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What are the Principle Modes of Action of Wetting Agents and How can they Aid Turfgrass Quality While Improving Water Conservation?

By

Thomas Paul Martin

In collaboration with

Agronomy Research Group LTD

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What are the Principle Modes of Action of Wetting Agents and How can they Aid Turfgrass Quality While Improving Water Conservation?

Wetting agents are a class of surfactant - organic chemicals that reduce the surface tension between two materials. The sandy soils that many golf courses are constructed on can develop water repellency due to an organic coating that forms around sand particles. Wetting agents allow water to effectively bind with sand particles and can significantly reduce the length of time that water sits on the soil surface. Along with potentially improving turf quality, this has positive implications in terms of reducing surface runoff and improving water use efficiency. To explore the effects of wetting agents on grass physiology, development and soil interactions, investigations were conducted using three different wetting agent treatments on two cool-season turfgrass species - Highland bentgrass *Agrostis castellana* and Annual meadow grass *Poa annua*. Assessments of the effects of wetting agent treatments on seed germination, plant growth rate, nutrient uptake, rhizosheath properties, rooting characteristics and drought interactions were made over the course of three experiments carried out in controlled conditions. It was found that plant and turf growth rate can be affected in the two species to different extents depending on the wetting agent used, with positive implications for turf quality in the field for a newly developed wetting agent. The same treatment also significantly improved germination success in *A. castellana* and resulted in significant differences between the two species in terms of rhizosheath size and root diameter, with *A. castellana* being positively affected. The evidence presented in this study shows that wetting agents can affect the distribution of water resources in the soil by shifting the rhizosheath water content to bulk soil water content ratio to potentially maximise water and nutrient uptake by the roots. The results also show that another wetting agent treatment has the potential to improve drought tolerance in drought susceptible species through analysis of plant growth rate under drought conditions, stomatal conductance and leaf relative water content. It is hypothesised that the combination of effects caused by a wetting agent treatment may lead to improved water use efficiency and a more desirable sward composition for golf course owners in the field, resulting in water conservation and a reduction in the need to use herbicides to combat weed species.

Keywords: Wetting agent, water conservation, germination, plant growth rate, rhizosheath, rooting, plant-soil-water interactions, drought

Word Count: 18478

Introduction

1.1 – The importance of water

Plant productivity and growth are negatively impacted by a range of environmental stresses, with water deficit being amongst the most problematic (Vasseur *et al*., 2014). Water deficit stress can result in the water potential and turgor of plant cells being reduced to such a level that key biological functions cannot take place at normal capacity, most notably cell expansion (Taiz and Zeiger, 2010). The results of several studies have suggested that it may be the chief factor limiting global primary productivity due to its impact on carbon fixation and growth (Boyer, 1982; Schulze, 1986; Dawson, 1993). In both agricultural and amenity situations, irrigation is used to combat the effects of water deficit by applying water to plants in controlled amounts during periods of water stress. The level of irrigation required is determined by the amount of rainfall a plant is exposed to and the soil type. At the point at which free drainage of water from the soil ceases (which varies based on the relative amounts of sand, clay, salt and organic matter in the soil) the soil is said to be at 'field capacity' (Buckmaster, 2004). Irrigation is used to maintain this field capacity – excess watering will cause 'waterlogging' (when air is completely excluded from the soil) and under watering will fail to alleviate the stress (Buckmaster, 2004).

With predictions that years with extreme summer droughts will become more common and that the number of people living in countries with physical water scarcity may double in the future, it will be wise to develop systems that use water as conservatively as possible to maintain plant health (Maraldo *et al*., 2008; Roson and Damania., 2017). Large-scale droughts and drying trends in the Southern Hemisphere are heavily implicated as the cause of regional reductions in terrestrial net primary production – should this decline continue, the global terrestrial carbon sink would be severely weakened (Zhao and Running, 2011). There is an expectation that climate change will progressively increase the frequency and severity of drought events across the Northern Hemisphere (Carnicer *et al*., 2011). As a result of this prediction, there is a huge amount of research being undertaken to understand and improve drought tolerance in plants, including genetic engineering-based solutions (Peleg *et al*., 2011; Ahn *et al*., 2018), the use of arbuscular mycorrhizal fungi (Zarik *et al*., 2016;), evaluating physiological traits (Khan *et al*., 2007; Zhan *et al*., 2015), seed priming (Samota *et al*., 2017) and the use of ultraviolet-B radiation (Robson *et al*., 2015). In addition to this, it is important that even when water is readily available it is used as efficiently as possible – 70% of the fresh water on Earth is currently being used for irrigation which is unlikely to be sustainable (Oliver, 2017).

1.2 - Surfactants

Surfactants, or surface-active agents, are organic chemicals that reduce the interfacial tension between a liquid and another liquid, gas or solid (Llenado and Jamieson, 1981; Yan *et al*., 2009). Soils can exhibit varying levels of water repellency, a phenomenon that is believed to be caused by a hydrophobic organic coating that can arise on soil particles if the right conditions are met (Tucker *et al*., 1990; Hallett *et al*., 2001; Oostindie *et al*., 2008). This organic coating can be produced by changes in soil structure due to root growth, rhizodeposition and repeated drying cycles (Bengough, 2012). Rhizodeposits may coat particles with material that becomes hydrophobic when it dries beyond a critical water content, which will vary depending on the soil type (Naveed *et al*., 2019). Sand particles are the most susceptible to acquiring a soil repellent property due to their low specific surface area compared with particles of other soil types, meaning that they are more readily coated by organic material (Wallis and Horne, 1992). If a soil dries beyond the critical soil water content level (the water content below which the soil will not wet spontaneously when a water droplet meets its surface and above which the soil is wetTable), it will become water repellent (Ritsema and Dekker, 1994; Panina, 2010). Therefore, if a soil is moist, this hydrophobicity does not occur and thus frequent irrigation can prevent it, but this is unlikely to be a sustainable water use practice. An estimated 30% of the global annual water withdrawal used for irrigation is supplied from overused surface waters and non-renewable groundwater resources (Cisar *et al*., 2000; Alexandratos and Bruinsma, 2012; Liu *et al*., 2017).

Wetting agents are a class of surfactant that can temporarily overcome the hydrophobic property of these soils by binding to both the organic coatings and water molecules, effectively joining them together and allowing the soil particles to become ‘wet’ (Karnok *et al*., 2004). Wetting agent products are only likely to have significant positive effects when used at the correct concentrations and in appropriate conditions – how they move and behave in soils is influenced by solute concentration, adsorption isotherms, hydraulic conductivity and the chemical and physical characteristics of the medium (Miller *et al*., 1975). If used correctly, they have potential to allow for a reduced irrigation frequency and therefore increased water conservation (Moore *et al*., 2010; Park *et al*., N.D.).

