To reconcile this discrepancy, further studies are needed to determine how root exudation and surfactant application interact to determine rhizosphere water repellency.

Moreover, enhanced rhizosheath formation did not alter transpiration response to VPD (Fig. 3.8), independent of whether surfactant application or the presence of root hairs boosted rhizosheath mass. While there were no genotypic differences in daily transpiration between *brb* and WT plants grown at relatively stable VPDs (Dodd & Diatloff, 2016) or in momentary transpiration response to abrupt changes in VPD (Table 3.1), under some circumstances individual *brb* plants were unable to sustain transpiration (Carminati *et al.,* 2017), suggesting that root hairs (and associated rhizosheath development) are important in maintaining water uptake. Although barley root hairs increase soil-to-root hydraulic conductivity (Carminati *et al.,* 2017) and surfactant application maintains Ψ_{soil} higher as the soil dried (Fig. 3.1; 3.2 b), neither were required to sustain transpiration as VPD increases in our experiments. This

focuses attention on hydraulic conductance upstream of roots in the soil-plant-atmosphere continuum in mediating transpiration, with effects of rhizosheath development on plant physiological responses determined by changes in soil hydraulic conductivity as the soil dries (Hayat *et al*., 2020).

3.5. Conclusions

Taken together, applying surfactants increased soil water availability in drying soil, by decreasing the matric forces holding soil water. Additionally, surfactants augmented rhizosheath formation, but these effects did not affect soil and plant water availability or leaf growth and transpiration responses to soil drying. Furthermore, neither surfactant nor root-hair mediated changes in rhizosheath formation affected transpiration response to abrupt changes in atmospheric VPD. Further studies are needed to investigate the long-term effect of different surfactants when plants are grown in different substrates, especially as the soil dries, since surfactants increase soil water availability.

Chapter 4: Surfactants and rehydration technique do not affect leaf gas exchange response of different cereal species to rewatering.

4.1. Introduction

Surfactants have been widely used in the turfgrass industry as adjuvants that reduce SWR, enhancing turfgrass quality and colour (Kostka *et al.,* 2007; Alvarez *et al.,* 2016). A few studies demonstrated agronomical benefits of surfactants as enhanced biomass accumulation, plant height or yield in diverse crop species, especially under water-limiting conditions, and increased water use efficiency (WUE; the ratio of biomass accumulation expressed as CO_2 assimilation or total biomass/yield, to water consumed, expressed as transpiration) (Sinclair *et al.,* 1984; Chaichi *et al.,* 2015; Daneshnia *et al.,* 2016). Plant growth and gas exchange are inextricably linked. Transpiration through stomata accounts for more than 95% of water losses, however, is essential for plant cooling since the loss of water vapour decreases leaf temperature (Cook *et al.,* 1964). Stomatal opening also allows CO_2 uptake for photosynthesis, which is essential for plant growth and development (Buckley, 2005; Galmés *et al.,* 2007). However, whether soil-applied surfactants affect leaf gas exchange has received little attention.

Previous work has shown that rewatering dried soil with dilute surfactant solutions (as opposed to water controls) decreased lupin transpiration (Ahmed *et* al, 2017). This response was attributed to reduced soil-to-root hydraulic conductivity, with cross-linking between mucilage and surfactant molecules increasing mucilage viscosity. Limited mucilage swelling after rewatering decreases soil-to-root hydraulic conductivity, that may explain lower transpiration of plants irrigated with surfactant solution than controls (Ahmed *et al.,* 2017).

Nevertheless, transpirational responses to rewatering may differ between rehydration techniques, different species and whether surfactants are applied to the soil.

Rewatering from the top or base of the pot may alter soil rehydration kinetics, and therefore affect transpirational responses differently. In previous experimental work (Fig. 2.1), surfactant application to soil had no consistent effect on barley daily transpiration during drying and rewetting cycles, when irrigation was applied to the top of the soil column. In contrast, when lupin was rewatered by capillarity from the base of the pot, applying surfactant delayed the recovery of plant transpiration (Ahmed *et al.,* 2017). By decreasing the surface tension of water, surfactants may inhibit capillary rise (Gross *et al.,* 2011), thereby hindering rehydration of upper soil layers, restricting plant water uptake and delaying the recovery of transpiration. Further investigation is needed to determine whether surfactant affects gas exchange according to whether plants are rewatered by surface or sub-surface irrigation.

Soil rehydration kinetics may be affected differently by applying surfactants to soil (Chapters 2, 3) or irrigating with surfactant solutions during basal rewatering (Ahmed *et al.,* 2017). Putative differences in soil rehydration following basal rewatering could affect transpiration recovery, but this has not been investigated. To reconcile apparent discrepancies between studies, it is essential to determine whether soil moisture recovery following basal rewatering significantly differs depending on whether surfactant is previously incorporated in the soil or applied as an irrigation solution.

Moreover, species differences in mucilage properties may alter surfactant effects on plant transpiration. Root exudates of barley differed from those of maize (and lupin), with barley rhizodeposits acting as surfactants, drying the rhizosphere at smaller suctions. In contrast,

maize rhizodeposits and mucilage from chia seeds behaved as hydrogels that held more water in the rhizosphere, but with slower rewetting (Naveed *et al.,* 2019). Therefore, surfactants may affect soil-to-root hydraulic conductivity and hence transpiration differently, depending on mucilage properties. However, it remains unknown whether the root mucilage properties of different species interact with surfactants to influence transpiration response to soil rehydration.

To our knowledge, no comprehensive and integrated evaluation of surfactant effects on gas exchange has been undertaken, utilizing plant species with contrasting mucilage properties, and applying different rehydration techniques. Hence, this Chapter reports the results of a fully factorial experiment that varied species (barley *versus* maize), application of surfactant (control and surfactant-treated soil) and rehydration method (water applied to the top of the soil column *versus* from the base by partially submerging the pot in water). This study aimed to investigate surfactant and root mucilage effects on whole plant gas exchange, following rewatering, using two different rehydration methods. It was hypothesised that (a) applying surfactant will inhibit rehydration of the soil column, thereby limiting whole plant gas exchange, only when plants are rewatered by partially submerging the pot in water, and (b) species differences in mucilage properties could explain different responses of gas exchange to rehydration; with exogenous surfactants decreasing maize gas exchange but not affecting barley gas exchange following rewatering.

4.2. Materials and Methods

4.2.1. Plant materials, growth conditions and application of surfactant

Barley (cv. *Pallas*) and maize (cv. *Lark* F1) seeds were germinated on moistened tissue paper and kept under dark conditions for 3 days at room temperature (approximately 20^{o}C). After

germination, seeds were cultivated as detailed in Section 2.2.1. All plants were kept WW for three weeks and maintained in a naturally lit glasshouse compartment with an average daytime temperature of 27 ± 1.5°C, with a RH of 30–40% and supplementary lighting providing a PPFD at bench height of 250–400 µmol m^{-2} s^{1} for a 12 h photoperiod (0800 h-2000 h). An Ektron II sensor (HortiMax, Pijnacker, The Netherlands) monitored environmental conditions in the centre of the glasshouse. Surfactant solution (Surfactant 1; 25 ml) was prepared and applied as detailed in Section 2.2.1. Saucers were placed underneath each pot to capture any drainage water, which was re-incorporated into the pots.

4.2.2. Daily transpiration and rehydration treatments

Prior to starting soil drying, plants were watered at 1600 h until water drained from the base of the pot, and next morning weighed using a precision balance to 0.1 g (Scout Pro Portable balance, Ohaus, Switzerland), to establish pot capacity. The surface of each pot was covered with black duct tape to limit evaporation losses (less than 5% of plant evapotranspiration; determined by weighing pots without plants that were similarly covered with duct tape). Plants were subjected to one drying and rewetting cycle that lasted 3 days. Pot weight was determined daily (between 1000 h and 1300 h) during the drying cycle to calculate water losses between successive days. Two rehydration methods replaced the cumulative water loss from the past 3 days (approx. 160 mL): (a) watering from the top of the pot in equivalent doses to avoid any drainage and (b) watering by capillarity from a saucer (21.5 diameter x 4.5 cm height) placed beneath the pot. Saucers were covered with aluminium foil to prevent evaporation losses. Preliminary results showed that basal rewatering of surfactant-treated soil with water or untreated soil with surfactant solution (prepared according to

manufacturer's recommendations) did not alter soil moisture kinetics (Fig. C-3). Thus, surfactant-treated soil was rehydrated with tap water in the main study.

4.2.3. Gas exchange and soil moisture measurements

Transpiration rate and photosynthesis responses following rehydration were determined using a novel whole plant gas exchange system, as detailed in Section 3.2.6. Inside the chamber, air relative humidity (RH; i.e. the ratio of the current absolute humidity to the highest possible absolute humidity) was controlled by diverting air through a water bath and mixing it with dry air (5% RH) to maintain 40% RH (similar to average RH in glasshouse) for all measurements, at a constant temperature of 25°C. Differences in [CO_2] and [H_2O] between air entering and exiting the chamber were measured and recorded using an infra-red gas analyser, every 1 min (Model LI-6400XT, Li-Cor Inc., Lincoln, Nebraska, USA). To determine plant rehydration kinetics, transpiration and photosynthesis values were recorded 1 h before and for 4-5 h following rehydration. Preliminary work showed that gas exchange recovered 4-5 h after rewatering. Two plants were measured per day. Transpiration and photosynthesis were calculated per unit leaf area.

Two soil moisture sensors (ML3 Thetaprobe, Delta-T Devices, Cambridge, UK) were inserted in each pot through holes made on the pot wall. The central rod of the lower sensor was inserted 7 cm above the base of the soil column (6cm above the water table), while the upper sensor was inserted 17 cm above the base of the soil column (16cm above the water table). The dielectric constant of the soil was recorded every 15 min by a data-logger (DL2e, Delta-T Devices, Cambridge, UK) and transformed into volumetric soil moisture using the default calibration factor for mineral soils provided by the manufacturer.

4.3.3. Leaf water status, leaf area and biomass

Leaf water potential was measured before rewatering (after 1 h of gas exchange measurements) and 4-5 h after rewatering using a Scholander pressure chamber (Soil Moisture Equipment Corp., Santa Barbara, CA, USA). The two youngest fully expanded leaves used for Ψ_{leaf} measurements were immediately frozen in liquid nitrogen and stored at –20°C for ABA analysis. Total leaf area was measured using a leaf area meter (Model Li-3100, Li-Cor Inc., Lincoln, Nebraska, USA). Shoot and root tissues were weighted to obtain fresh weight and dried at 80°C for at least 72 h to obtain dry biomass weight.

4.3.4. Foliar ABA quantification

Leaf samples were freeze-dried and ground into powder. Dry leaf tissues were mixed with deionized water (extraction ratio 1:50; dry sample(g): water(g)) and then shaken at 4°C overnight to extract ABA. The extracts were centrifuged at 15,000 rpm for 5 min, and the supernatant was directly used for ABA assay. Foliar ABA concentration was measured by radioimmunoassay method, using the monoclonal antibody AFRC MAC 252 (Quarrie *et al.,* 1988).

4.3.5. Statistical analysis

Data were tested for normality using Shapiro-Wilk test. Time of the measurements (morning/afternoon) did not significantly affect values and therefore data for each species, rehydration technique and surfactant treatment were pooled and analysed together. Statistical differences ($P<0.05$) in fresh and dry shoot and root biomass between species and surfactant treatments were determined by two-way ANOVA. Leaf water potential and foliar ABA concentration data were analysed using repeated measures ANOVA, with rewatering

time (before/following rewatering) as within-subjects factor and species, irrigation, and surfactant treatments as between-subject factors. Gas exchange variables were grouped in 30 min intervals, means were calculated within intervals and were analysed by repeated measures ANOVA, with time (after rewatering) as within-subjects factor and species, irrigation, and surfactant treatments as between-subject factors. Soil moisture variables were grouped in 30 min intervals, means were calculated within intervals and were analysed using a normal linear mixed-effects model, with Restricted Maximum Likelihood Estimation, using plant as a random factor and time (after rewatering), soil layer (upper/lower) irrigation and surfactant treatments as fixed factors. Statistical analyses were performed using SPSS 25 (SPSS Statistics 25, IBM, Armonk, New York, USA) and R (Version 4.0.3, R Foundation for Statistical Computing, Vienna, Austria).

4.3. Results

4.3.1. Shoot and root biomass

Shoot dry biomass of both species was ca. 20% higher in plants growing in surfactant-treated soil than untreated controls (Fig. 4.1). Applying surfactant tended to increase fresh shoot biomass in both species, but those differences were not statistically significant ($P = 0.06$; Fig. C-1). Root fresh and dry biomass were not significantly different between surfactant treatments of both species (Fig. C-2). Thus, applying surfactant significantly increased shoot dry weight of barley and maize.

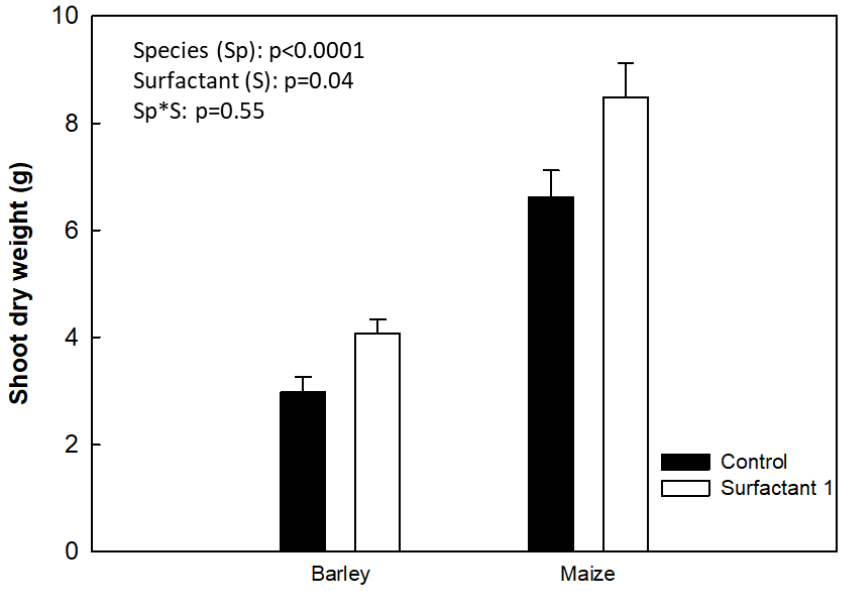

Figure 4.1: Shoot dry biomass for barley and maize plants. Black bars: plants were growing in untreated (control) soil; White bars: plants were growing in surfactant-treated soil. Bars are means ± SE of 8 replicates. P values are reported for species (Sp), surfactant treatments (S) and their interaction.

4.3.2. Effect of rehydration technique and surfactant treatments on soil moisture

Rewatering from the top rapidly recovered soil moisture of both upper and lower soil layers, within 30 minutes (Fig. 4.2 a, c). However, average soil moisture of the lower soil layer between 30 and 210 minutes after rewatering from the top was ca. 31% and 23% lower in surfactant-treated soils compared to untreated, in barley and maize, respectively (Table 4.1). Rewatering from the base resulted in significant differences in soil moisture between upper and lower soil layers (Table 4.2). Thus, soil moisture was always higher in the lower than the upper soil layer (Fig. 4.2 b, d). Basal rewatering of surfactant-treated soil decreased local (upper layer) soil moisture by approx. 20%, in both species (averaged between 30 min and 210 min; Table 4.2). In conclusion, previous surfactant application impeded upper layer

rehydration or moisture depletion when watered from the base or the top of the pot, respectively.

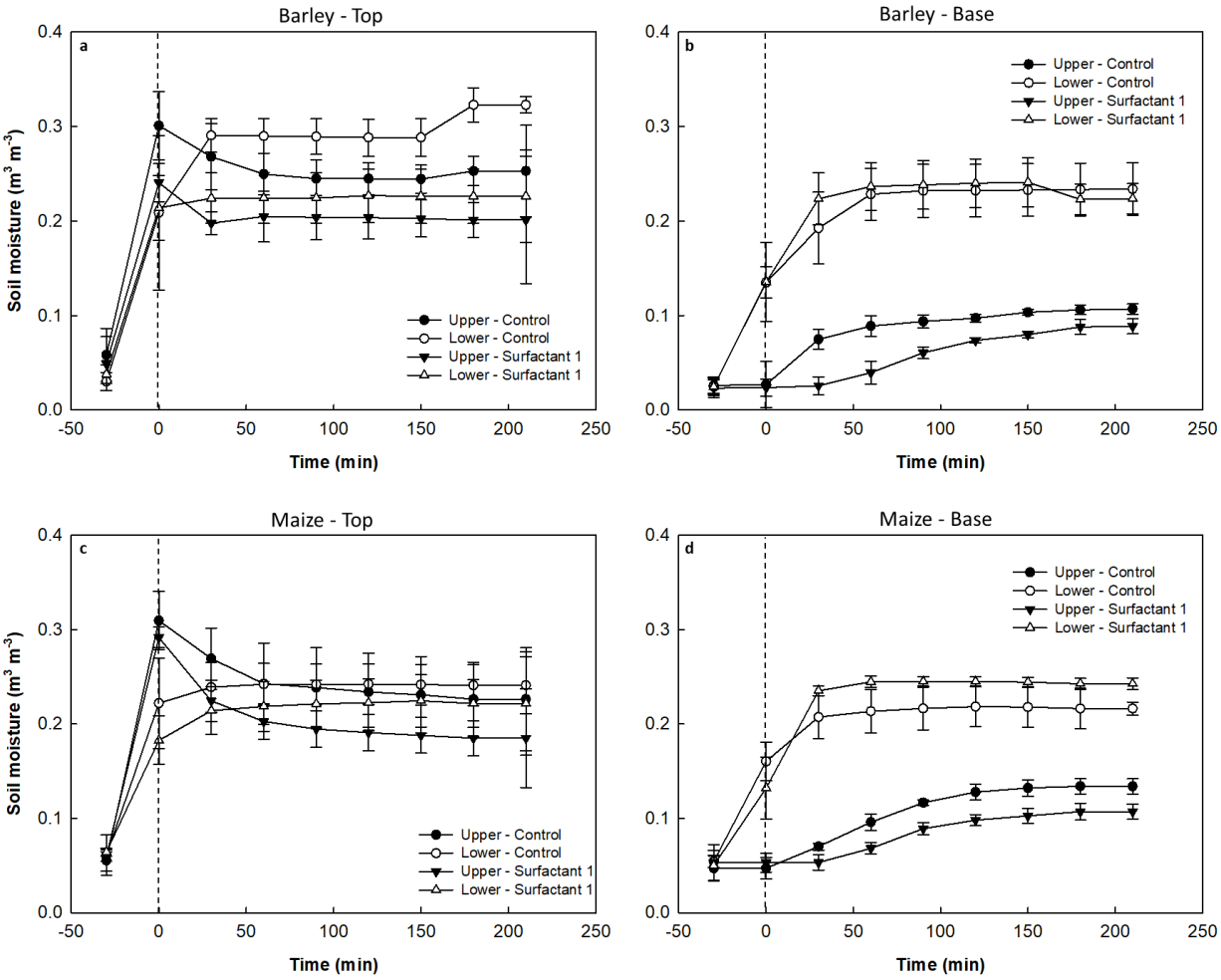

Figure 4.2: Soil moisture of upper and lower soil layer over time, for barley (a, b) and maize (c, d). Soil was rehydrated at 0 min (indicated by vertical medium-dashed line), from top (a, c) or from the base (b, d). Symbols are means ± S.E of 4 replicates. Filled and hollow circles denote upper and lower layer of untreated (control) soil, respectively. Filled and hollow triangles denote upper and lower layer of surfactant-treated soil, respectively.

Table 4.1: Soil moisture (expressed in m^3 m^{-3}) of upper and lower soil layer (C and S denote plants grown in untreated and surfactant-treated soil, respectively), 210 min following rewatering from the top. Different letters within a row indicate significant ($P<0.05$) differences.

Species	**C- Upper**	**C- Lower**	**S - Upper**	**S - Lower**
Barley	0.25 ± 0.010 b	0.32 ± 0.010 a	0.20 ± 0.014 b	0.22 ± 0.012 b
Maize	0.23 ± 0.011 a	0.26 ± 0.011 b	0.18 ± 0.015 b	0.20 ± 0.010 b

Table 4.2: Repeated measures ANOVA (P values reported) examining the effects of time (after rewatering, T), soil layer (SL), irrigation location (I) and surfactant treatments (S) and their interactions on soil moisture, in barley and maize. Significant effects (P<0.05) are in bold text.

	Barley	Maize
Time (T)	**<0.0001**	**<0.0001**
Soil layer (SL)	**<0.0001**	**<0.0001**
Surfactant (S)	**0.03**	**0.02**
Irrigation location (I)	**<0.0001**	**<0.0001**
T*SL	**<0.0001**	**<0.0001**
T*S	0.89	0.68
T*I	**<0.0001**	**<0.0001**
SL*S	**0.02**	**0.02**
SL*I	**<0.0001**	**<0.0001**
S*I	0.14	0.28
SL*S*I	**<0.001**	**<0.001**
T*SL*S	0.71	0.80
T*SL*I	**<0.0001**	**<0.0001**
T*S*I	0.57	0.82
T*SL*S*I	0.97	0.82

4.3.3. Effect of rehydration techniques, surfactant treatments and species on gas exchange

Irrespective of species or rewatering technique, whole plant gas exchange increased with time (Table 4.3) and reached a plateau after 210 min (Fig. 4.3; 4.4). Rehydration technique significantly affected the recovery time of transpiration rate, as indicated by a significant Time x Irrigation interaction ($P<0.001$; Table 4.4). Rewatering plants from the top hastened the recovery of transpiration compared to rewatering from the base (Fig. 4.3). Transpiration rate did not vary between species, or whether the soil had been surfactant-treated, at any measurement occasion (Tables 4.3; 4.4). Photosynthesis followed a similar recovery pattern as transpiration rate (Fig. 4.4), with more rapid recovery in plants rewatered from the top than from the base, in both species ($P<0.001$ for the Time x Irrigation interaction; Table 4.3), without species or surfactant treatment affecting the response (Table 4.3). In conclusion, gas exchange recovered more rapidly when rewatering from the top than the base, in both species but previous surfactant application did not affect this recovery.

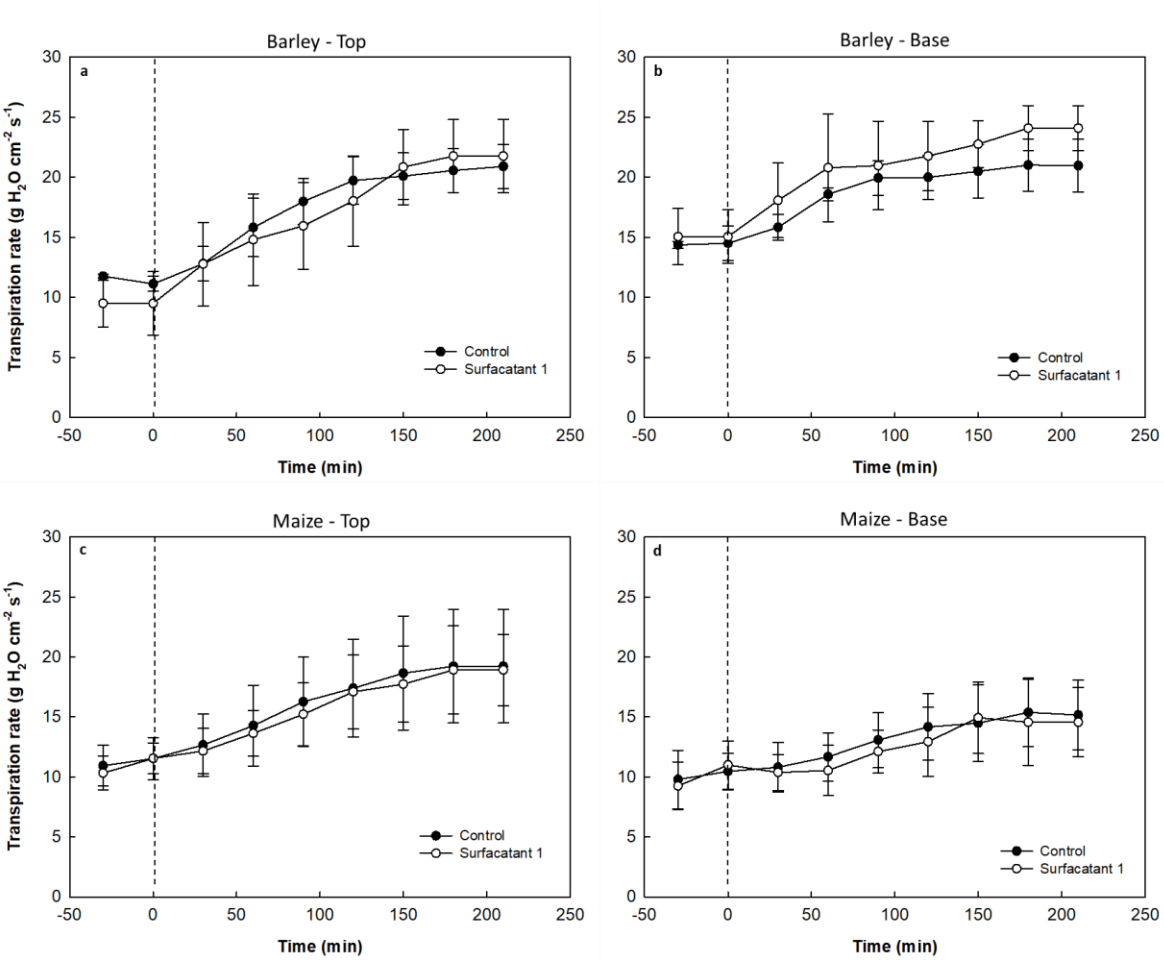

Figure 4.3: Transpiration rate of barley (a, b) and maize (c, d) over time. Plants were rewatered at 0 min (indicated by vertical medium-dashed line), from top (a, c) or from the base (b, d). Symbols are means ± S.E of 4 replicates. Plants were growing in control (untreated) soil (filled circles) and surfactant-treated soil (hollow circles), respectively.

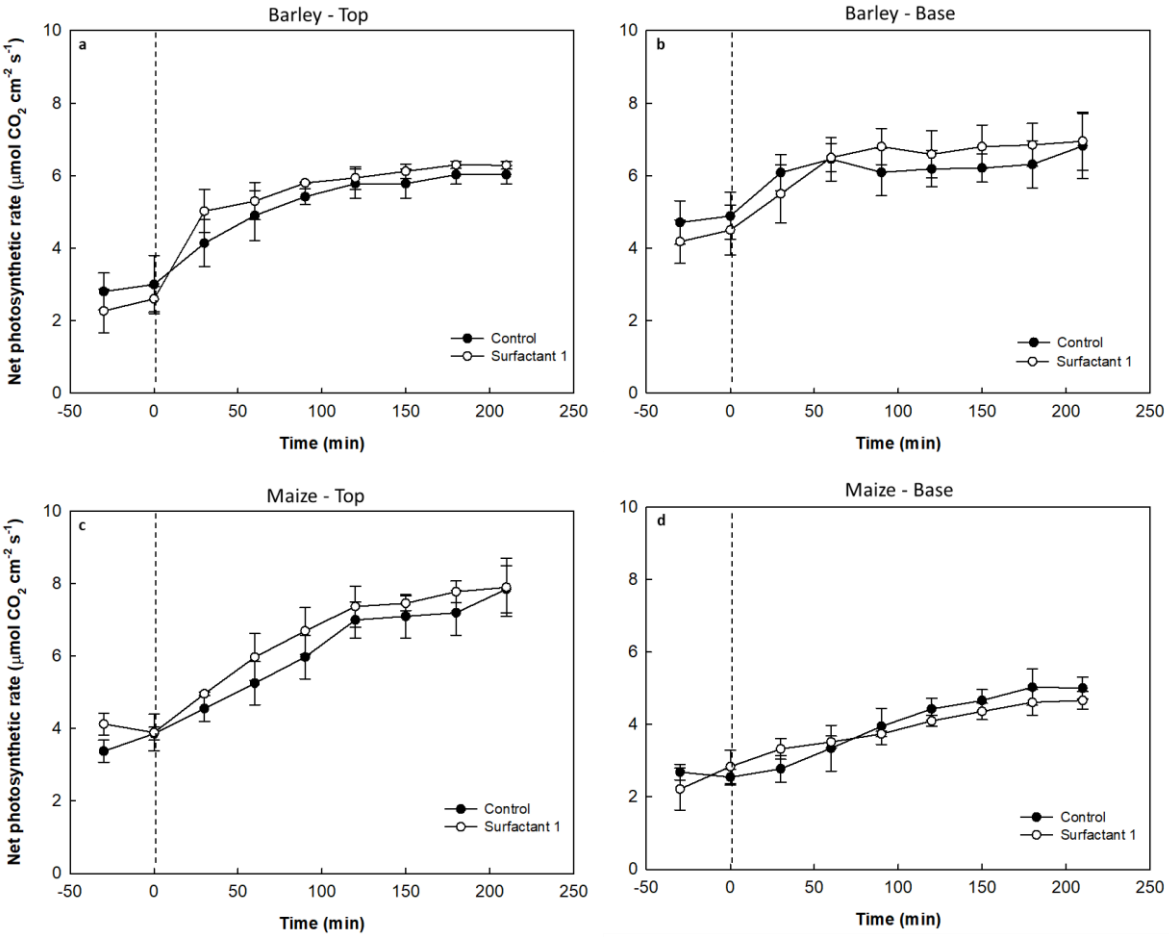

Figure 4.4: Net photosynthetic rate of barley (a, b) and maize (c, d) over time. Plants were rewatered at 0 min (indicated by vertical medium-dashed line), from top (a, c) or from the base (b, d). Symbols are means ± S.E of 4 replicates. Plants were growing in control (untreated) soil (filled circles) and surfactant-treated soil (hollow circles), respectively.

Table 4.3: Repeated measures ANOVA (P values reported) examining the effects of time (after rewatering, T), species (Sp), irrigation (I) and surfactant treatments (S) and their interactions on transpiration rate and photosynthesis. Significant effects (P<0.05) are in bold text.

	Transpiration	**Photosynthesis**
Time (T)	**<0.0001**	**<0.0001**
Species (Sp)	0.39	0.69
Surfactant (S)	0.38	0.65
Irrigation (I)	0.40	0.06
T*Sp	0.08	**0.001**
T*S	0.48	0.66
T*I	**0.002**	**<0.0001**
Sp*S	0.58	0.31
Sp*I	0.70	0.08
S*I	0.29	0.16
Sp*S*I	0.61	0.50
T*Sp*S	0.96	0.88
T*Sp*I	0.82	0.26
T*S*I	0.79	0.80
T*Sp*S*I	0.83	**0.01**

Table 4.4: Transpiration rate (expressed in g H_2O cm^{-2} s^{-1}) and net photosynthetic rate (expressed in µmol CO_2 cm^{-2} s^{-1}) of barley and maize (C and S denote plants grown in untreated and surfactant-treated soil, respectively), 210 min following rewatering from the top or base. There were no significant differences between surfactant treatments (P>0.05).

Species	Irrigation	E - C	E - S	An - C	An -S
Barley	Top	20.90 ± 1.81	21.77 ± 3.04	6.02 ± 0.25	6.28 ± 0.10
Barley	Base	20.97 ± 2.18	24.10 ± 1.86	6.82 ± 1.09	4.90 ± 0.07
Maize	Top	18.42 ± 4.73	20.10 ± 2.97	6.78 ± 1.38	7.90 ± 0.11
Maize	Base	15.15 ± 2.93	23.10 ± 8.13	5.00 ± 0.83	4.66 ± 0.25

4.3.4. Leaf water status and foliar ABA concentration

Leaf water potential of barley and maize was ca. 1.36 MPa and 0.9 MPa higher following rehydration than before (across two surfactant treatments), respectively. Surfactant application and rehydration method had no impact on Ψ_{leaf} (Fig. 4.5; Table 4.5). Overall, only rehydration significantly increased Ψ_{leaf} of both species.