Wetting agents have historically been used to greatest effect in the amenity turfgrass industry – for the maintenance of golf courses and sports pitches. For the reasons discussed above, soil water repellency is usually at its worst when the soil is sand based, which is largely the case for coastal/links golf courses and new or reconstructed soil profiles of tees and greens (Kostka, 2000). Sand-based soils drain rapidly, are resistant to compaction and although initially they will hold water they have a tendency to become hydrophobic (Beard, 1972; Wallis and Horne, 1992). This tendency has been exacerbated by irrigation technology that

just replaces a proportion of evapotranspiration, water restrictions and organic matter deposition from the turf (Cisar, 2004). This type of irrigation coupled with water use restrictions on amenity turf may lead to this exacerbation because soil moisture may be lower than the soil's critical water content level for extended periods of time (Doerr *et al*., 2000). In addition to this, as organic matter settles in a turf soil (composed mainly of dead and decaying plant tissue) it forms a layer of 'thatch' beneath the soil surface. A failure to manage this thatch layer is likely to contribute to a sandy soil becoming hydrophobic as a higher organic matter content has been shown to contribute to increased soil water repellency (de Jonge *et al*., 1999; Morley *et al*., 2005).

Studies have shown that non-ionic wetting agents (the class of surfactant that are used in most amenity grassland situations) have been effective in reducing soil water repellency and reducing soil water requirement by up to fifty percent (Cisar, 2004; Kostka *et al*., 2007; Scott *et al*., 2018). In addition to this, irrigation efficiency has been shown to be improved by a number of different wetting agent products in isolated test scenarios (Karnok and Tucker, 2008).

When a growth medium is somewhat water repellent, water can bypass the hydrophobic areas of the soil which leads to what those in the amenity turf industry would refer to as "localised dry spots" (York and Baldwin, 1992; Schlossberg, 2005; Song *et al*., 2018). Localised dry spots (LDS) are characterised by irregularly shaped areas of dead or wilted turfgrass and are a cause of concern to greenkeepers the world over due to reduced visual quality and surface smoothness (York, 1995; Soldat *et al*., 2010; Panina, 2010). Wetting agents are a useful tool to control LDS but must be applied regularly to prevent their formation – if the organic coating remains on the soil particles, repellency will return soon after a wetting agent treatment is ceased (Karnok *et al*., 2004). However, recent findings have shown that the application of certain wetting agents to water repellent soils followed by deep irrigation may be effective in removing the organic coatings from the sand profile, allowing the soil to become wet without repeated wetting agent applications (Song *et al*., 2018). This is an attractive finding, as it could reduce the financial cost of wetting agent use while still increasing irrigation efficiency. As is the case with any laboratory study though, it remains to be seen whether this observation will translate to the field.

1.3 – Water use in amenity turf

Despite the use of wetting agents alongside other management practices designed to conserve water, such as incorporation of drought-tolerant grasses and the use of soil moisture sensors to reduce overwatering, estimates suggest that between three and five thousand cubic metres of water per day may be consumed by an eighteen-hole golf course (Wheeler and Nauright, 2006; Scott *et al*., 2018). Globally, around nine and a half million cubic metres of water are used per day to irrigate golf courses and at present it is difficult to determine whether this Figure is sustainable (Wheeler and Nauright, 2006). Water demand by golf courses has been implicated as a cause of water shortages in some regions, especially during summers and in already stressed environments such as throughout the Mediterranean, with the potential to threaten agricultural development (Tapias and Salgot, 2006). However, whatever opinion one may hold regarding water use on golf courses, it cannot be ignored that the average economic productivity of the water used for golf dwarfs that of even the highest value agricultural crops (Diaz *et al*., 2007). That being said, with the competition between agriculture and other economic sectors likely to increase due to heightened water scarcity, climate change induced drought and an increasing population, the diversion of water resources to favour agriculture is to be expected (Mancosu *et al*., 2015). This emphasises the need for developing methods of improving the efficiency of water use in amenity situations.

1.4 – Grasses and drought

Grasses that populate temperate European ecosystems have weak stomatal control, which makes them more susceptible to drought than other flowering plants such as forbs (Bolling and Feller, 2014). While the species' that characterise sub-Mediterranean grasslands exhibit a range of xeromorphic leaf features to overcome regular summer droughts, temperate species that lack these adaptions may suffer greater ill effects if drought frequency in the region increases as expected (Wellstein *et al*., 2017). When under drought stress, grass species have been shown to suffer reductions in net photosynthesis, biomass and resource allocation to the roots (Weißhuhn *et al*., 2011; Bolling and Feller, 2014). As a result of lower root biomass due to reduced resource allocation, nitrogen availability in the soil has been shown to increase under drought conditions due to a reduced capacity for roots to take in nitrogen (de Vries *et al*., 2016). These findings will inform which variables are selected for analysis when the grasses used in this study are exposed to drought stress.

How a grassland system is managed also influences drought induced responses. For example, frequent mowing has been found to reduce the resistance of grasslands against drought (Vogel *et al*., 2012). This finding is important in any evaluation of amenity turf responses to drought, as they are frequently mown systems; the *Agrostis* species that are sown on temperate golf

greens are often mown to a height as low as five millimetres (Beard, 1972). Competition with other species can also influence the degree to which processes such as water and mineral uptake are affected by drought. Grasses may have a lower capacity to acquire water and minerals than their competitors, and thus a greater drought effect has been observed when grasses are grown in systems with increased species richness (Vogel *et al*., 2012; Miranda-Apodaca *et al*., 2014; Van den Berge *et al*., 2014). However, this effect may be reduced in turf grass situations, as these systems are often managed in such a way that species richness is comparatively low. Studies have shown that wetting agents can improve resource acquisition in lettuce (Baratella and Trinchera, 2018), increase grain yield and emergence in wheat and lupin (Crabtree and Henderson, 1999) and increase establishment of perennial ryegrass (Wallis *et al*., 1990). However, it is unclear whether these studies provide applicable results to the grass species associated with a golf course context.