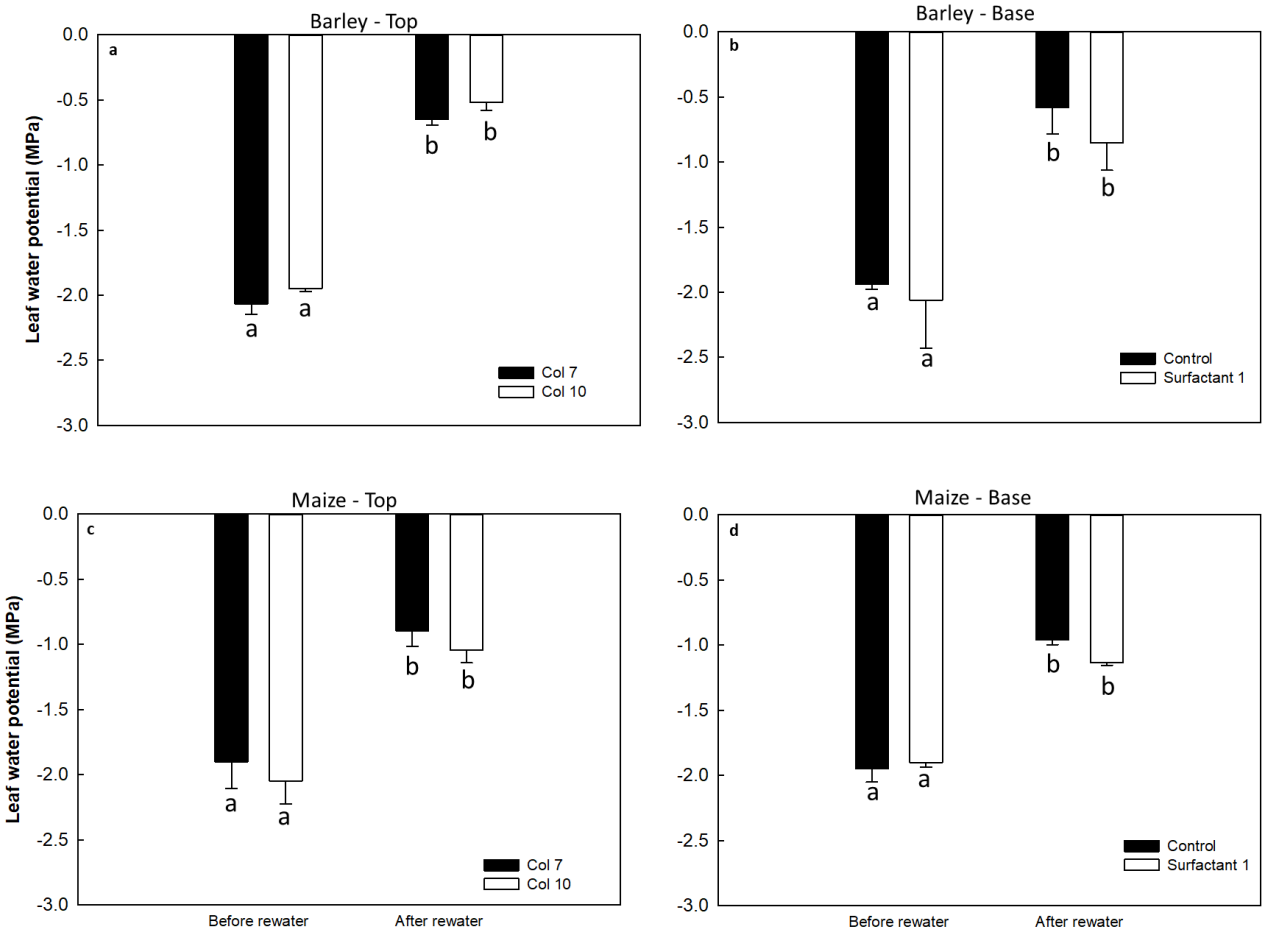

Figure 4.5: Leaf water potential of barley (a, b) and maize (c, d) before and after rehydration. Plants were rewatered from top (a, c) or from the base (b, d). Black bars: plants were growing in control (untreated) soil; White bars: plants were growing in surfactant-treated soil. Bars are means ± S.E of 4. Different letters denote significant differences between means ($P<0.05$).

Table 4.5: Repeated measures ANOVA (P values reported) examining the effects of time (before and following rewatering, R), irrigation (I) and surfactant treatments (S) and their interactions on leaf water potential, in barley and maize. Significant effects (P<0.05) are in bold text.

	Barley	**Maize**
Time (R)	**<0.0001**	**<0.0001**
Surfactant (S)	0.47	0.16
Irrigation (I)	0.36	0.72
R*S	0.71	0.97
R*I	0.38	0.33
S*I	0.13	0.24
R*S*I	0.66	0.24

Rehydration significantly decreased foliar ABA concentration in both species (Fig. 4.6; Table 4.6) but rehydration method did not alter ABA concentration on any measurement occasion (Table 4.6). In barley, ABA concentration was significantly higher in plants growing in surfactant treated soil than untreated control, following basal rewatering (Fig. 4.6 b), as indicated by a significant Irrigation x Surfactant interaction (P=0.028). Thus, foliar ABA concentration decreased following basal rewatering in barley, but this decrease was attenuated in surfactant-treated soil.

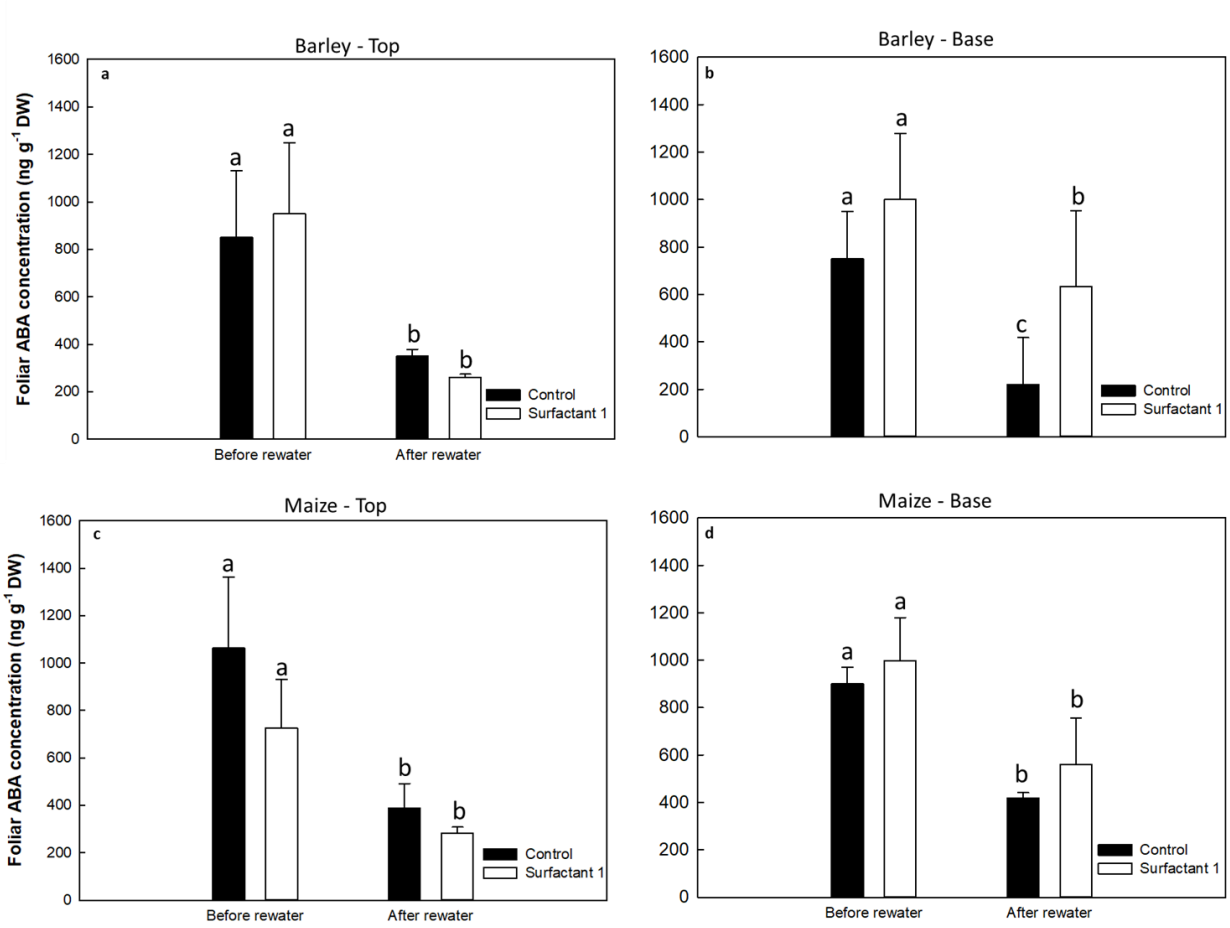

Figure 4.6: Foliar ABA concentration of barley (a, b) and maize (c, d) before and after rewatering. Plants were rewatered from top (a, c) or from the base (b, d). Black bars: plants were growing in control (untreated) soil; White bars: plants were growing in surfactant-treated soil. Bars are means ± S.E of 4 plants. Different letters denote significant differences between means ($P<0.05$).

Table 4.6: Repeated measures ANOVA (P values reported) examining the effects of rewatering time (before and following rewatering, R), irrigation (I) and surfactant treatments (S) and their interactions on foliar ABA concentration, in barley and maize. Significant effects (P<0.05) are in bold text.

	Barley	Maize
Time (R)	**<0.0001**	**0.02**
Surfactant (S)	0.82	0.61
Irrigation (I)	0.17	0.83
R*S	0.40	0.44
R*I	**0.01**	0.49
S*I	**0.02**	0.58
R*S*I	0.09	0.94

4.4. Discussion

4.4.1. Soil moisture and gas exchange responses to species, rehydration techniques and surfactant treatments

Species did not alter gas exchange responses to surfactant application (Table 4.3). Thus, any putative reductions in soil-to-root hydraulic conductivity due to surfactants and subsequent effects on stomatal aperture (Ahmed *et al.,* 2017) seemed to be independent of mucilage properties of different species (Naveed *et al.,* 2019) and did not occur in this study. Regardless of surfactant effects on soil moisture (Fig. 4.2), gas exchange did not differ between treatments (Table 4.4) while rehydration techniques significantly affected the speed at which gas exchange recovered (Table 4.3).

Soil moisture fully recovered following rewatering from the top, but soil moisture of the lower soil layer of surfactant-treated soil was lower in both species (Table 4.2). Likely, local differences in soil moisture between treatments are attributed to enhanced soil moisture distribution in the soil column in surfactant-treated soils. Several studies, mainly in turfgrass, have confirmed that surfactants can alleviate preferential flow paths and facilitate soil moisture distribution within the root-zone (Soldat *et al.,* 2010; Schiavon *et al.,* 2014; Alvarez *et al.,* 2016). Therefore, when soil was rehydrated from the top, water was likely distributed evenly throughout the soil column in surfactant-treated soil. On the contrary, in untreated controls, water might infiltrate more rapidly following preferential flow paths and accumulated in the lower soil layer, leading to higher local volumetric water contents (Fig. 4.2 a, c; Table 4.2). Nevertheless, presumable facilitation of soil moisture distribution due to surfactant application, following rehydration from the top (Table 4.1), did not enhance gas

exchange in the root-constrained pot environment; however, further investigation is needed under field conditions.

Basal rewatering resulted in pronounced soil moisture gradients, with full rehydration of lower soil layers, but only partial recovery of upper soil layers, irrespective of surfactant treatment (Fig. 4.2 b, d). Rehydration of upper soil layers was hindered in surfactant-treated soils for both species (Fig. 4.2 b, d), which may be attributed to surfactants diminishing capillarity. Previous workers found that surfactants decrease capillarity by reducing surface tension of water in columns of hydrophilic sand (Shafran *et al.,* 2005; Mobbs *et al.,* 2012) in the absence of any plants. Decreased capillarity and therefore restricted water uptake following basal rewatering of surfactant-treated soil did not limit further gas exchange (Fig. 4.3 b, d; 4.4 b, d). Lupin plants that were rewatered from the base with surfactant solution transpired significantly less than those that received water from the base, until stomata fully recovered, approx. after 40 h (Ahmed *et al.,* 2017). Surfactant likely further decreased capillarity and thus soil moisture of upper layers were diminished more than in our work. Although different irrigation solutions (± surfactant) may reconcile this discrepancy, preliminary results showed that rehydration technique (rewatering untreated soil with surfactant solution *versus* rewatering surfactant-treated soil with water) did not significantly affect local soil moisture (Fig. C-3). Overall, delayed rehydration of the upper layers of surfactant-treated soil, following basal rehydration, did not change gas exchange recovery, which was solely affected by the rehydration technique, with basal rewatering significantly restraining it in both treatments.

4.4.2. Surfactant effects on leaf water status and ABA concentration and stomatal regulation

Leaf water status can regulate stomatal aperture (Day *et al.,* 1981; Buckley, 2005). Before rehydration, Ψ_{leaf} was comparable between surfactant treatments, in both species (Fig. 4.5), in very dry soil. In Chapter 3, Ψ_{leaf} was significantly higher in barley plants that were growing in surfactant-treated soil that had experienced mild drying (Fig. 3.9 b); however, those differences were eliminated in very dry soil (Fig. 3.9 c), consistent with results of this study. Five hours after rehydration, leaf water status was similar between surfactant treatments and rehydration techniques (Fig. 4.5; Table 4.5), suggesting that partial soil moisture recovery following basal rehydration (Fig. 4.2 b, d) was adequate to enhance leaf water status. Gas exchange recovered (Fig. 4.3; 4.4) along with increased Ψ_{leaf} (Fig. 4.5) following rehydration in all measurement occasions, suggesting hydraulic regulation of stomatal aperture (Saliendra *et al*., 1995; Huber *et al.,* 2019). Overall, surfactant treatment and rehydration technique did not affect leaf water status before and after rewatering, with Ψ_{leaf} likely playing a dominant role in stomatal regulation.

Stomatal aperture can be also regulated by long-distance chemical signals (Davies *et al.,* 2002), and ABA is widely accepted as having a key role in stomatal regulation (Wilkinson and Davies, 2002; Dodd, 2005; Saradadevi *et al.,* 2017). Generally, bulk leaf ABA concentration was lower after rewatering than before (Fig. 4.6), but foliar ABA accumulation was higher in barley plants growing in surfactant-treated soil than in untreated controls, following basal rehydration (Fig. 4.6 b), due to partial soil moisture recovery (Fig. 4.2 b). Previous workers have speculated that application of surfactants to the soil, due to enhanced rhizosphere water status, induces a convective flux of water that transports ABA via the xylem to the shoot,

temporarily inhibiting transpiration (Ahmed *et al.,* 2017). Since this study did not conduct xylem ABA measurements, further support for this idea is required. Alternatively, surfactants may be considered as antitranspirants (Sibley *et al.,* 2018), although there is considerable uncertainty whether they enter the xylem. Taken together, basal rewatering of barley delayed the expected decline in ABA concentration in surfactant-treated soil, but further work is needed to investigate whether surfactants affect xylem ABA concentration and determine whether chemical signals are involved in stomatal regulation.