While it appears that appropriate use of wetting agents will have positive effects on turfgrass quality and density, it is important to acknowledge any negative environmental impacts that may arise from their use. Although primarily non-ionic wetting agents are used which are less phytotoxic than cationic or anionic surfactants, heavy irrigation is still recommended after their application to reduce potential phytotoxic effects to the grasses (Müller and Deurer, 2011). However, as they can result in reduced irrigation following this initial wetting this is unlikely to be viewed as a net-negative impact. It has been suggested that wetting agents may de-stabilise the soil structure and increase evapotranspiration from the soil surface, but there is little evidence supporting these claims (Hallett and Gaskin, 2007). Conversely, there is evidence to suggest that the use of wetting agents may reduce leaching of fungicides - which are regularly applied to golf greens and have often been detected in corresponding drainage water - resulting in less contamination of water bodies and surrounding soils (Larsbo *et al*., 2008). In contrast to this, one study found that a non-ionic surfactant had the potential to increase groundwater pollution, due to increasing the leaching potential of a cattle antibiotic when it was present during irrigation (ElSayed *et al*., 2013). However, results to support these findings are limited and potential long-term ecological impacts of wetting agent use (for example, impacts on the soil microbial community) are yet to be formally assessed (Müller and Deurer, 2011).

1.5 – Possible modes of Action

Exactly how surfactants impact plant and soil interactions positively is somewhat unknown, with many studies providing inconsistent results. Plants and microorganisms produce their own exudates with surfactant-like properties which may provide insight into how man-made wetting agents affect plant growth (Banat, 1995; Dunbabin *et al*., 2006). In plants, these biosurfactants have been shown to be released from the root tip and are found in the root

mucilage (Read *et al*., 2003; Bais *et al*., 2004). At present it is unclear whether they are secreted actively or are released by cell membrane damage, but what we do know is that, due to being composed of phospholipids, they are powerful surfactants (Read *et al*., 2003). The suggestion is that the maintenance of these surfactants in the rhizosphere would allow water and nutrients to be drawn from smaller soil pores and they have been shown to increase the amount of phosphorus in the rhizosphere which is immediately available to the plant (Read *et al*., 2003; Bais *et al*., 2004). This effect may be further magnified by the phospholipids released by the soil microfauna, which have been shown to affect phosphorus bioavailability in the soil (Bais *et al*., 2004; Deubel and Merbach, 2005). If a wetting agent could be shown to influence microbial communities, they may be affecting phosphorus availability and therefore impacting plant growth - microbial biomass has been shown to decrease when a surfactant is applied to a soil with arbuscular mycorrhizal fungi (Wu *et al*., 2008). Likewise, an interaction with plant derived surfactants would likely influence water and nutrient availability.

Surfactants may affect plant-nutrient interactions, as has been shown under high salinity conditions. Non-ionic surfactant application has been shown to increase uptake of sodium, phosphorus, copper, manganese and zinc in fenugreek plants and help maintain ionic balance in tomato plants, which promotes growth (Dadresan *et al*., 2015; Chaichi *et al*., 2017). Again, the results show inconsistencies, for example in a study using potatoes, nitrogen use efficiency was improved by surfactant application in the first year but not the next, whereas tuber nitrogen uptake was significantly increased in the second year but not the first (Kelling *et al*., 2003). The notion that surfactants can increase crop yield is also supported by inconsistent data. Yield has been maintained when using less fertiliser than controls (Kelling *et al*., 2003, Lowery *et al*., 2002), but likewise some studies have shown non-ionic surfactants to have no positive effects on yield or nutrient availability at any fertiliser application rate (Wolkowski *et al*., 1985). It is probable that positive effects are due to more uniform wetting of the soil allowing for increased nutrient uptake as opposed to a direct effect on the mode of nutrient acquisition used by the plant, though the latter cannot be dismissed completely.

Due to their principle function, we would expect positive effects on growth from surfactants to be linked to increased water uptake due to more uniform water retention in the soil. Indeed, non-ionic wetting agents can improve soil water distribution in the field, which would be expected to result in more consistent growth across a plant population (Cooley *et al*., 2009). In turfgrass, a non-ionic organosilicone surfactant has been shown to improve uptake of water which has been suggested to reduce the symptoms of turfgrass diseases such as those caused by *basidiomycetes* fungi (Nadeau *et al*., 1993). Hydraulic conductivity,

which impacts flow rate through soils, has been shown to be affected by non-ionic surfactants (Miller *et al*., 1975), but likewise in other studies no significant impacts on soil hydraulic characteristics have been observed (Abu-Zreig *et al*., 2003).

Soil compaction results in lower air and water holding capacity in a soil, while also making it more difficult for roots to penetrate downwards. It was shown in an American study that non-ionic surfactants can significantly increase root depth and corn yield in soils with a compacted zone below the soil surface (Brumbaugh and Peterson, 2001). In turfgrass systems, soil compaction has been shown to reduce visual quality, clipping yield, nitrogen use, evapotranspiration, and root growth (Sills and Carrow, 1983). If some wetting agents have the potential to alleviate these negative impacts of compaction (which is often common in areas of a golf course with heavy customer traffic), they could be a desirable option for compaction management on golf courses (Carrow and Petrovic, 1992).

Following on from this, an effect on rooting was observed in a study of twenty-two different non-ionic surfactant products, with some being shown to repress elongation but others being shown to stimulate growth of roots, leaves and/or coleoptiles (Parr and Norman, 1964). In these cases, it was shown that the concentration that different surfactants are applied at can have a substantial influence on the observed effects – effects are often noted at concentrations as low as 0.01% v/v. Different surfactants, but also different concentrations of the same surfactant have been shown to both inhibit and stimulate growth (Stowe, 1958; Stowe, 1960).

In terms of germination, it has been suggested that surfactants may act on cellular membranes to influence the imbibing process (Endo *et al*., 1969). Non-ionic surfactants have been shown to stimulate, inhibit or have no effect on germination in a range of plant species and this effect varies with both surfactant concentration and environmental conditions (Hurtt and Hodgson, 1987). Germination assays have also recently been carried out with turfgrass species where non-ionic surfactants have been shown to stimulate germination and improve germination synchrony when applied directly to the seed (Madsen *et al*., 2016). In the same study, turfgrass establishment under drought simulation was also shown to be enhanced by direct application of surfactant to the seed using a film coating (Madsen *et al*., 2016). However, although these findings are useful in that they show surfactants can influence turfgrass germination, effects on germination have not yet been documented in *A. castellana* or *P. annua*. Results that demonstrate such effects in these species are highly desirable due to their central role in influencing the management of temperate golf greens.

Despite the inconsistencies in the literature and some of the studies being somewhat dated now, there is enough evidence to suggest that the effect of surfactants in biological systems are not limited to only surface tension reduction - biochemical effects are also possible (Parr and Norman, 1964). However, the inconsistencies do indicate that the modes of action are likely to vary markedly between products. This is because different chemical blends are expected to have different modes of action and variable effects (Parr and Norman, 1964; Stevens *et al*., 1991). Many of the studies discussed in this section took place in the field, where environmental conditions are likely to greatly impact results between measurements. They are also often based on species that physiologically differ from the turfgrass species' that will be used in this study, or turfgrass species that have limited relevance to the industry in the UK.

1.6 – Turfgrass species

Grasses that have a temperature optimum ranging from around 15.6 ºC to 23.9 ºC are known as cool season grasses. Species that fall into this category are utilised by the amenity turfgrass industry across the upper Northern Hemisphere, with most species originating from the fringe forests of Eurasia (Beard, 1972; Martiniello and D'Andrea, 2006). A huge range of cool season varieties have been selected and bred from both introduced and native species to be suited to specific microenvironmental conditions (Zhou and Abaraha, 2007). This study focuses on two species of cool season grasses that can be found on amenity turf throughout the region – *Agrostis castellana* (highland bentgrass) and *Poa annua* (annual meadowgrass). *A. castellana* forms a dense turf and is believed to have originated from the Mediterranean coast, as such it tends to perform better in warmer, drier areas than other *Agrostis* species. This potentially indicates it also has increased drought tolerance, making it a more suiTable species for the golf greens of the future (Hubbard, 1984; Brede and Sellmann, 2003).

P. annua is almost always treated as a pest species and has been described as "the most problematic weed of temperate zone golf putting greens" (Gange *et al*., 2001). Although the species exhibits many traits that are desirable for turf grasses (tolerance of close mowing, a dense growth pattern and self-regeneration) it is less tolerant of many stresses than other turf grasses (Peel, 1982; Lush, 1988). It is highly susceptible to moisture and heat stress, as well as to all diseases of turf, for example Fairy Ring (caused by many species of *basidiomycetes* fungi) and Anthracnose (caused by the fungus *Colletotrichum cereale*) (Neal, 1994). Upon its infestation of *Agrostis* greens, the aesthetic value of the turf is decreased by its erratic colour and texture and its presence can lead to unpredicTable ball trajectory following a golf putt (Beard *et al*., 1978; Rana *et al*., 2016; Askew, 2017). As a result, its seed heads will often be supressed by the application of chemical herbicides such as mefluidide and growth regulators such as ethephon, with varying degrees of effectiveness (Eggens *et al*., 1989; Askew, 2017).

Due to it often being targeted with herbicides that have the same modes of action, several populations of the species have developed herbicide resistance (Cross *et al*., 2015). As a result of the problems *P. annua* causes and of regulations in countries such as the United Kingdom controlling the application of many types of pesticide, research has been conducted to develop other methods of control for the species, for example using arbuscular mycorrhizal fungi to provide conditions that favour *Agrostis* species and negatively impact *P. annua* abundance (Gange *et al*., 2001).

1.7 – Why this study is being conducted

A large-scale study by Henie *et al*., 2007 in the USA using ten wetting agent products failed to ascertain which product was the most effective when tested at nine different locations. As this was a field study, the activity of the products generally depended on location and weather (Panina, 2010). Interestingly, the products that appeared to cause short-term damage to grasses and produce a lower quality turf colour were those that were most effective in reducing water drop penetration time (Panina, 2010). Studies to support these results are limited and, to the authors knowledge, no study has yet been conducted assessing the relative impact the use of a wetting agent may have on a desirable turfgrass species and one that is considered a weed.

This study owes its origins to another MSc project that was conducted within Lancaster Environment Centre (Baldwin, 2019). In recent field trials comparing the effects of three different wetting agents, the data suggests that a new experimental formulation of a wetting agent may significantly improve the growth and development of *A. castellana* in situations where it is competing with *P. annua*, whereas other wetting agents have been observed having a lesser effect. The other surfactant formulations that have been tested in the study have a wetting effect on the surface layers of the soil or have limited longevity of performance, whereas the product that has the most significant effects penetrates much deeper into the soil profile and has been shown to have long-lasting positive effects in terms of alleviating soil water repellency.

The researcher has suggested that due to most *P. annua* roots being confined to the surface layers of the root-zone, products that target this area of the soil profile will favour the growth of this species (Beard, 1972). As *Agrostis* species often root deeper in the root-zone, a wetting agent that penetrates further into the soil may favour its establishment and growth (Beard, 1972). The rooting characteristics of the two species are also likely to influence drought tolerance; drought-induced root mortality in grasses has been shown to have the greatest effect in the surface soil layer (Huang and Gao, 2000). This may mean that we expect a surfactant that moves through the surface layers of the soil (where the *P. annua* roots are mostly

confined) and has its main effect deeper in the soil profile (where *Agrostis* roots are present) to give *Agrostis* a competitive advantage during drought periods (York, 1995).

1.8 – Aims and Hypotheses

The primary aim of this study is to establish what biological and biochemical mechanisms are underpinning the observations made in the field. An understanding will be gained of whether they are solely based on moisture effects (as would be expected of a wetting agent) or whether other factors are involved, for example wetting agents causing changes to plant physiology or soil-plant interactions. The effects are expected to differ between the two grass species and vary depending on the wetting agent product in use. The following hypotheses have been constructed, reflecting preliminary results from the field trials (Baldwin, 2019):

1. *H_1 = There will be a positive effect on overall germination success when seeds are sown with different wetting agent treatments. This effect will be more pronounced in A. castellana than P. annua under the deep penetrating wetting agent formulation.*
2. *H_2 = There will be a positive effect on plant growth rate and above-ground biomass accumulation when plants are grown with wetting agent treatments under both well-watered and drought conditions. This effect will be more pronounced in A. castellana than P. annua under the deep penetrating wetting agent formulation.*
3. *H_3 = Wetting agents will affect the distribution of water resources in the soil and have a positive impact on rhizosheath size. This effect will be more pronounced in A. castellana than P. annua under the new experimental wetting agent formulation.*
4. *H_4 = Wetting agents may have effects under both well-watered and drought conditions that can be observed by measuring shoot relative water content, evapotranspiration and stomatal conductance of A. castellana and P. annua. These measurements could help explain any growth rate differences that are observed.*
5. *H_4 = An effect on plant nutrient uptake will be observed in A. castellana and P. annua when grown with different wetting agent treatments under well-watered conditions.*

Methods

2.1 - Germination Assay

To assess any effects on germination success rate in a given time period caused by the wetting agent treatments, germination assays were carried out. The assays incorporated five treatment groups – a control (deionised water only), wetting agent 1, wetting agent 2, wetting agent 3 and then wetting agents 1 and 2 in combination with one another. Wetting agents will be referred to as treatments 1, 2, 3 and 1&2 from hereafter.