4.4.3. Surfactant effects on biomass accumulation and putative regulatory mechanisms

Shoot dry biomass of both species was greater in surfactant-treated soil (Fig. 4.1), as in other studies (Chaichi *et al.,* 2015; Daneshnia *et al.,* 2016; Jafarian *et al.,* 2016). However, the mechanism(s) involved remain unclear. Previous work has emphasised the role of cell turgor or tissue water status in determining leaf growth rates, suggesting that leaf water relations regulate leaf growth over timescales of minutes to hours (Munns *et al.,* 2000). Leaf water potential was similar between surfactant treatments (Fig. 4.5; Table 4.5), indicating that leaf water relations might not explain shoot growth differences. Further work such as continuously measuring Ψ_{leaf} after the application of surfactant or pressurising the roots to maintain leaf xylem on the point of bleeding (Passioura & Munns, 2000), is needed to determine whether leaf water relations regulate shoot growth in different surfactant treatments.

Photosynthesis provides the raw materials for vegetative biomass accumulation (Beadle & Long, 1985; Demura & Ye, 2010). In this study, applying surfactant did not affect photosynthetic rate (Fig. 4.4) and therefore, enhanced biomass accumulation might not be attributed to photosynthetic activity. Nevertheless, whether short-term gas exchange

measurements reflect time-integrated carbon gain has not been investigated. Thus, future work should focus on whether surfactants increase biomass accumulation by enhancing carbon gain during plant growth and at different developmental stages.

4.5. Conclusions

Taken together, basal rewatering delayed rehydration of upper soil layers, especially in surfactant-treated soils. This resulted in foliar ABA concentration of barley plants decreasing more slowly than after rewatering from the top of the pot; yet Ψ_{leaf} was not correlated with foliar ABA concentration. These differences did not alter whole plant gas exchange, which recovered quicker when plants were watered from the top. Shoot dry biomass of both species was greater when grown in surfactant-treated soil, but these differences were not associated with enhanced leaf water status or gas exchange.

Chapter 5: General Discussion

Numerous studies have investigated effects of surfactants on soil moisture distribution, turfgrass quality and colour (e.g., Serena *et al.,* 2018; Hutchens *et al.,* 2020). Additionally, a few agronomical studies have determined surfactant effects on biomass accumulation, plant height, grain yield, as well as nutrient availability (Table A-1; B-1). However, a review of the available literature identified that effects of surfactants on plant physiological responses to water deficit, as well as mechanistic effects on nutrient acquisition, remain largely unknown. Thus, this research aimed to determine the impact of surfactant application on soil-plant water relations, plant water use and nutrient uptake.

Initially, effects of surfactants on rhizosheath formation and nutrient uptake in drying soil were determined in two barley genotypes (WT and *brb*) with contrasting rhizosheath traits (Chapter 2). To investigate surfactant effects on plant water availability, soil moisture release curves were constructed and Ψ_{base} of plants growing in surfactant-treated and untreated soil was measured in drying soil (Chapter 3). Since surfactant application enhanced rhizosheath formation, whether surfactant-induced rhizosheath formation improved soil-plant water relations and water uptake was tested in drying soil and/or elevated evaporative demand. Lastly, since application of surfactant did not affect daily plant transpiration or transpiration rate under elevated evaporative demand, Chapter 4 focused on surfactant effects on transpiration rate following rewatering.

5.1. Applying surfactants facilitated plant water availability in drying soil

At the same SWC, application of surfactant increased soil-water relations as indicated by constructing soil moisture release curves with thermocouple psychrometers (Fig. 3.1) and

Ψ_{base} measurements in non-transpiring plants (Fig. 3.2 b). Differences between surfactant treatments commenced when SWC was ca. 0.10 g g^{-1} (Ψ_{soil} ~ -0.1 MPa), and became more pronounced as the soil dried, which was consistent in the two measurement methods. Enhanced Ψ_{soil} in surfactant-treated soils may be attributed to the fundamental action of surfactant molecules decreasing the surface tension of water and thereby reducing the contact angle between water molecules and soil particles (Karagunduz *et al.,* 2001). Previous workers have shown that surfactants reduce capillarity of sandy substrates (Gross *et al.,* 2011; Mobbs et *al.,* 2012), likely retaining water less firmly to soil particles. To determine surfactant effects on capillarity, soil moisture was measured in upper and lower soil layers following basal rewatering. Indeed, applying surfactant hindered rehydration of upper soil layers (Fig. 4.2 b, d), implying that matric forces were decreased. However, *in situ* measurements of rate of capillary rise (Lu & Likos, 2004) were not conducted in this research. Taken together, utilizing two methods of water relations measurements (thermocouple psychrometry and pressure chamber) demonstrated that application of surfactant increased Ψ_{soil} for the same SWC, below a threshold soil moisture.

5. 2. Enhanced plant water availability by applying surfactant had minimal effects on leaf water relations and water uptake, independently of rhizosheath formation

Since applying surfactant maintained higher Ψ_{soil} for any given SWC (Fig. 3.1; 3.2 b), surfactant effects on water status of transpiring leaves were investigated. Higher plant water availability in surfactant-treated soils was predicted to enhance leaf water status. Indeed, under mild soil drying (SWC ~ 0.10 g g^{-1}, Ψ_{soil} ~ 0.1 MPa), plants that grown in surfactant-treated soil had higher Ψ_{leaf} (ca. 0.1 MPa) than untreated controls (Fig. 3.9 b). However, those differences were eliminated in very dry soil (SWC ~ 0.35 g g^{-1}, Ψ_{soil} ~ -0.4 MPa), similarly in two species

(Fig. 3.9 c; 4.5). Thus, surfactant effects on plant water availability enhanced leaf water status only with moderate soil drying. Further research is needed to explore the long-term effect of surfactants on plant-water relations when they are grown in different substrates, especially as the soil dries.

Whether higher soil water status in surfactant-treated soil affects water uptake was determined. Initially, plants were subjected to drying/rewetting cycles and surfactant application did not consistently affect daily transpiration (Fig. 3.6). Nonetheless, putative effects of surfactants on transpiration rate were investigated under elevated VPD (Fig. 3.8). Although WW plants growing in surfactant-treated soil transpired 14% more than untreated plants (across 4 VPDs; albeit non-significant), no differences were found when plants subjected to soil drying (Fig. 3.8 a, b, c). Previous workers have suggested that surfactant application increased plant transpiration (compared to water controls) following rewatering (Ahmed *et al.,* 2017). Thus, surfactant effects on whole plant gas exchange of different species, following rewatering either from the top or base of the pot, were investigated. Even though basal rewatering hindered transpiration recovery (Fig. 4.2 b, d), surfactant application did not affect whole plant gas exchange in both species (Fig. 4.3). Taken together, exhaustive transpiration measurements suggest that surfactant application did not consistently affect plant water use in this study.

Since few studies have considered whether the rhizosheath alters plant water relations, Ψ_{base} (in non-transpiring plants) was measured in two genotypes of barley that differed in rhizosheath size, due to the presence or absence of root hairs. Both genotypes had a similar Ψ_{base} (Fig. 3.3) despite greater rhizosheath size in WT plants (Fig. 3.7), implying that more rhizosheath did not enhance plant water availability. Nonetheless, it was important to

investigate whether surfactant-induced rhizosheath formation affected plant-water relations when plants were transpiring, when exposed to drying soil and/or increased evaporative demand.

As discussed above, application of surfactant did not affect daily plant transpiration during drying and rewetting cycles (Fig. 3.6), nor transpiration response to elevated VPD (Fig. 3.8), indicating that rhizosheath formation did not affect water uptake. In contrast, wheat cultivars with larger rhizosheaths maintained higher transpiration rates in drying soil, compared to cultivars that formed thinner rhizosheaths and wilted at a higher SWC. These genotypic differences in transpiration were attributed to greater rhizosheath size facilitating root access to water (Basirat *et al.,* 2019). Possibly these drought tolerant wheat cultivars (which produced larger rhizosheaths) developed longer or denser root hairs to cope with water-limited conditions (Zhang *et al.,* 2019), ultimately enhancing rhizosheath formation. Indeed, rhizosheath development was highly correlated with root hair length in foxtail millet (*Setaria italica*) in drying soil, suggesting that soil drying stimulated root hair elongation, thereby enhancing soil binding (Liu *et al.,* 2018). Although root hairs facilitate water acquisition by increasing soil volume that can be reached (Wasaya *et al.,* 2018), their impact on plant transpiration remains ambiguous. Daily transpiration of *brb* and WT plants grown at relatively stable VPDs was similar (Dodd & Diatloff, 2016), as was momentary transpiration response to abrupt changes in VPD (Fig. 3.8). However, under some circumstances WT barley plants maintained transpiration at high VPDs unlike *brb* (Carminati *et al.,* 2017), suggesting that root hairs (and thus, greater rhizosheath size) sustain water uptake. While root hairs may sustain transpiration under water-limited conditions, whether longer and/or denser root hairs or consequential greater rhizosheath formation affect water relations remain ambiguous.

5.3. Surfactant application and rhizosheath formation did not affect nutrient acquisition

Although rhizosheath formation did not affect plant water relations (Fig. 3.3), it is considered a favourable habitat for microbial growth. These microorganisms produce enzymes (such as phosphatase and sulfatase) that solubilize nutrients during mineralization (Ortíz-Castro *et al.,* 2009; Marasco *et al.,* 2018). Furthermore, the rhizosheath may act as a niche for nitrogen-fixing bacteria (Wullstein *et al.,* 1979; Wullstein, 1991; Othman *et al.,* 2004). Since the rhizosheath might be important in nutrient acquisition, whether surfactant-mediated rhizosheath formation facilitates nutrient uptake was investigated. Application of surfactant did not affect cumulative N or P uptake (Tables 2.7; 2.8), implying that surfactant-induced rhizosheath formation did not affect nutrient acquisition. Nonetheless, by applying surfactants to genotypes with contrasting rhizosheath traits (due to presence or absence of root hairs), root hairs were demonstrated to determine N and P uptake (Tables 2.7; 2.8), as in studies where root hairs substantially contributed to N (Canales *et al.,* 2017) and P uptake (Gahoonia & Nielsen, 1998; Haling *et al.,* 2013).

Although surfactant-induced rhizosheath formation did not affect nutrient uptake in this study, previous research suggests that surfactant application can enhance enzymes activity (such as phosphatase, sulfatase and chitinase) in rhizosheath soil, likely by increasing wettability of the rhizosphere (Ahmadi *et al.,* 2017; Ahmadi *et al.,* 2018). Whether enhanced enzymes activity in rhizosheath soil could facilitate nutrient uptake needs to be tested, since these studies did not determine nutrient status of plant tissues. Thus, further studies on surfactants should measure both enzymes activity in bulk soil and rhizosheath soil (e.g., soil zymography; Razavi *et al.,* 2016) and nutrient analysis in plant tissues, to determine whether

putative increments of enzymes activity in surfactant-induced rhizosheath formation could be associated with facilitated nutrient uptake and/or greater biomass accumulation.

5.4. Surfactant-induced biomass accumulation cannot be explained by enhanced nutrient or water uptake

Surfactant application sometimes increased biomass accumulation of both barley and maize (Table 2.3; Fig. 4.1). This could not be attributed to plant nutrient status, since neither enhanced rhizosheath formation (Fig. 2.3) nor more homogeneous distribution of soil moisture (Table 4.1) affected nutrient acquisition (Tables 2.7; 2.8). Moreover, enhanced biomass accumulation might not be associated with greater photosynthetic rate (Fig. 4.4) and seemed to be independent of leaf water status (Fig. 4.5), in agreement with previous observations in drying soil (Passioura, 1988). Overall, this research suggests that surfactant application facilitated biomass accumulation in pot-scale experiments (Fig. 5.1), but relevant mechanisms remain ambiguous.

5. 5. Future work

Two main areas for future studies are highlighted. Since the work in this thesis used a sandy soil that is mainly used by the turfgrass industry, it may be appropriate to determine the impact of surfactants in substrates of agronomical value (such as loamy or silty substrates). Since applying surfactants increased soil water availability and the shape of the relationship between Ψ_{soil} and SWC depends on soil characteristics (soil texture), it would be necessary to determine whether this response is more pronounced in substrates with larger percentage of soil-micropores (and thus higher water holding capacity) and investigate putative effects on plant physiology. Thus, further studies should examine surfactants effects on soil- plant water relations and water uptake in different substrates.

Further work on plant physiological responses to surfactant-treated soils should be conducted on a larger scale, as the findings of this study apply to pot experiments. Specifically, long-term field trials should investigate effects of applying surfactants on predawn water potential ($\Psi_{predawn}$), Ψ_{leaf} and g_s in different developmental stages, in order to determine whether response is stable throughout plant life cycle as well as their effects on biomass accumulation and/or yield.

5. 6. Concluding remarks

Taken together, this research has shown:

- In drying soil, plant water availability increased because surfactants decreased capillary forces.
- Plants that were growing in surfactant-treated soil had higher leaf water status than untreated controls when exposed to mild soil drying, but those differences were eliminated in very dry soil.
- Surfactant application amplified rhizosheath formation independent of root hairs.
- Nevertheless, surfactants did not improve plant water availability by enhancing rhizosheath size, as WT and *brb* plants (with ca. 56% more rhizosheath in WT than in *brb* plants) had a similar Ψ_{base}.
- Application of surfactant did not affect water uptake when barley plants were exposed to drying soil and/or elevated VPD, indicating no discernible role of the rhizosheath in determining plant water relations.
- Plant nutrient acquisition was facilitated by root hairs, but not surfactant-induced rhizosheath formation.

- Application of surfactant hindered rehydration of upper soil layers following basal rewatering; however, transpiration rate was solely affected by rehydration technique and recovered more rapidly when rewatering from the top than the base of the pot.
- Shoot dry biomass of barley and maize was sometimes greater in plants grown in surfactant-treated soil than untreated controls.
- However, plant growth differences were not associated with greater nutrient uptake, leaf water status or photosynthetic rate, suggesting that further research is needed to investigate how surfactants affect plant growth.

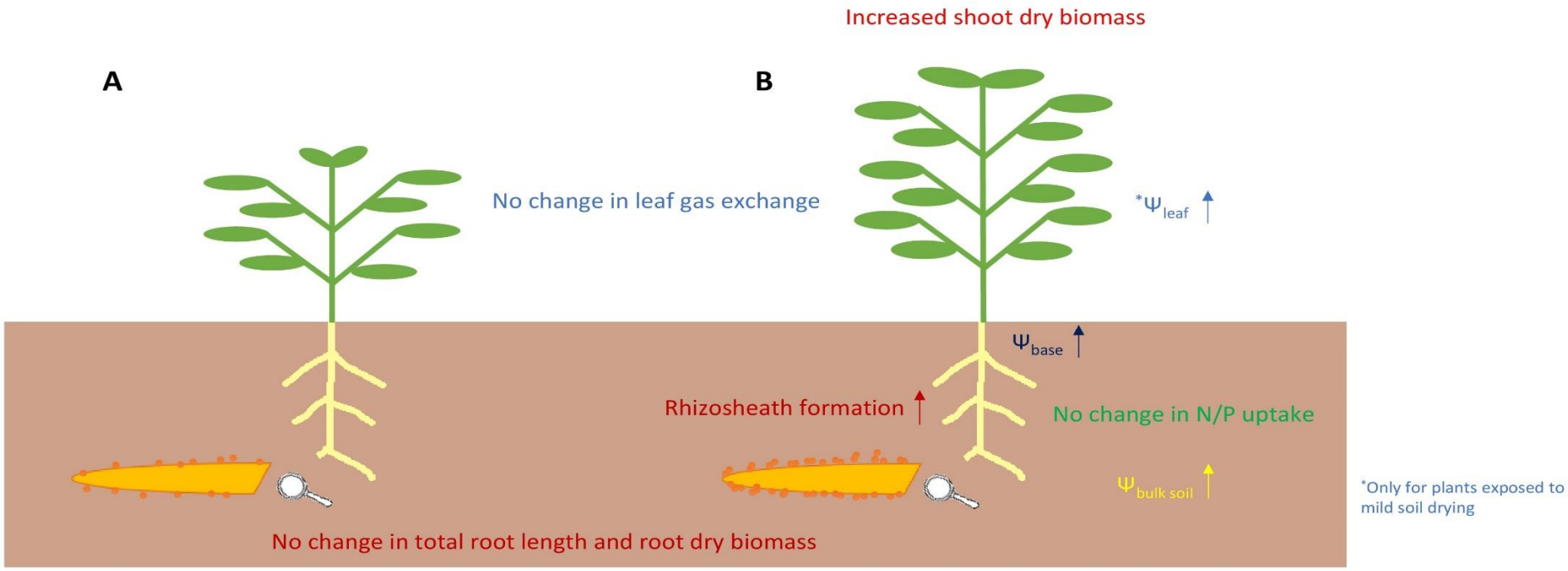

Figure 5.1: The main findings of this thesis are summarised: Plants growing in untreated soil (A) and surfactant treated soil (B).

References

Aamlid, T., Espevig, T. and Kvalbein, A. (2009). The potential of a surfactant to restore turfgrass quality on a severely water-repellent golf green. *Biologia*, 64(3), 620-623.

Aamlid, T., Larsbo, M. and Jarvis, N. (2009). Effects of surfactant use and peat amendment on leaching of fungicides and nitrate from golf greens. *Biologia*, 64(3), 419-423.

Ahmadi, K., Razavi, B., Maharjan, M., Kuzyakov, Y., Kostka, S., Carminati, A. and Zarebanadkouki, M. (2018). Effects of rhizosphere wettability on microbial biomass, enzyme activities and localization. *Rhizosphere*, 7, 35-42.

Ahmadi, K., Zarebanadkouki, M., Ahmed, M., Ferrarini, A., Kuzyakov, Y., Kostka, S. and Carminati, A. (2017). Rhizosphere engineering: Innovative improvement of root environment. *Rhizosphere*, 3, 176-184.

Ahmed, M., Holz, M., Woche, S., Bachmann, J. and Carminati, A. (2015). Effect of soil drying on mucilage exudation and its water repellency: a new method to collect mucilage. *Journal of Plant Nutrition and Soil Science*, 178(6), 821-824.

Ahmed, M., Kroener, E., Benard, P., Zarebanadkouki, M., Kaestner, A. and Carminati, A. (2015). Drying of mucilage causes water repellency in the rhizosphere of maize: measurements and modelling. *Plant and Soil*, 407(1-2), 161-171.

Ahmed, M., Kroener, E., Holz, M., Zarebanadkouki, M. and Carminati, A. (2014). Mucilage exudation facilitates root water uptake in dry soils. *Functional Plant Biology*, 41(11), 1129-1137.

Ahmed, M., Zarebanadkousi, M., Ahmadi, K. Kostka, S., Kaestner, A. and Carminati, A. (2017). Engineering rhizosphere hydraulics: Pathways to improve plant adaption of drought. *Vadose Zone Journal*, 17(1), 1-12.

Ali, M. and Talukder, M. (2008). Increasing water productivity in crop production - A synthesis. *Agricultural Water Management*, 95(11), 1201-1213.

Allen, S., Grimshaw, H., Parkinson, J. and Quarmby, C., (1974). *Chemical analysis of ecological materials*. Oxford: Blackwell.

Alvarez, G., Sevostianova, E., Serena, M., Sallenave, R. and Leinauer, B. (2016). Surfactant and polymer-coated sand effects on deficit irrigated bermudagrass turf. *Agronomy Journal*, 108(6), 2245-2255.

Arriaga, F. J., Lowery, B. and Kelling, K.A. (2009). Surfactant impact on nitrogen utilization and leaching on potatoes. *American Journal of Potato Research*, 86 (3), 383-390.

Bailey, C. and Scholes, M. (1997). Rhizosheath occurrence in South African grasses. *South African Journal of Botany*, 63(6), 484-490.

Baligar, V.C. and Fageria, N. (2015). Nutrient use efficiency in plants: An Overview. In: Rakshit A., Singh H.B., Sen A. (eds) *Nutrient Use Efficiency: from Basics to Advances*. New Delhi: Springer.

Baligar, V., Fageria, N. and He, Z., 2001. Nutrient use efficiency in plants. *Communications in Soil Science and Plant Analysis*, 32(7-8), 921-950.

Banks, M., Kremer, R., Eivazi, F., Motavalli, P. and Nelson, K. (2014). Effects of selected surfactants on nutrient uptake in corn (*Zea mays L.*). *Journal of Plant Nutrition*, 38(7), 1036-1049.

Baratella, V., Renzaglia, M. and 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. and 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.

Barton, L. and Colmer, T.D. (2011). Granular wetting agents ameliorate water repellency in turfgrass of contrasting soil organic matter content. *Plant soil*, 348 (1), 411–424.

Basirat, M., Mousavi, S., Abbaszadeh, S., Ebrahimi, M. and Zarebanadkouki, M. (2019). The rhizosheath: a potential root trait helping plants to tolerate drought stress. *Plant and Soil*, 445(1-2), 565-575.

Bates, L. and Hall, A., 1981. Stomatal closure with soil water depletion not associated with changes in bulk leaf water status. *Oecologia*, 50(1), 62-65.

Bates, T. and Lynch, J. 2000. Plant growth and phosphorus accumulation of wild type and two root hair mutants of *Arabidopsis thaliana* (*Brassicaceae*). *American Journal of Botany*, 87(7), 958-963.

Beadle, C. and Long, S., 1985. Photosynthesis — is it limiting to biomass production?. *Biomass*, 8(2), 119-168.

Benard, P., Kroener, E., Vontobel, P., Kaestner, A. and Carminati, A. (2016). Water percolation through the root-soil interface. *Advances in Water Resources*, 95, 190-198.

Benard, P., Zarebanadkouki, M. and Carminati, A. (2018). Physics and hydraulics of the rhizosphere network. *Journal of Plant Nutrition and Soil Science*, 182 (1), 5-8.

Bergmann, D., Zehfus, M., Zierer, L., Smith, B. and Gabel, M. (2009). Grass rhizosheaths: associated bacterial communities and potential for nitrogen fixation. *Western North American Naturalist*, 69(1), 105-114.

Bois, J., Couchat, P. and Lasceve, G. (1985). Relationships between transpiration and photosynthesis during a water stress. *Acta Horticulturae*, (171), 297-314.

Brown, L., George, T., Neugebauer, K. and White, P. (2017). The rhizosheath – a potential trait for future agricultural sustainability occurs in orders throughout the angiosperms. *Plant and Soil*, 418(1–2), 115-128.

Brown, L., George, T., Thompson, J., Wright, G., Lyon, J., Dupuy, L., Hubbard, S. and White, P. (2012). What are the implications of variation in root hair length on tolerance to phosphorus deficiency in combination with water stress in barley (*Hordeum vulgare*)?. *Annals of Botany*, 110(2), 319-328.

Brumbaugh, E. and Peterson, M. (2001). The use if a non-ionic surfactant to alleviate the effects of compacted soil on corn (*Zea mays L*.) yield and root growth. *Proceedings of the 6th International Symposium on Adjuvants for Agrochemicals,* Amsterdam, Netherlands, 1-4.

Buckley, T. (2005). The control of stomata by water balance. *New Phytologist*, 168(2), 275-292.

Bueckert, R. A. (2013). General principles of plant water relations. *Prairie Soils & Crops Journal*, 6, 107-118.

Burak, E., Quinton, J. and Dodd, I. (2021). Root hairs are the most important root trait for rhizosheath formation of barley (*Hordeum vulgare L.*), maize (*Zea mays L.*), and Lotus japonicus (*Gifu*). *Annals of Botany*, 128(1), 45-57

Canales, J., Contreras-López, O., Álvarez, J. and Gutiérrez, R. (2017). Nitrate induction of root hair density is mediated by TGA1/TGA4 and CPC transcription factors in *Arabidopsis thaliana.* *The Plant Journal*, 92(2), 305-316.

Carminati, A., Moradi, A., Vetterlein, D., Vontobel, P., Lehmann, E., Weller, U., Vogel, H. and Oswald, S. (2010). Dynamics of soil water content in the rhizosphere. *Plant and Soil*, 332(1-2), 163-176.

Carminati, A., Passioura, J., Zarebanadkouki, M., Ahmed, M., Ryan, P., Watt, M. and Delhaize, E. (2017). Root hairs enable high transpiration rates in drying soils. *New Phytologist*, 216(3), 771-781.

Carminati, A. and Vetterlein, D. (2012). Plasticity of rhizosphere hydraulic properties as a key for efficient utilization of scarce resources. *Annals of Botany*, 112(2), 277-290.

Carminati, A., Vetterlein, D., Koebernick, N., Blaser, S., Weller, U. and Vogel, H. (2013). Do roots mind the gap?. *Plant and Soil*, 367(1-2), 651-661.

Čereković, N., Pagter, M., Kristensen, H., Brennan, R. and Petersen, K. (2014). Effects of deficit irrigation during flower initiation of two blackcurrant (*Ribes nigrum L.*) cultivars. *Scientia Horticulturae*, 168, 193-201.

Chaichi, M., Keshavarz-Afshar, R., Lu, B. and 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(4), 457-466.

Chaichi, M., Nurre, P., Slaven, J. and Rostamza, M. (2015). Surfactant application on yield and irrigation use efficiency in corn under limited irrigation. *Soil Science*. 55 (1), 386-393.

Cid-Ballarin, C., Muñoz-Carpena, R., Socorro-Monzón, A. and González-Tamargo, G. (1998). Wetting agent effects on peat properties related to nutrient solution losses and plant growth. *Acta Horticulturae*, (458), 161-170.

Cisar, J.L., Williams, K.E, Vivas, H.E., Haydu, J.J. (2000). The occurrence and alleviation by surfactants of soil-water repellency on sand-based turfgrass systems. *Journal of Hydrology*, 231-232, 352-358.

Claeys, H. and Inze, D. (2013). The agony of choice: How plants balance growth and survival under water-limiting conditions. *Plant Physiology*, 162(4), 1768-1779.

Comstock, J. (2002). Hydraulic and chemical signalling in the control of stomatal conductance and transpiration. *Journal of Experimental Botany*, 53(367), 195-200.

Cook, G., Dixon, J. and Leopold, A. (1964). Transpiration: its effects on plant leaf temperature. *Science*, 144(3618), 546-547.

Cooley, E.T., Lowery, B., Kelling, K.A., Speth, P.E., Madison, F.W., Bland, W.L. and Tapsieva, A. (2009). Surfactant use to improve soil water distribution and reduce nitrate leaching in potatoes. *Soil Science*, 174, 321-329.

Dadresan, M., Luthe, D., Reddivari, L., Chaichi, M. and 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(22), 2807-2820.

Daneshnia, F., Amini, A. and Chaichi, M. R. (2016). Berseem clover quality and basil essential oil yield in intercropping system under limited irrigation treatments with surfactant. *Agricultural Water Management*, 164, 331-339.

Datta, S., Kim, C., Pernas, M., Pires, N., Proust, H., Tam, T., Vijayakumar, P. and Dolan, L. (2011). Root hairs: development, growth and evolution at the plant-soil interface. *Plant and Soil*, 346(1-2), 1-14.

Davies, W., Wilkinson, S. and Loveys, B. (2002). Stomatal control by chemical signalling and the exploitation of this mechanism to increase water use efficiency in agriculture. *New Phytologist*, 153(3), 449-460.

Day, W., Lawlor, D. and Legg, B. (1981). The effects of drought on barley: soil and plant water relations. *The Journal of Agricultural Science*, 96(1), 61-77.

Debaeke, P., Pellerin, S. and Scopel, E. (2017). Climate-smart cropping systems for temperate and tropical agriculture: mitigation, adaptation, and trade-offs. *Cahiers Agricultures*, 26, 1-12.

Dekker, L.W., Ritsema, C.J. and Oostindie, K. (2004). Dry spots in gold courses: Occurrence, Amelioration and Prevention. *Acta Horticulturae*, 661, 99-104.

Delhaize, E., James, R. and Ryan, P. (2012). Aluminium tolerance of root hairs underlies genotypic differences in rhizosheath size of wheat (*Triticum aestivum*) grown on acid soil. *New Phytologist*, 195(3), 609-619.

Delhaize, E., Rathjen, T. and Cavanagh, C. (2015). The genetics of rhizosheath size in a multiparent mapping population of wheat. *Journal of Experimental Botany*, 66(15), 4527-4536.

Demura, T. and Ye, Z. (2010). Regulation of plant biomass production. *Current Opinion in Plant Biology*, 13(3), 298-303.

de Ollas, C. and Dodd, I. (2016). Physiological impacts of ABA–JA interactions under water-limitation. *Plant Molecular Biology*, 91(6), 641-650.

Dodd, I. C. (2005). Root-To-Shoot Signalling: Assessing the roles of 'up' in the up and down world of long-distance signalling in *Planta. Plant and Soil*, 274(1-2), 251-270.

Dodd, I.C. and Diatloff, E. (2016). Enhanced root growth of the *brb* (bald root barley) mutant in drying soil allows similar shoot physiological responses to soil water deficit as wild-type plants. *Functional Plant Biology*, 43(2), 199-206.

Doussan, C., Pierret, A., Garrigues, E. and Pagès, L. (2006). Water uptake by plant roots: II – Modelling of water transfer in the soil root-system with explicit account of flow within the root system – comparison with experiments. *Plant and Soil*, 283 (1-2), 99-117.

Drinkwater, L. and Snapp, S. (2007). Nutrients in agroecosystems: Rethinking the management paradigm. *Advances in Agronomy*, 92, 163-186.

Dube, A., Stevensoni, R., Thurtell, W. and Neumann, H. (1975). Steady state resistance to water flow in corn under well watered conditions. *Canadian Journal of Plant Science*, 55, 941-948.

Elliott, G. (1992). Imbibition of water by rockwool-peat container media amended with hydrophilic gel or wetting agent. *Journal of the American Society for Horticultural Science*, 117(5), 757-761.

Ericksen, P., Ingram, J. and Liverman, D., 2009. Food security and global environmental change: emerging challenges. *Environmental Science & Policy*, 12(4), 373-377.

Fan, J., Du, Y., Turner, N., Wang, B., Fang, Y., Xi, Y., Guo, X. and Li, F. (2015). Changes in root morphology and physiology to limited phosphorus and moisture in a locally-selected cultivar and an introduced cultivar of *Medicago sativa L.* growing in alkaline soil. *Plant and Soil*, 392(1-2), 215-226.

Fan, M., Shen, J., Yuan, L., Jiang, R., Chen, X., Davies, W. and Zhang, F. (2011). Improving crop productivity and resource use efficiency to ensure food security and environmental quality in China. *Journal of Experimental Botany*, 63(1), 13-24.

FAO. (2017). Global database of GHG emissions related to feed crops: Methodology. Version 1. Livestock environmental assessment and performance partnership. FAO, Rome, Italy.

FAO, IFAD, UNICEF, WFP and WHO. (2020). The state of food security and nutrition in the world 2020. Transforming food systems for affordable healthy diets. FAO, Rome, Italy.

Feng, G. L., Letey, J and Wu, L. (2002). The influence of two surfactants on infiltration into a water-repellent soil. *Soil Science Society of America Journal*,66 (3), 361–367.

Foehse, D. and Jungk, A. (1983). Influence of phosphate and nitrate supply on root hair formation of rape, spinach and tomato plants. *Plant and Soil*, 74(3), 359-368.

Fróna, D., Szenderák, J. and Harangi-Rákos, M., 2019. The challenge of feeding the world. Sustainability, 11, 1-18.

Gahoonia, T. and Nielsen, N., 1998. Direct evidence on participation of root hairs in phosphorus (32P) uptake from soil. *Plant and Soil*, 198, 147-152.

Galmés, J., Medrano, H. and Flexas, J. (2007). Photosynthetic limitations in response to water stress and recovery in Mediterranean plants with different growth forms. *New Phytologist*, 175(1), 81-93.

Garg, B. (2003). Nutrient uptake and management under drought: nutrient-moisture interaction. *Current Agriculture*, 27(1-2), 1-8.