2.1.1 – Treatment descriptions

Treatment 1 is a commercial block co-polymer turfgrass wetting agent, which is known to be an efficient wetting agent for the thatch and surface layers of sports turf. It has a 20 litres / hectare application rate which, according to the manufacturer, should be applied monthly to give consistent effects. Treatment 2 is an experimental surfactant formulation which has been shown to reduce water surface tension significantly and to have good soil penetrant properties, but no longevity in the soil. It is a highly active formulation and known from previous studies to give good soil penetration at an application rate of 1 litre / hectare. Treatment 3 is another experimental soil wetting agent that has been shown in preliminary tests to penetrate deep into the soil profile. It contains a lethicin / polyglucoside / linear alcohol / long chain polymeric surfactant which has longer lasting effects than treatment 2. It has been shown to have wetting effects on both surface and sub-surface layers of the root-zone. The same application rate as treatment 1 (20 litres / hectare) was recommended so that a direct comparison to what is an industry standard product could be made. In each case, the required amount of wetting agent was added to 15 ml of deionised water to produce stock solutions at the desired concentrations (Table 1). Treatment descriptions are based on those provided by Baldwin, 2019.

2.1.2 – Details of the Assay

The concentrations used were equivalent to 25% v/v of the recommended field application rates for each wetting agent. The field application rate accounts for further dilution via rain water and moisture present in the soil and thus a reduced concentration was deemed to be more representative of what a seed would be in contact with in a field scenario. For the combined treatment (1&2), the ratio between the constituent wetting agent parts is shown in Table 1. This ratio was based on recommendations provided by the manufacturer of the wetting agents.

The treatments were applied in assays for both *P. annua* and *A. castellana*. Two layers of Whatman No.1 filter paper were placed inside 90 mm petri dishes and soaked with 2 ml of treatment solution. 50 individual seeds were placed directly onto the wetted filter paper

equidistant from one another in a grid arrangement (Figure 1). Dishes were triplicated for each treatment. The decision was made not to sterilise the seeds to avoid any interaction effects between the wetting agents and sterilant.

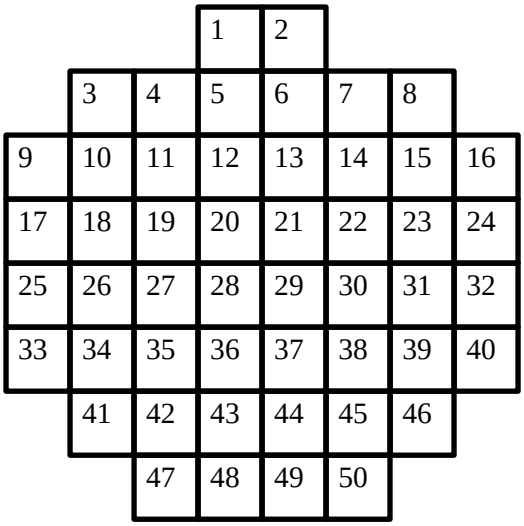

Figure 1*: The grid arrangement that seeds were placed within inside each petri dish. One seed occupied each square.*

Table 1: The percentages of wetting agent present in the wetting agent-water mix used to soak the filter paper. For the combined treatment 1&2, the ratio between the constituent wetting agent parts is shown. The control treatment is composed of only deionised water. The concentrations are equivalent to twenty-five percent of the recommended field application rates.

Treatment	Percentage of Wetting Agent in Water-Agent Mix (%)
C	0
1	0.715
1 & 2 (19:1)	0.680
2	0.0358
3	0.715

The Petri dishes were sealed using electrical tape immediately after the 50th seed was placed. The dishes were stored in the dark in an incubator with the temperature set to a constant 20 ± 1 ºC. Dishes were randomly arranged inside the incubator using random numbers assigned to each dish. Dishes were removed from the incubator to assess germination at three intervals during the day, with six hours between each interval during the rapid germination phase. After each removal, dishes were placed randomly in the incubator to avoid position effects. The assay was carried out over a period of seven days for *P. annua* and eight days for *A. castellana*. The experiment ran for a shorter period for *P. annua* due to no increase in the germination percentage after 168h, while *A. castellana* seeds were still germinating up to 192h after the start of the experiment. Seeds were considered germinated as soon as an emerged radical could be observed under ten times magnification. Once deemed germinated, a

mark was made with marker pen above the seed on the lid of the dish so that it would not be re-counted.

2.2 - Turf Assessments

To assess growth rate, evapotranspiration rate and plant nutrient uptake, turfs were maintained in 2L square based pots. The growth medium was composed of a 1:1 v:v mix of washed dune sand and soil collected from Powfoot golf course in South-West Scotland (NY141657). Prior to mixing, the soil was sieved to remove any particles with a diameter greater than 3.35 mm. Once mixed, sixty pots were filled with the medium which was then compacted consistently by pressing the soil with a 2.5 kg weight when pots were half-filled, and again when fully filled. The pH of the final sand-soil mix was 5.17 ± 0.0612 with a water holding capacity of 0.26 g of water per g of dry soil. Water holding capacity (WHC) was determined by watering three pots filled with the sand-soil mix until water started to drain from the bottom. When the draining stopped (when the soil was assumed to be at drained field capacity), the soil was weighed before being oven-dried at 80 ºC for one week. The dry soil was then weighed and WHC was calculated using the following equation:

$$WHC = (S_w - S_d) / S_d$$

Where S_w is the weight of the soil at drained field capacity and S_d is the weight of the oven-dried soil. This calculation was performed for the three replicates and the mean was taken to give the WHC.

The pots were watered to drained field capacity and then regularly watered for a period of two weeks. Any seedlings that had emerged from seeds already present in the soil were removed. *A. castellana* and *P. annua* seed were then sown (using thirty pots for each species, seeds from the respective species were sown on 08/01/19 and 09/01/19) and pots were watered and covered with foil to allow germination to take place. *A. castellana* seed was sown at a rate of 0.11 ± 0.005 g per pot and *P. annua* seed at a rate of 0.275 ± 0.025 g per pot – these rates were calculated based on the recommended sowing rates employed at Powfoot golf club (NY141657) and meant each pot was sown with approximately the same amount of seeds. Germination was observed on 14/01/19, with over-seeding taking place the following day to fill any gaps that were present due to seeds failing to germinate. An NPK (10:1:7) liquid fertiliser was then applied to the soils at a rate equivalent to 40L in 700L of water per hectare, as this is the standard application rate on a golf course. Additional over-seeding took place on 27/01/19. Once the grasses were established, they were cut to a mean ± SE height of 11.9 ± 0.19 mm on 13/02/19.

2.2.1 - Treatment groups

The trial incorporated the same five treatment groups as the germination assay – a control (water only), wetting agent 1, wetting agent 2, wetting agent 3 and then wetting agents 1 and 2 in combination. In each case, the required amount of wetting agent was added to 15 ml of water to produce stock solutions at the desired concentrations (Table 2). The concentrations are based on the recommended field application rate for each wetting agent. Each dose consisted of 1.08 ml of the wetting agent-water mix followed by 30 ml of water (apart from treatment 2, which consisted of 1.0515 ml of the wetting agent-water mix followed by 30 ml of water). The control treatment only contained 30 ml of water. These quantities were calculated based on pot area, as in the field the wetting agents are applied by hectare (see Appendix 1).

Sixty pots meant that there were six repeats for each treatment group for both species. The pots were kept in a controlled environment room with a day-time temperature of 20 ± 1 ºC (07:00-19:00), night-time temperature of 15 ± 1 ºC (19:00-07:00), a light level at canopy height of 196 ± 16.5 µmol m^{-2} s^{-1} and humidity of between 50-60%. The pots were arranged in a fully-randomised block design, with the blocks being repositioned each time turfs were watered. Plants were kept well-watered during the treatment application phase which consisted of four doses applied over a period of six weeks from 14/02/19 to 21/03/19. Treatments were applied using a new, clean syringe for each treatment. After this period, the plants were subjected to a period of drought from 30/03/19 to 12/04/19. It was hoped that a 'recovery' phase would also be incorporated into the experiment when turfs were re-watered after the completion of the drought. However, the plants were very slow to recover and thus, due to time constraints, the experiment had to be terminated before any meaningful recovery data could be collected.

Table 2: The percentages of wetting agent present in the wetting agent-water mix used to treat each pot. For the combined treatment 1&2, the ratio between the constituent wetting agent parts is shown. The control treatment is composed of only tap water.

Treatment	Percentage of Wetting Agent in Water-Agent Mix (% v/v)
C	0
1	2.86
1&2 (19:1)	2.86
2	0.143
3	2.86

2.2.2 - Water Infiltration

An infiltration rate assessment was made to determine any changes in soil hydrophobicity caused by treatment applications. After three doses of treatment had been applied, the time taken for 40 ml of water to be fully absorbed into the soil in each pot was recorded (a method adapted from Letey, Pelishek and Osborn, 1961). This was repeated five times for each pot (five technical replicates), from which the median infiltration time was used to give a water infiltration time for each pot. The mean of the infiltration time for each treatment could then be calculated for both grass species. This process was then repeated after all doses had been applied and the soils had been subjected to the drought period. For the post-drought tests, technical replicates were not used due to the soil no longer being 'droughted' after water was added.

2.2.3 - Growth rate

Grasses were cut every 5-6 days to a mean (± SE) height of 6.66 ± 0.075 mm. To measure the change in sward height that had taken place during the period following each cut, a square piece of light cardboard was placed on top of the turfs. The distance between each edge of the card and the soil surface was then measured, and then the median of these four measurements was calculated to give an estimate of sward height (Figure 2).

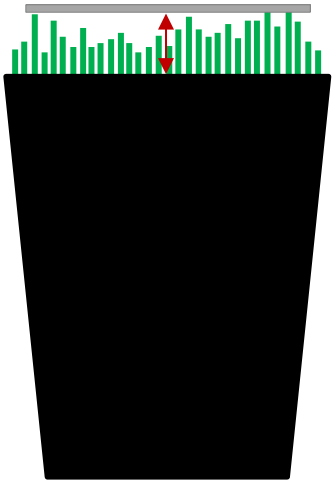

***Figure 2**: A diagram of one of the sixty pots containing turf. The red arrow represents the distance under the cardboard that would be measured. This measurement was taken from the middle of each side of the square and then the median of these four measurements was calculated, giving the sward height.*

Growth rate (GR) was then estimated using the following formula:

$$GR = (H_g - H_c) / N$$

Where H_g is the sward height post-growth period, H_c is the sward height measured immediately after the proceeding cut and N is the number of days (measured to the nearest minute) that elapsed between the post-cut measurement and the post-growth measurement.

2.2.5 - Evapotranspiration

An estimate for evapotranspiration rate was calculated using a gravimetric method. Empty pot weight, the soil weight in each pot and the soil water holding capacity were used to determine an estimate of the water weight in each pot at drained field capacity.

Pots were watered to drained field capacity every two to three days and received an additional 40 ml of water daily in between these events to ensure pots were maintained as close to field capacity as possible. Each pot was also weighed every two to three days, with the weight of the water added in between pot weighing events being subtracted from this value. These values were then used to determine the weight loss from each pot over a two to three-day period, from which estimates for gravimetric water content (GWC) and evapotranspiration rate (E) could be calculated using the following formulae:

$$GWC = (W_f - W_w) - (W_p) - (W_d)$$

$$E = [\ W_c - (W_f - W_w)\ ] / N$$

Where W_f is the pot weight after the two to three-day period, W_w is the weight of water added in between pot weighing events (including that which is added during treatment application), W_p is the empty pot weight, W_d is the weight of dry soil in a pot, W_c is the pot weight when the soil is watered to drained field capacity and N is the number of days (measured to the nearest minute) between weighing events.

2.2.7 - Nutrient Uptake Analysis

Above-ground tissue nutrient analysis was carried out using a microwave assisted acid digestion and inductively coupled plasma - optical emission spectrometry (ICP-OES). Each time turfs were cut during the well-watered phase of the experiment, the removed tissue was placed in a labelled paper bag and oven dried at 80 ºC.

A dried tissue sample from each turf of between ~50-150 mg was ball-milled for 1 minute at a vibrational frequency of 30 Hz (1800 min^{-1}). The sample was then transferred into a digestion tube and the sample weight was recorded before 5 ml of 70% v/v Aristar nitric acid (HNO_3)

was added. Samples were left for between 20 minutes and 1h while the initial digestion reaction took place. Tubes were then sealed with a screwcap and polytetrafluoroethylene (PTFE) pressure release bung, before being microwaved in a CEM Mars 5 digestion oven at 200 ºC for 15 minutes. Samples were then left to cool overnight before being diluted with Milli-Q water to 20% v/v HNO_3 and stored at room temperature. Finally, samples were diluted to 2% v/v HNO_3 before being refrigerated at 3-10 ºC in preparation for analysis by ICP-OES. All digestion tubes and centrifuge tubes were washed with detergent, acid washed and rinsed before any samples were added or diluted into them.