George, T., Brown, L., Ramsay, L., White, P., Newton, A., Bengough, A., Russell, J. and Thomas, W. (2014). Understanding the genetic control and physiological traits associated with rhizosheath production by barley (*Hordeum vulgare*). *New Phytologist*, 203(1), 195-205.

Giorio, P., Sorrentino, G. and d'Andria, R. (1999). Stomatal behaviour, leaf water status and photosynthetic response in field-grown olive trees under water deficit. *Environmental and Experimental Botany,* 42(2), 95-104.

Godfray, C., Beddington, J., Crute, I., Haddad, L., Lawrence, D., Muir, J., Pretty, J., Robinson, S., Thomas, M. and Toulmin, C. (2010). Food security: the challenge of feeding 9 billion people. *Science*, 327(5967), 812-818.

Gollan, T., Passioura, J. and Munns, R. (1986). Soil water status affects the stomata conductance of fully turgid wheat and sunflower leaves. *Functional Plant Biology*, 13(4), 459-464.

Good, A., Shrawat, A. and Muench, D. (2004). Can less yield more? Is reducing nutrient input into the environment compatible with maintaining crop production?. *Trends in Plant Science*, 9(12), 597-605.

Green, R. (1962). *Infiltration of water into soils as influenced by antecedent moisture*. Ph.D. Iowa State University.

Gross, A., Mohamed, R., Anda, M. and Ho, G. (2011). Effectiveness of wetting agents for irrigating sandy soils. *Water*, 38 (2).

Gu, D., Zhen, F., Hannaway, D., Zhu, Y., Liu, L., Cao, W. and Tang, L. (2017). Quantitative Classification of Rice (Oryza sativa L.) Root length and diameter using image analysis. *PLOS ONE*, 12(1).

Haling, R., Brown, L.K, Bengough, A., Valentine, T.A., White, P.J., Young, I. and George, T. (2014). Root hair length and rhizosheath mass depend on soil porosity, strength and water content in barley genotypes. *Planta,* 239, 643-651.

Haling, R., Brown, L., Bengough, A., Young, I., Hallett, P., White, P. and George, T. (2013). Root hairs improve root penetration, root–soil contact, and phosphorus acquisition in soils of different strength*. Journal of Experimental Botany*, 64(12), 3711-3721.

Haling, R., Richardson, A., Culvenor, R., Lambers, H. and Simpson, R. (2010). Root morphology, root-hair development and rhizosheath formation on perennial grass seedlings is influenced by soil acidity. *Plant and Soil*, 335(1-2), 457-468.

Hartman, S. (2020). Trapped in the rhizosheath: root-bacterial interactions modulate ethylene signaling*. Plant Physiology*, 183(2), 443-444.

Hartnett, D., Wilson, G., Ott, J. and Setshogo, M. (2013). Variation in root system traits among African semi-arid savanna grasses: Implications for drought tolerance. *Austral Ecology.* 38, 383-392.

Hayat, F., Ahmed, M. A., Zarebanadkouki, M., Javaux, M., Cai, G. and Carminati, A. (2020). Transpiration reduction in maize (*Zea mays L.*) in response to soil drying. *Frontiers in Plant Science*,10, (1695).

Hellkvist, J., Richards, G. and Jarvis, P. (1974). Vertical gradients of water potential and tissue water relations in sitka spruce trees measured with the pressure chamber. *The Journal of Applied Ecology*, 11(2), 637-667.

Hodge, A. (2004). The plastic plant: root responses to heterogeneous supplies of nutrients. *New Phytologist*, 162(1), 9-24.

Hou, E., Chen, C., McGroddy, M. and Wen, D. (2012). Nutrient limitation on ecosystem productivity and processes of mature and old-growth subtropical forests in China. *PLoS ONE*, 7(12).

Hsiao, T. (1973). Plant responses to water stress. *Annual Review of Plant Physiology*, 24(1), 519-570.

Htoon, W., Jogloy, S., Vorasoot, N., Toomsan, B., Kaewpradit, W., Puppala, N. and Patanotahi, A. (2014). Nutrient uptakes and their contributions to yield in peanut genotypes with different levels of terminal drought resistance. *Turkish Journal of Agriculture and Forestry*, 38, 781-791.

Huber, A., Melcher, P., Piñeros, M., Setter, T. and Bauerle, T. (2019). Signal coordination before, during and after stomatal closure in response to drought stress. *New Phytologist*, 224(2), 675-688.

Hutchens, W., Gannon, T., Shew, H., Ahmed, K. and Kerns, J. (2020). Soil surfactants influence fungicide movement in United States Golf Association putting green soil. *Journal of Environmental Quality*, 49(2), 450-459.

IPCC. (2019). Climate change and land: an IPCC special report on climate change, desertification, land degradation, sustainable land management, food security, and greenhouse gas fluxes in terrestrial ecosystems [Shukla, P.R., Skea, J., Calvo Buendia, E., Masson-Delmotte, V., Pörtner, H.O., Roberts, D.C., Zhai, P., Slade, R., Connors, S., van Diemen, R., Ferrat, M., Haughey, E., Luz, S., Neogi, S., Pathak, M., Petzold, J., Portugal Pereira, J., Vyas, P., Huntley, E., Kissick, K., Belkacemi, M., Malley, J., (eds.)]. In press.

Ishiguro, M. and Koopal, L. (2016). Surfactant adsorption to soil components and soils. *Advances in Colloid and Interface Science*, 231, 59-102.

Jafarian, S., Chaichi, M.R, Mehrdad, M.G. (2016). Effects of surfactant and limited irrigation on forage yield and quality of alfalfa (*Medicago sativa L*.). *Australian Journal of Crop Science*, 10 (1), 386-393.

James, R., Weligama, C., Verbyla, K., Ryan, P., Rebetzke, G., Rattey, A., Richardson, A. and Delhaize, E. (2016). Rhizosheaths on wheat grown in acid soils: phosphorus acquisition efficiency and genetic control. *Journal of Experimental Botany*, 67(12), 3709-3718.

Jauregui, I., Rothwell, S., Taylor, S., Parry, M., Carmo-Silva, E. and Dodd, I.C. (2018). Whole plant chamber to examine sensitivity of cereal gas exchange to changes in evaporative demand. *Plant Methods*, 14(1), 1-13.

Jezek, M. and Blatt, M. (2017). The membrane transport system of the guard cell and its integration for stomatal dynamics. *Plant Physiology*, 174(2), 487-519.

Jin, J. (2012). Changes in the efficiency of fertiliser use in China. *Journal of the Science of Food and Agriculture*, 92(5), 1006-1009.

Jungk, A. (2001). Root hairs and the acquisition of plant nutrients from soil. *Journal of Plant Nutrition and Soil Science*, 164(2), 121-129.

Junjittakarn, J., Pimratch, S., Jogloy, S., Htoon, W., Singkham, N., Vorasoot, N., Toomsan, B., Holbrook, C. and Patanothai, A. (2013). Nutrient uptake of peanut genotypes under different water regimes. *International Journal of Plant Production*, 7(4), 677-692.

Karagunduz, A., Pennell, K. and Young, M. (2001). Influence of a non-ionic surfactant on the water retention properties of unsaturated soils. *Soil Science Society of America Journal*, 65, 1392-1399.

Karnok, K.J. and Tucker, K.A. (2001). Fight localized dry spots through the roots: Using wetting agents to treat an entire green may be the best way to combat localized dry spots. *Golf Course Management*, 69(7), 58-60.

Karnok, K.J. and Tucker K.A. (2001). Wetting agent treated hydrophobic soil and its effect on colour, quality and root growth of creeping bentgrass. *International Turfgrass Society Research Journal,* 9, 537-541.

Kirkham, M. (2004). Principles of Soil and Plant Water Relations. California: Elsevier Academic Press.

Klamer, F., Vogel, F., Li, X., Bremer, H., Neumann, G., Neuhäuser, B., Hochholdinger, F. and Ludewig, U. (2019). Estimating the importance of maize root hairs in low phosphorus conditions and under drought. *Annals of Botany*, 124(6), 961-968.

Koebernick, N., Schlüter, S., Blaser S. and Vetterlein, D. (2018). Root-soil contact dynamics of *Vicia faba* in sand. *Plant and Soil*, 431(1–2), 417-431.

Kostka, S.J. (2000). Amelioration of water repellency in highly managed soils and the enhancement of turfgrass performance through the systematic application of surfactants. *Journal of Hydrology*, 231-232, 359-368.

Kostka, S. J., Cisar, J.L., Mitra, S., Park, D., Ritsema, C., Dekker, L. and Franklin, M. (2007). Irrigation efficiency. Soil surfactants can save water and help maintain turfgrass quality. *Golf Course Industry*, 2007, 91-95.

Kostka, S.J. Dekker, L.W., Ritsema, C.J. Cisar, J.L. and Franklin M. K. (2007). Surfactants as management tools for ameliorating soil water repellency in turfgrass systems. *Proceedings of*

the 8th International Symposium on Adjuvants for Agrochemicals. International Society for Agrochemical Adjuvants (ISAA).

Lambers, H., Shane, M., Cramer, M., Pearse, S. and Veneklaas, E. (2006). Root structure and functioning for efficient acquisition of phosphorus: Matching morphological and physiological traits. *Annals of Botany*, 98(4), 693-713.

Lee, J. and Mudge, K. (2013). Water deficit affects plant and soil water status, plant growth, and ginsenoside contents in American ginseng. *Horticulture, Environment, and Biotechnology*, 54(6), 475-483.

Lehrsch, G. (2013). Surfactant effects on the water-stable aggregation of wettable soils from the continental USA. *Hydrological Processes*, 27, 1739-1750.

Lehrsch, G. A., Sojka, R.E., Reed, J.L., Henderson, R.A., and Kostka, S.J. (2011). Surfactant and irrigation effects on wettable soils: runoff, erosion, and water retention responses. *Hydrological Prosseces*, 25, 766–777.

Leitner, D., Klepsch, S., Ptashnyk, M., Marchant, A., Kirk, G., Schnepf, A. and Roose, T. (2009). A dynamic model of nutrient uptake by root hairs. *New Phytologist*, 185(3), 792-802.

Liu, G., Li, Y. and Alva, A. (2012). Water potential vs. pressure in relation to water movement and transpiration in plants. *International Journal of Agronomy and Plant Production*, 3(10), 369-373.

Liu, T., Ye, N., Song, T., Cao, Y., Gao, B., Zhang, D., Zhu, F., Chen, M., Zhang, Y., Xu, W. and Zhang, J. (2018). Rhizosheath formation and involvement in foxtail millet (*Setaria italica*) root growth under drought stress. *Journal of Integrative Plant Biology*, 61(4), 449-462.

Liu, X., Zhang, W., Wang, X., Cai, Y. and Chang, J. (2015). Root–soil air gap and resistance to water flow at the soil–root interface of *Robinia pseudoacacia*. *Tree Physiology*, 35(12), 1343-1355.

Lobet, G., Couvreur, V., Meunier, F., Javaux, M. and Draye, X. (2014). Plant water uptake in drying soils. *Plant Physiology*, 164(4), 1619-1627.

Lu, N. and Likos, W. (2004). Rate of capillary rise in soil. *Journal of Geotechnical and Geoenvironmental Engineering*, 130(6), 646-650.

Lyons, E. M., Jordan, K.S, and Carey, K. (2009). Use of wetting agents to relieve hydrophobicity in sand rootzone putting greens in a temperate climate zone. *International Turfgrass Society Research Journal*, 11(2), 1131-1138.

Madsen, M.D., Coronel, E.G. and Hopkins, B.G. (2013). Soil surfactant products for improving hydrologic function in post-fire water-repellent soil. *Soil Science Society of America Journal*, 77(5), 1825-1830.

Marasco, R., Mosqueira, M., Fusi, M., Ramond, J., Merlino, G., Booth, J., Maggs-Kölling, G., Cowan, D. and Daffonchio, D. (2018). Rhizosheath microbial community assembly of sympatric desert speargrasses is independent of the plant host. *Microbiome*, 6(215), 1-18.

McAvoy, R. (1994). Nitrate nitrogen movement through the soil profile beneath a containerized greenhouse crop irrigated with two leaching fractions and two wetting agent levels. *Journal of the American Society for Horticultural Science*, 119(3), 446-451.

Mehrvarz, S., Chaichi, M., Hashemi, M. and Parsinejad, M. (2013). Yield and growth response of corn (*Zea Mays L*. S.C. 704) to surfactant under deficit irrigation. *Scientific Journal of Agronomy and Plant Breeding*. 1(1), 41-51.

Mengel, K. (1982). Factors of plant nutrient availability relevant to soil testing. In: Németh K. *Application of Electro-Ultrafiltration (EUF) in Agricultural Production*. Dordrecht: Springer.

Mishra, S. (2004). Photosynthesis in plants. New Delhi: Discovery Publishing House.

Mobbs, T., Peters, R., Davenport, J., Evans, M. and Wu, J. (2012). Effects of four soil surfactants on four soil-water properties in sand and silt loam. *Journal of Soil and Water Conservation*, 67(4), 275-283.

Moradi, A., Carminati, A., Lamparter, A., Woche, S., Bachmann, J., Vetterlein, D., Vogel, H. and Oswald, S. (2012). Is the rhizosphere temporarily water repellent?. *Vadose Zone Journal*, 11(3), 1-8.

Moradi, A., Carminati, A., Vetterlein, D., Vontobel, P., Lehmann, E., Weller, U., Hopmans, J., Vogel, H. and Oswald, S. (2011). Three-dimensional visualization and quantification of water content in the rhizosphere. *New Phytologist*, 192(3), 653-663.

Moradi, P., Ford-Lloyd, B. and Pritchard, J. (2014). Plant-water responses of different medicinal plant thyme (*Thymus spp*.) species to drought stress condition. *Australian Journal of Crop Science*, 8(5), 666-673.

Moreno-Espíndola, I., Ferrara-Guerrero, M., De León-González, F., Rivera-Becerril, F., Mayorga-Reyes, L. and Pérez, N. (2018). Enzymatic activity and culturable bacteria diversity in rhizosphere of amaranth, as indicators of crop phenological changes. *Botanical Sciences*, 96(4), 640-649.

Mouat, M. and Nes, P. (1985). Soil water content affects the availability of phosphate. *Proceedings of the New Zealand Grassland Association*, 46, 185-189.

Munns, R., Passioura, J., Guo, J., Chazen, O. and Cramer, G. (2000). Water relations and leaf expansion: importance of time scale. *Journal of Experimental Botany*, 51(350), 1495-1504.

Nambiar, E., 1976. Uptake of Zn65 from dry soil by plants. *Plant and Soil*, 44(1), 267-271.

Naveed, M., Ahmed, M., Benard, P., Brown, L., George, T., Bengough, A., Roose, T., Koebernick, N. and Hallett, P. (2019). Surface tension, rheology and hydrophobicity of rhizodeposits and seed mucilage influence soil water retention and hysteresis. *Plant and Soil*, 437(1-2), 65-81.

Naveed, M., Brown, L., Raffan, A., George, T., Bengough, A., Roose, T., Sinclai, I., Koebernick, N., Cooper, L., Hackett, C. and Hallett, P. (2017). Plant exudates may stabilize or weaken soil depending on species, origin and time. *European Journal of Soil Science*, 68(6), 806-816.

Nemmati, M.R. and Yeong, K.Y. (2017). Wetting agent effects on plant growth. *Acta Horticulturae*, 1168, 63-70.

Nobel, P. and Cui, M. (1992). Shrinkage of attached roots of *Opuntia ficus-indica* in response to lowered water potentials-Predicted consequences for water uptake or loss to soil. *Annals of Botany*, 70(6), 485-491.

North, G. and Nobel, P. (1997). Drought-induced changes in soil contact and hydraulic conductivity for roots of *Opuntia ficus-indica* with and without rhizosheaths. *Plant and Soil*, (191), 249–258.

North, G. and Nobel, P. (1997). Root-soil contact for the desert succulent *Agave deserti* in wet and drying soil. *New Phytologist*, 135(1), 21-29.