After samples had been analysed by ICP-OES, the values for each data point were converted from parts per million (ppm) to mg of the nutrient per g of dry plant matter (N) using the following formula:

$$N = [(C * A) / W] / D$$

Where *C* is the concentration of the nutrient detected by the ICP-OES in ppm, *A* is the volume of acid in litres added to each sample before microwave digestion took place, *W* is the weight of the dry plant tissue sample and *D* is the dilution factor of the sample after preparation.

2.3 - Single Plant Assessments

To assess growth rate, biomass accumulation, water use, rhizosheath development, drought stress responses and rooting characteristics, single grass plants were maintained in modified 50 ml centrifuge tubes. Four holes were drilled into the bottom of each tube to allow any excess water to drain from the soil. Each tube was also double-wrapped in opaque tape to prevent light from penetrating the sides of the tubes which would promote the growth of mosses. Before adding any soil, the weight of each empty tube was recorded.

The growth medium was composed of a 3:1 v:v mix of washed dune sand and soil collected from Powfoot golf course. A higher percentage of sand in the mix was chosen for this experiment as this would allow the plants to be harvested more easily – i.e. the soil would more readily fall away from the roots. Prior to mixing, the soil was sieved to remove any particles with a diameter greater than 3.35 mm. Once mixed, 160 labelled tubes were filled with 75 g of the medium. The final sand-soil mix had a water holding capacity of 0.21 g of water per g of dry soil, which was determined using the same method employed for the turf assessments. The tubes were watered to drained field capacity and then regularly watered for one week. Following this, any seedlings that had emerged from seeds present in the soil were removed. *A. castellana* and *P. annua* seed were then allowed to germinate inside petri dishes (sowing dates were 05/06/19 and 06/06/19, respectively) and kept in the dark before being transplanted into the soil on 11/06/19. Each seedling was planted in an individual tube, giving

a total of eighty plants for each species. NPK (10:1:7) liquid fertiliser was then applied to the soils at a rate equivalent to 40L in 700L of water per hectare on 25/06/19.

2.3.1 - Treatment groups

The trial again incorporated five different treatment groups – a control (water only), wetting agent 1, wetting agent 2, wetting agent 3 and wetting agents 1 and 2 in combination with one another. Treatments were applied prior to transplanting in 10 ml of water using a separate syringe for each treatment - the concentrations of the treatments were the same as those used for the turf assessments which are shown in Table 2. The amount of solution that was applied to the soil was calculated based on the field 'per hectare' application rates (see Appendix 1). This amount (Table 3) was the equivalent of six doses of wetting agent – in the field up to six doses would be applied throughout the year, but a decision was made to add all the doses in one application due to the short duration of the experiment. This would hopefully give enough loading of wetting agent in the soil to observe effects without the need for further applications. For each species, half the plants were well-watered throughout the experiment and half were subjected to a deficit irrigation regime after establishment (two weeks of growth).

In order to determine what level of deficit irrigation should be imposed a pilot experiment was conducted with ten plants from each species, grown in identical conditions to those which were to be grown for the formal experiment. Half of the plants from each species were grown under the control treatment and half under wetting agent treatment 1. After a two-week establishment phase throughout which plants were kept well-watered, water was withheld. The water loss was then monitored by recording the weight of each tube over a three-week period from 01/05/19 to 21/05/19. Each time a weight measurement was taken, a measurement of stomatal conductance was also made using a Delta-T AP4 dynamic porometer. Two measurements were taken from the oldest living leaf on each plant and then the average of these two measurements was taken as a value for stomatal conductance.

The data from this pilot study supported the idea that *A. castellana* has a higher drought tolerance than *P. annua* and therefore can maintain a higher stomatal conductance as the plants start to experience water stress. However, as a blanket treatment was needed for both species a deficit irrigation level was chosen that would hopefully still allow stomatal resistance to be detected in *P. annua* – i.e. a soil gravimetric water content above which no zero values for stomatal conductance were recorded. As such, the gravimetric water content which resulted in around a 40% decrease in stomatal conductance when compared to readings at field capacity was chosen as the deficit irrigation target (see Appendix 2 for details of these calculations).

The tubes were kept in a controlled environment room with a day-time temperature of 20 ± 1 ºC (07:00-19:00), night-time temperature of 15 ± 1 ºC (19:00-07:00), a light level at canopy height of 202 ± 13.3 µmol m^{-2} s^{-1} and humidity of between 50-60%. The tubes were arranged in a fully-randomised block design, with the blocks being repositioned each time they were watered. After 2 weeks of growth (27/06/19) water was withheld from half of the plants until the deficit irrigation target was reached, after which water was added appropriately to maintain the desired tube weight (see Appendix 2, Table 2). The largest and smallest plant from each treatment group were also removed from the experiment at this point so that the plants were as uniform as possible and to minimise the chance that anomalous growth would skew the results. This left a total of one hundred and forty plants with six replicates in each treatment group. All plants were harvested between 07/07/19 and 08/07/19.

Table 3: The amount of solution used to treat each tube. The amount of solution applied to the soil is the equivalent of six doses of wetting agent in the field.

Treatment	Amount of Solution applied to soil (µl)
C	247
1	247
1&2 (19:1)	247
2	240
3	247

2.3.2 - Growth Rate

Growth rate was calculated by measuring tiller length at regular intervals after the establishment phase. Tiller length was chosen to monitor growth rate as due to the large number of measurements that needed to be taken, the process needed to be relatively quick and easy. The method used was adapted from Chapman *et al*. (1983) with tiller length being measured from a fixed reference point (the top edge of the tube) to the tip of the longest living leaf. An initial height measurement was taken before deficit irrigation was implemented on 26/06/19 with subsequent measurements taken on 28/06/19, 30/06/19 and 02/07/19. Growth rate could then be calculated by dividing the length increase throughout this period by the number of days (measured to the nearest minute) that had elapsed.

2.3.3 - Above-ground Characteristics

Estimates for leaf relative water content were made based on the methods used by Garnier and Laurent (1994), Munné-Bosch and Peñuelas (2004) and Saura-Mas and Lloret (2007). Immediately after the shoots had been cut from the roots during harvesting, they were blotted with paper towel to remove any dew and weighed to determine shoot fresh weight. They were then immediately sealed in 15 ml centrifuge tubes filled with water and stored at room

temperature for 24h. After this period, shoots were removed from the water, blotted with paper towel to remove any excess surface water and then weighed again to determine the turgid/hydrated weight. They could then be oven dried at 80 ºC for one week and weighed a final time to give the shoot dry biomass. These three weights could then be used to calculate the shoot percentage relative water content (RWC) using the following formula:

$$RWC = 100 \times [(W_f - W_d) / (W_t - W_d)]$$

Where W_f is the fresh weight, W_t is the turgid weight after rehydrating the plant (the saturated weight), and W_d is the dry weight after oven-drying the plant. The RWC can be defined as the proportion of the leaf water content related to the maximum water content that can potentially be achieved by the leaf (Saura-Mas and Lloret, 2007). The shoot dry weight could also be used to calculate the root to shoot ratio by dividing it by the root dry weight, which would be determined later in the harvesting process.