Nye, P.H. (1994). The effect of root shrinkage on soil water inflow. *Philosophical Transactions of the Royal Society of London*. Series B: Biological Sciences, 345(1314), 395–402.

O'Geen, A. T. (2013) Soil Water Dynamics. *Nature Education Knowledge*, 4(5), 9.

Oostindie, K., Dekker L., Wesseling J. G., Moore, D. (2012). Influence of a single soil surfactant application on potato ridge moisture dynamics and crop yield in a water repellent sandy soil. *Acta Horticulturae*, 938, 341-346.

Oostindie K., Dekker, L.W., Wesseling, J.G., and Ritsema, C.J. (2008). Soil surfactant stops water repellency and preferential flow paths. *Soil Use and Management*, 24 (1), 409–415.

Ortíz-Castro, R., Contreras-Cornejo, H., Macías-Rodríguez, L. and López-Bucio, J. (2009). The role of microbial signals in plant growth and development. *Plant Signaling & Behavior*, 4(8), 701-712.

Othman, A., Amer, W., Fayez, M. and Hegazi, N. (2004). Rhizosheath of sinai desert plants is a potential repository for associative diazotrophs. *Microbiological Research*, 159(3), 285-293.

Pang, J., Ryan, M., Siddique, K. and Simpson, R. (2017). Unwrapping the rhizosheath. *Plant and Soil*, 418(1-2), 129-139.

Park, D., Cisar, J.L., Williams, K. and Snyder, G. (2004). Alleviation of soil water repellency in sand based bermudagrass in South Florida. *Acta Horticulturae*, (661), 111-115.

Parry, M. and Hawkesford, M. (2010). Food security: increasing yield and improving resource use efficiency. *Proceedings of the Nutrition Society*, 69(4), 592-600.

Passioura, J.B. (1988). Root signals control leaf expansion in wheat seedlings growing in drying soil. *Australian Journal of Plant Physiology*, 15, 687-693.

Passioura, J.B. and Munns, R., 2000. Rapid environmental changes that affect leaf water status induce transient surges or pauses in leaf expansion rate. *Functional Plant Biology*, 27(10), 941-948.

Pou, A., Flexas, J., Alsina, M., Bota, J., Carambula, C., de Herralde, F., Galmés, J., Lovisolo, C., Jiménez, M., Ribas-Carbó, M., Rusjan, D., Secchi, F., Tomàs, M., Zsófi, Z. and Medrano, H. (2008). Adjustments of water use efficiency by stomatal regulation during drought and recovery in the drought-adapted Vitis hybrid Richter-110 (*V. berlandieri × V. rupestris*). *Physiologia Plantarum*, 134(2), 313-323.

Quarrie, S., Whitford, P., Appleford, N., Wang, T., Cook, S., Henson, I. & Loveys, B. (1988). A monoclonal antibody to (S)-abscisic acid: its characterisation and use in a radioimmunoassay for measuring abscisic acid in crude extracts of cereal and lupin leaves. *Planta*, 173(3), 330-339.

Razaq, M., Zhang, P., Shen, H. and Salahuddin (2017). Influence of nitrogen and phosphorous on the growth and root morphology of Acer mono. *PLOS ONE*, 12(2), 1-13.

Razavi, B., Blagodatskaya, E. and Kuzyakov, Y. (2016). Temperature selects for static soil enzyme systems to maintain high catalytic efficiency. *Soil Biology and Biochemistry*, 97, 15-22.

Reinikeinen, O. and Herranen, M. (1997). The influence of wetting agent on physical properties of peat. *Acta Horticulturae*, 450, 375-380.

Reinson, J., Fredlund, D. and Wilson, G. (2005). Unsaturated flow in coarse porous media. *Canadian Geotechnical Journal*, 42(1), 252-262.

Robinson, D. and Rorison, I. (1987). Root hairs and plant growth at low nitrogen availabilities. *New Phytologist*, 107(4), 681-693.

Rosegrant, M. (2003). Global food security: Challenges and policies. *Science*, 302(5652), 1917-1919.

Ruemmele, B. and Amador, J. (1999). Turfgrass and soil responses to soil wetting agents. *Journal of Turfgrass Management*, 2(4), 71-82.

Saliendra, N., Sperry, J. and Comstock, J. (1995). Influence of leaf water status on stomatal response to humidity, hydraulic conductance, and soil drought in *Betula occidentalis*. *Planta*, 196(2), 357-366.

Saradadevi, R., Palta, J. and Siddique, K. (2017). ABA-mediated stomatal response in regulating water use during the development of terminal drought in wheat. *Frontiers in Plant Science*, 8(1251), 1-13.

Schiavon, M., Leinauer, B., Serena, M., Maier, B. and Sallenave, R. (2014). Plant growth regulator and soil surfactants' effects on saline and deficit irrigated warm-season grasses: I. Turf quality and soil moisture. *Crop Science*, 54 (6), 2815-2826.

Schmidhalter, U., Evequoz, M., Camp, K. and Studer, C. (1998). Sequence of drought response of maize seedlings in drying soil. *Physiologia Plantarum*, 104(2), 159-168.

Schmidhuber, J. and Tubiello, F. (2007). Global food security under climate change. *Proceedings of the National Academy of Sciences*, 104(50), 19703-19708.

Sellin, A. (1996). Base water potential of *Picea abies* as a characteristic of the soil water status. *Plant and Soil*, 184(2), 273-280.

Serena, M., Schiavon, M., Sallenave, R. and Leinauer, B., 2020. Drought avoidance of warm-season turfgrasses affected by irrigation system, soil surfactant revolution, and plant growth regulator trinexapac-ethyl. *Crop Science*, 60(1), 485-498.

Serena, M., Sportelli, M., Sevostianova, E., Sallenave, R. and Leinauer, B., 2018. Combining trinexapac-ethyl with a soil surfactant reduces bermudagrass irrigation requirements. *Agronomy Journal*, 110(6), 2180-2188.

Shafran, A., Gross, A., Ronen, Z., Weisbrod, N. and Adar, E. (2005). Effects of surfactants originating from reuse of greywater on capillary rise in the soil. *Water Science and Technology*, 52(10-11), 157-166.

Sibley, J., Yang, X., Lu, W. and Eakes, D. (2018). Effects of a nonionic surfactant on growth, photosynthesis, and transpiration of New Guinea impatiens in the greenhouse. *Journal of Environmental Horticulture*, 36(2), 73-81.

Simonneau, T., Barrieu, P. and Tardieu, F. (1998). Accumulation rate of ABA in detached maize roots correlates with root water potential regardless of age and branching order. *Plant, Cell and Environment*, 21(11), 1113-1122.

Sinclair, T., Tanner, C. and Bennett, J. (1984). Water use efficiency in crop production. *Bioscience*, 34(1), 36-40.

Singer, M. and Munns, D. (2002). Soils: An Introduction: Pearson.

Singh, B. and Singh, G. (2004). Influence of soil water regime on nutrient mobility and uptake by *Dalbergia sissoo* seedlings. *Tropical Ecology*, 45(2), 337-340.

Snyder, G. H. and Cisar, J. L. (2004). Evaluation of physical and chemical methods for alleviating soil-water repellency in turfgrass in Florida. *Soil and Crop Society of Florida*, 63, 76-83.

Soldat, D., Lowery, B. and Kussow, W. (2010). Surfactants increase uniformity of soil water content and reduce water repellency on sand-based golf putting greens. *Soil Science*, 175(3), 111-117.

Sorgonà, A., Abenavoli, M., Gringeri, P., Lupini, A. and Cacco, G. (2007). Root architecture plasticity of citrus rootstocks in response to nitrate availability. *Journal of Plant Nutrition*, 30(11), 1921-1932.

Sullivan, D.G., Nuti, R. C. and Truman, C.C. (2009). Evaluating a non-ionic surfactant as a tool to improve water availability in irrigated cotton. *Hydrological Processes*, 23, 2326–2334.

Taiz, L. and Zeiger, E. (2002). Plant Physiology. USA: Sinauer Associates Inc.

Tardieu, F., Parent, B. and Simonneau, T. (2010). Control of leaf growth by abscisic acid: hydraulic or non-hydraulic processes?. *Plant, Cell & Environment*, 33(4), 636-647.

Torres-Ruiz, J., Diaz-Espejo, A., Perez-Martin, A. and Hernandez-Santana, V. (2015). Role of hydraulic and chemical signals in leaves, stems and roots in the stomatal behaviour of olive trees under water stress and recovery conditions. *Tree Physiology*, 35(4), 415-424.

Trinchera, A. and Baratella, V. (2018). Use of a non-ionic water surfactant in lettuce fertigation for optimizing water use, improving nutrient use efficiency, and increasing crop quality, *Water*, 10(5), 1-15.

Trubat, R., Cortina, J. and Vilagrosa, A. (2006). Plant morphology and root hydraulics are altered by nutrient deficiency in *Pistacia lentiscus (L.). Trees,* 20(3), 334-339.

Trubat, R., Cortina, J. and Vilagrosa, A. (2012). Root architecture and hydraulic conductance in nutrient deprived *Pistacia lentiscus L*. seedlings. *Oecologia*, 170(4), 899-908.

Tuteja, N. and Sopory, S. (2008). Chemical signalling under abiotic stress environment in plants. *Plant Signalling & Behaviour*, 3(8), 525-536.

Tyree, M. (2003). Matric Potential. In: Encyclopaedia of water science. New York: Marcel Dekker, 615-617.

Vatter, T., Neuhäuser, B., Stetter, M. and Ludewig, U. (2015). Regulation of length and density of Arabidopsis root hairs by ammonium and nitrate. *Journal of Plant Research*, 128(5), 839-848.

Voroney, R. and Heck, R. (2021). The soil habitat. In: E. Paul, ed., *Soil microbiology, ecology and biochemistry*, 4th ed. Academic Press.

Wang, Y., Thorup-Kristensen, K., Jensen, L. and Magid, J. (2016). Vigorous root growth is a better indicator of early nutrient uptake than root hair traits in spring wheat grown under low fertility. *Frontiers in Plant Science*, 7,1-9.

Wasaya, A., Zhang, X., Fang, Q. and Yan, Z. (2018). Root phenotyping for drought tolerance: A review. *Agronomy*, 8(11),1-19.

Watt, M., McCully, M. and Canny, M. (1994). Formation and stabilization of rhizosheaths of *Zea mays L.* (Effect of soil water content). *Plant Physiology*, 106(1), 179-186.

Wheeler, T. and von Braun, J. (2013). Climate change impacts on global food security. *Science*, 341(6145), 508-513.

Wiel-Shafran, A., Ronen, Z., Weisbrod, N., Adar, E. and Gross, A. (2006). Potential changes in soil properties following irrigation with surfactant-rich greywater. *Ecological Engineering*, 26(4), 348-354.

Wilkinson, S. (1999). PH as a stress signal. *Plant Growth Regulation*, 29, 87-99.

Wilkinson, S. and Davies, W. J. (2002). ABA-based chemical signalling: the co-ordination of responses to stress in plants. *Plant Cell and Environment*, 25, 195-210.

Wolkowski, R., Kelling, K. A. and Oplinger, E. (1985). Evaluation of three wetting agents as soil additives for improving crop yield and nutrient availability. *Agronomy Journal*, 77(5), 695-698.

Wu, A., Fang, Y., Liu, S., Wang, H., Xu, B., Zhang, S., Deng, X., Palta, J., Siddique, K. and Chen, Y., 2021. Root morphology and rhizosheath acid phosphatase activity in legume and graminoid species respond differently to low phosphorus supply. *Rhizosphere*, 19, 100391, 1-13.

Wullstein, L. (1980). Nitrogen fixation (acetylene reduction) associated with rhizosheaths of Indian ricegrass used in stabilization of the Slick Rock, Colorado tailings pile. *Journal of Range Management*, 33(3), 204-206.

Wullstein, L. (1991). Variation in N_2 fixation (C_2H_2 reduction) associated with rhizosheaths of indian ricegrass (*Stipa hymenoides*). *American Midland Naturalist*, 126(1), 76-81.

Wullstein, L., Bruening, M. and Bollen, W. (1979). Nitrogen fixation associated with sand grain root sheaths (rhizosheaths) of certain xeric grasses. *Physiologia Plantarum*, 46(1), 1-4.

Xinchun, C., Mengyang, W., Xiangping, G., Yalian, Z., Yan, G., Nan, W. and Weiguang, W. (2017). Assessing water scarcity in agricultural production system based on the generalized

water resources and water footprint framework. *Science of The Total Environment*, 609, 587-597.

Yang, X., 2008. *Effects of a nonionic surfactant on plant growth and physiology*. Ph.D. Auburn University.

Yang, H., Wang, L., Abbaspour, K. and Zehnder, A. (2006). Virtual water highway: water use efficiency in global food trade. *Hydrology and Earth System Sciences Discussions*, 3(1), 1-26.

York, C.A. and Baldwin, N.A. (1993). Localized dry spot of UK golf greens, field characteristics, and evaluation of wetting agents for alleviation of localized dry spot symptoms. *International Turfgrass Society Research Journal*, 7, 476–483.

Young, I. (1995). Variation in moisture contents between bulk soil and the rhizosheath of wheat (*Triticum aestivum L. cv. Wembley*). *New Phytologist*, 130(1), 135-139.

Zarebanadkouki, M., Ahmed, M., Hedwig, C., Benard, P., Kostka, S., Kastner, A. and Carminati, A. (2018). Rhizosphere hydrophobicity limits root water uptake after drying and subsequent rewetting. *Plant and Soil*, 428(1-2), 265-277.

Zarebanadkouki, M. and Carminati, A. (2013). Reduced root water uptake after drying and rewetting. *Journal of Plant Nutrition and Soil Science*, 177(2), 227-236.

Zhang, J. and Davies, W., 1989. Sequential response of whole plant water relations to prolonged soil drying and the involvement of xylem sap ABA in the regulation of stomatal behaviour of sunflower plants. *New Phytologist*, 113(2), 167-174.

Zhang, Z., Liu, G., Zhang, F., Zheng, C., Ni, F., Kang, Y. and Zeng, Y. (2014). Effects of nitrogen content on growth and hydraulic characteristics of peach (*Prunus persica L.*) seedlings under different soil moisture conditions. *Journal of Forestry Research*, 25(2), 365-375.

Zhang, X., Mi, Y., Mao, H., Liu, S., Chen, L. and Qin, F. (2019). Genetic variation in ZmTIP1 contributes to root hair elongation and drought tolerance in maize. *Plant Biotechnology Journal*, 18(5), 1271-1283.

Zuchi, S., Cesco, S., Gottardi, S., Pinton, R., Römheld, V. and Astolfi, S. (2011). The root-hairless barley mutant *brb* used as model for assessment of role of root hairs in iron accumulation. *Plant Physiology and Biochemistry*, 49(5), 506-512.

Appendices

Appendix 1

Table A-1: Summary of significant effects of surfactants on plant growth and nutrient concentrations/contents in diverse crops. Symbols (+/-), ns and nd denote positive effect of surfactant treatment, negative effect of surfactant treatment, non-statistically significant difference and not determined, respectively.

Publication	Species	Biomass	N	P	K	S	Ca	Cu	Na	Fe	Mg	Mn	Zn
Banks *et al.,* 2014	*Zea mays*	ns	ns	ns	ns	-	-	ns	ns	nd	ns	ns	ns
Baratella & Trinchera, 2018	*Lactuca sativa*	ns	ns	-	+	nd	-	-	ns	ns	-	ns	-
Chaichi *et al.,* 2017	*Lycopersicon esculentum*	+	+	-	-	ns	-	-	-	+	-	+	+
Dadresan *et al.,* 2015	*Trigonella foenum graecum*	nd	nd	+	+	nd	ns	+	+	-	ns	+	+
Trinchera & Baratella, 2018	*Lactuca sativa*	ns	ns	-	-	nd	ns	+	ns	ns	ns	-	ns

Table A-2: Repeated measures ANOVA (P values are reported) examining the effects of soil type (bulk soil, rhizosheath soil, S), surfactant treatments (T), genotype (G) and nutrient level (NL) on soil moisture.

	P value
Soil type (S)	0.33
Treatment (T)	0.90
Genotype (G)	0.40
Nutrient Level (NL)	0.37
TxG	0.33
TxNL	0.57
GxNL	0.33
TxGxNL	0.30
SxT	0.37
SxG	0.32
SxNL	0.40
SxTxNL	0.33
SxTxG	0.33
SxGxNL	0.36
SxGxTxNL	0.46

Table A-3: Repeated measures ANOVA (P values are reported) examining the effects of time (days after withholding water, D), surfactant treatments (T), genotype (G) and nutrient level (NL) on transpiration and leaf elongation. Significant effects (P<0.05) are in bold text.

	Transpiration	**Leaf elongation**
Day (D)	**<0.001**	**<0.001**
Treatment (T)	0.61	0.37
Genotype (G)	**0.02**	**0.02**
Nutrient Level (NL)	**0.04**	0.49
TxG	0.22	0.14
TxNL	0.83	0.30
GxNL	0.53	0.82
TxGxNL	0.24	0.98
DxT	0.20	0.44
DxG	**<0.001**	0.82
DxNL	**0.03**	0.83
DxTxG	0.72	0.29
DxTxNL	0.51	0.78
DxGxNL	0.10	0.10
DxTxGxNL	0.53	0.72

Table A-4: Three-way ANOVA (P values are reported) examining the effects of treatment (T), genotype (G) and nutrient level (NL) on N concentrations and N contents. Significant effects (P<0.05) are in bold text.

	Treatment (T)	**Genotype (G)**	**Nutrient Level (NL)**	**TxG**	**TxN**	**GxN**	**TxGxN**
Shoot [N]	0.77	**0.02**	**<0.0001**	0.92	0.33	0.65	0.14
Root [N]	0.14	0.70	**0.04**	0.58	0.78	0.88	0.35
Total [N]	0.70	**0.02**	**<0.0001**	0.92	0.33	0.65	0.14
Shoot N content	0.34	**<0.0001**	**<0.0001**	0.35	0.20	**<0.0001**	0.48
Root N content	0.50	0.06	**<0.0001**	0.06	0.06	0.13	0.15
Total N content	0.74	**<0.0001**	**<0.0001**	0.68	0.45	0.02	0.25

Table A-5: Three-way ANOVA (P values are reported) examining the effects of treatment (T), genotype (G) and nutrient level (NL) on P concentrations and P contents. Significant effects (P<0.05) are in bold text.

	Treatment (T)	**Genotype (G)**	**Nutrient Level (NL)**	**TxG**	**TxN**	**GxN**	**TxGxN**
Shoot [P]	0.56	**<0.001**	**<0.001**	0.86	0.57	0.60	0.40
Root [P]	0.14	0.06	0.81	0.58	0.10	0.49	0.48
Total [P]	0.79	**<0.001**	**<0.001**	0.81	0.63	0.58	0.42
Shoot P content	0.87	**<0.001**	**<0.001**	0.87	0.59	0.22	0.06
Root P content	0.39	0.32	0.87	0.62	0.63	0.32	0.67
Total P content	0.91	**<0.05**	**<0.001**	0.61	0.21	0.44	0.07

Table A-6: Soil physical and chemical properties for the two soils (sandy soil irrigated with 100% and 10% strength of Hoagland's Solution) used in this study. Analyses were carried out by Fruit Advisory Services Team LLP.

Soil property	Low nutrient level	High nutrient level	Units	Method
Texture class	Loamy sand	Loamy sand		
Sand	90.00	90.00	% w/w	Particle size distribution via laser diffraction
Silt	7.00	7.00	% w/w	
Clay	3.00	3.00	% w/w	
Organic Matter	0.70	0.84	% w/w	Loss on ignition
pH	7.10	7.10		In water (1:2.5)
Total N	0.22	0.25	% w/w	Combustion catalytic oxidation
Total C	0.24	0.34	% w/w	
P	8.50	18.00	mg L^{-1}	Aqua-regia soluble elements: HCl and HNO_3 digestion with analysis via ICP-OES
K	40.00	52.50	mg L^{-1}	
Ca	560.50	564.00	mg L^{-1}	
Mg	25.00	25.00	mg L^{-1}	
Na	14.00	16.50	mg L^{-1}	
S	9.50	9.50	mg L^{-1}	
Zn	1.40	1.50	mg L^{-1}	
Cu	0.80	0.80	mg L^{-1}	
Mn	0.10	0.10	mg L^{-1}	
Fe	39.10	31.00	mg L^{-1}	
B	0.50	0.60	mg L^{-1}	

Appendix 2

Table B-1: Summary of significant positive effects of surfactants on plant growth, yield and irrigation water use efficiency (IWUE = Total dry matter/amount of water applied) in diverse crops, substrates and irrigation treatments. ET, EC, W, W+S, nd denote evapotranspiration, electrical conductivity, water and water + surfactant, not determined, respectively.

Publication	Species	Substrate	Irrigation treatment	Field/Pot experiment	Dry matter	Plant height	Yield	IWUE
Jafarian *et al.*, 2016	*Medicago sativa L.*	Loam	75%, 50% ET	Field	nd		W+S>W	nd
Daneshnia *et al.,* 2015	*Trifolium alexandrinum, Ocimum basilicum*	Clay loam	100%, 75%, 50% ET	Field	nd	W+S>W	W+S>W	W+S>W
Sibley *et al.,* 2018	*Impatiens hawkeri*	Bark-based potting mix	20%, 40%, 60% ET	Pot	W+S>W	W+S>W	nd	nd
Chaichi *et al.,* 2016	*Zea mays L.*	Clay loam	70%, 100%, 130% ET	Field	nd	nd	W+S>W	nd
Mehrvarz *et al.,* 2013	*Zea mays L.*	Clay loam	30%, 60%, 90% ET	Pot	nd	W+S>W	W+S>W	nd
Chaichi *et al.,* 2015	*Zea mays L.*	Sandy loam	40%, 60%, 80%, 100% ET	Field	W+S>W	nd	W+S>W	W+S>W
Oostindie *et al.,* 2012	*Solanum tuberosum L.*	Clay sand	100%, 0% ET	Field	nd	nd	W+S>W	nd
Chaichi *et al.,* 2017	*Solanum lycopersicum*	Compost	Fresh water, diluted seawater (EC= 6 dS m^{-1})	Pot	W+S>W	W+S>W	nd	nd

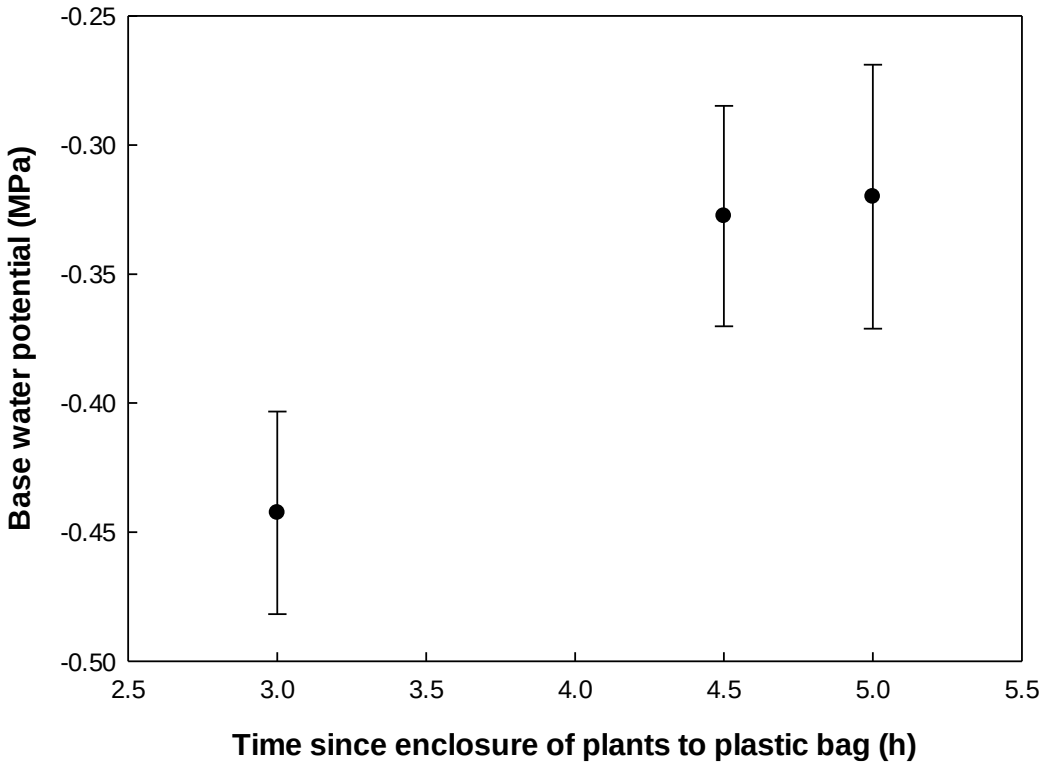

Figure B-2: Changes in base water potential of barley plants following enclosure. Data are means ± S.E of 6 replicate plants.

Table B-3: Repeated measures ANOVA (P values reported) examining the effects of surfactant treatments (T) and genotype (G) and their interactions on plant growth and soil water content in well-watered (Day 0) and drying soil (Days 5 and 9), in Experiment 3.

Day	Treatment			Genotype			Treatment x Genotype		
	Whole plant leaf area	Total biomass	Soil water content	Whole plant leaf area	Total biomass	Soil water content	Whole plant leaf area	Total biomass	Soil water content
0	0.73	0.75	0.11	0.53	0.58	0.16	0.83	0.92	0.31
5	0.61	0.49	0.64	0.42	0.37	0.54	0.87	0.59	0.86
9	0.99	0.98	0.93	0.90	0.64	0.49	0.89	0.84	0.21

Appendix 3

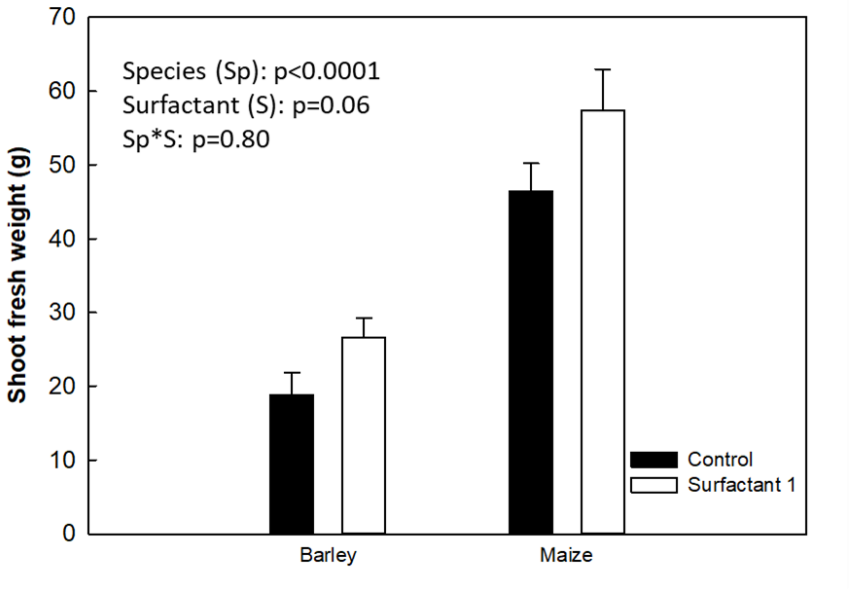

Figure C-1: Shoot fresh biomass for barley and maize plants grown in untreated (black bars) and surfactant-treated soil (white bars). Data are means ± SE of 8 replicates. P values are reported for species (Sp), surfactant treatments (S) and their interaction.

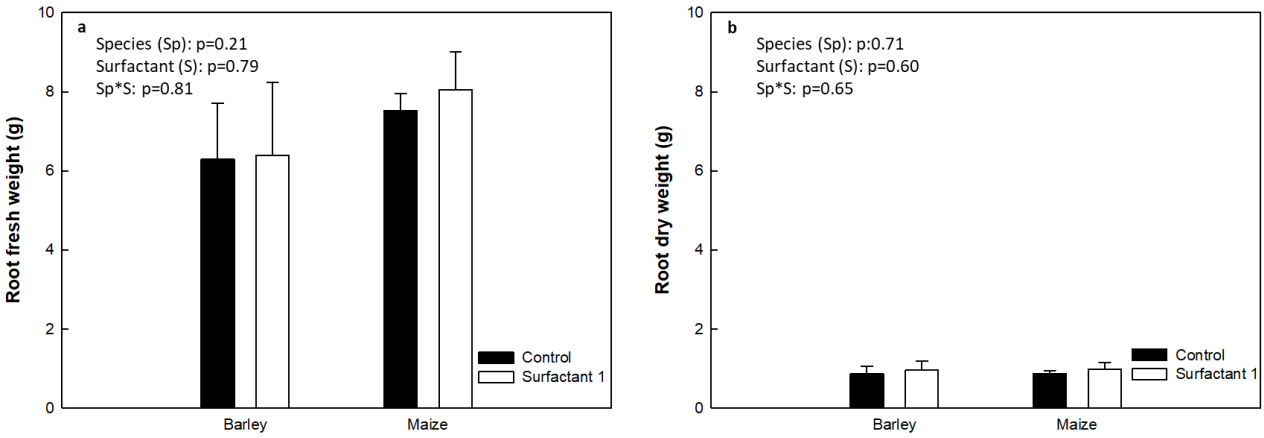

Figure C-2: Root fresh (a) and dry (b) biomass for barley and maize plants grown in untreated (black bars) and surfactant-treated soil (white bars). Data are means ± SE of 8 replicates. P values are reported for species (Sp), surfactant treatments (S) and their interaction.

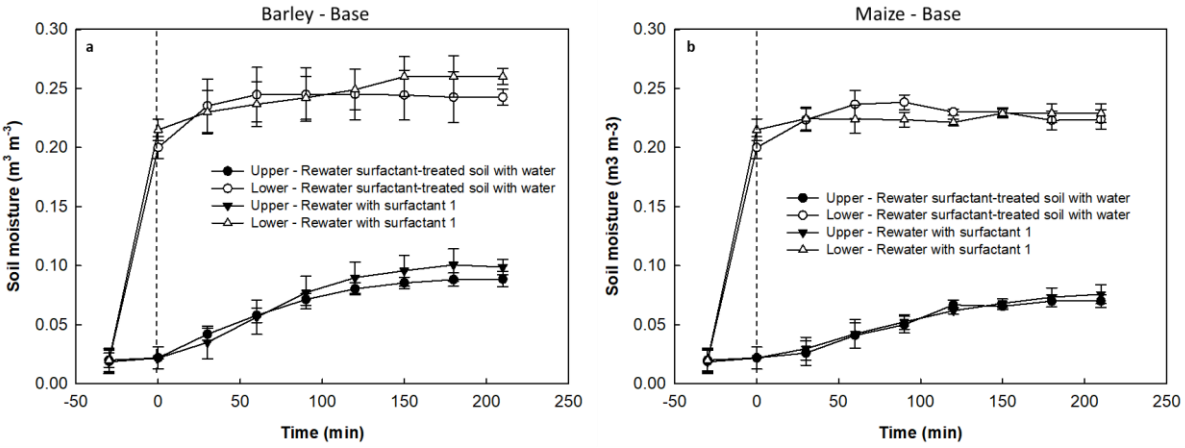

Figure C-3: Soil moisture of upper and lower soil layer over time, for barley (a) and maize (b). Soil was rehydrated at 0 min from the base (indicated by vertical dashed line). Symbols are means ± S.E of 4 replicates. Filled and hollow circles denote upper and lower layer of surfactant-treated soil rewatered with water. Filled and hollow triangles denote upper and lower layer of untreated soil rewatered with surfactant solution.