2.3.4 - Stomatal Conductance

Stomatal conductance was measured using a Delta-T AP4 dynamic porometer which was re-calibrated before each measurement session with a moulded polypropylene calibration plate. Readings were taken on 01/07/19, 02/07/19, 05/07/19 and 06/07/19 as by 01/07/19 the deficit irrigation group of plants had reached the target weight. Measurements were always taken between 10:00 and 16:00 in order to minimise variation due to the diurnal rhythm of the plants. Readings were taken from the oldest living leaf of each plant. This method was chosen rather than using the youngest fully expanded leaf due to these leaves often being much smaller in width than the oldest leaf. The leaves of the greatest width were needed to fill the cup space of the sensor head as much as possible which would allow for the most reliable stomatal conductance values to be recorded. On each date that readings were taken, the width of ten randomly selected leaves from each species was measured, the mean of which was used to calculate an estimate for the area of the cup space that was covered by each species. This area was then used to adjust the stomatal conductance values at the end of the experiment, for example if only 80% of the cup space was covered then the stomatal conductance values would be increased by twenty percent to give a more accurate estimate of stomatal conductance. A mean stomatal conductance value was calculated for each plant using the four technical replicates.

2.3.5 - Rhizosheath

The rhizosheath can be defined as the soil that physically adheres to the root system and binding materials such as mucigel (McCully, 1999; George et al., 2014; York et al., 2016; Pang et al., 2017). Rhizosheath size was estimated using a method adapted from Haling *et al*.

(2010). Plants were harvested by using a hammer to knock the bottom of the tube until the whole plant and soil column was released. The plant was then gently swung into a hard surface to remove any excess soil until only that which had adhered directly to the root remained attached to the plant. The remaining adhered soil was assumed to represent an estimate of the rhizosheath. The shoot was then removed by cutting the plant at the soil line, and the fresh rhizosheath and root were weighed together.

The whole root was then placed in a pre-weighed, labelled 50 ml centrifuge tube which was half-filled with water. The tube was shaken vigorously with the cap on to remove the soil from the root. Once all the soil was suspended in the water the root was removed, blotted dry with paper towel and then weighed to give the root fresh weight. The tubes could then have their caps removed and placed in an oven at 90 ºC. The water was allowed to completely evaporate from the tubes over a four-day period. The tubes were then weighed with the dry rhizosheath inside, from which the dry rhizosheath weight could be calculated by subtracting the empty tube weight. The fresh rhizosheath weight could also be calculated by subtracting the root fresh weight from the weight of the root with fresh rhizosheath still attached.

2.3.6 - Gravimetric Water Content

The final tube weight was recorded before harvesting to determine the final soil gravimetric water content. This was calculated on a grams per gram basis using the following formula:

$$GWC = (W_f - W_e) - W_s / W_s$$

Where W_f is the final tube weight, W_e is the empty tube weight and W_s is the amount of dry soil each tube contained (seventy-five grams). The gravimetric water content in grams of water per gram of dry soil could then be determined for the rhizosheath and bulk soil respectively:

$$GWC_{bulk} = (B_f - (W_s - R_d)) / (W_s - R_d)$$

$$GWC_{rhizosheath} = (R_f - R_d) / R_d$$

Where B_f is the final bulk soil weight (calculated by subtracting the fresh root and rhizosheath weight from the final soil weight), W_s is the amount of dry soil each tube contained (75 g), R_d is the dry rhizosheath weight and R_f is the fresh rhizosheath weight. The bulk to rhizosheath water content ratio could then be calculated by dividing GWC_{bulk} by $GWC_{rhizosheath}$.

2.3.7 - Roots

After the root fresh weight had been determined, roots were stored at 7-10 ºC in 20% v/v ethanol solution. An Epson Expression 1680 scanner was then used to quantify root

characteristics. For each plant's roots, a plastic tray was filled with water into which the root system was placed. The root system was then spread out across the tray such that no roots were joined parallel to each other. The roots were then scanned and the image data processed using WinRhizo© software (Regent Instruments Inc., Québec, Canada). A selection of the images can be viewed in Appendix 3. Total root length and average root diameter were determined for each plant. Other root traits that were analysed can be viewed in Appendix 5. Root length was used to normalise the dry rhizosheath weights by dividing the later by the former. This method of normalisation was chosen as it has been suggested to provide the most informative comparisons between different species (Pang *et al*., 2017). Upon completion of image analysis, roots were oven dried at 80 ºC for one week, after which they were weighed to give the dry root biomass.

2.3.8 - Statistical Analysis

Data analysis primarily involved using two-way analyses of variance (ANOVA's) to compare differences between means and identify any interaction effects between treatments and other factors. In the results section, interaction effects are only stated if a significant result was obtained. For comparing rate of change over time, ANCOVA's were used. Linear regression was used alongside the ANCOVA when appropriate. Where direct comparisons were required between species for a given treatment, Tukey post-hoc tests were used. Correlation analysis was also performed for variables for which a relationship might be expected. However, as no tests revealed strong correlations these were not included in the results section (Appendix 4).

Each time a model was constructed, diagnostic plots were used to confirm that the residuals of the model followed an approximately normal distribution, there was homogeneity of variance and that there were no extreme outliers skewing the results. If any of these assumptions were violated data was transformed or a different statistical test was employed – this is outlined in the results section wherever it is the case. For the germination data (results section 3.1), the ability to properly evaluate the assumptions of the ANOVA test is reduced by the fact that there were only three replicates per treatment for each species. P-values are quoted wherever they are important in explaining the key results from each statistical test. A threshold of $p < 0.05$ was used to determine whether differences were statistically significant or not. In all Figures that contain error bars these represent the standard error of the mean. For box and whisker plots, the horizontal line within the boxes is the median and the dot associated with each box is the mean. The space within the upper and lower edge of each box is the inter-quartile range and the whiskers show the complete range of the data.

Results

3.1 – Germination

There was no significant difference ($p = 0.967$) between treatments in terms of final germination success for *P. annua*, but it was found to be significantly higher for seeds under treatment 3 compared to the control group ($p = 0.0223$) for *A. castellana* (Figure 3). *P. annua* had a significantly higher germination success than *A. castellana* ($p < 0.05$) across all treatments (Figure 3).

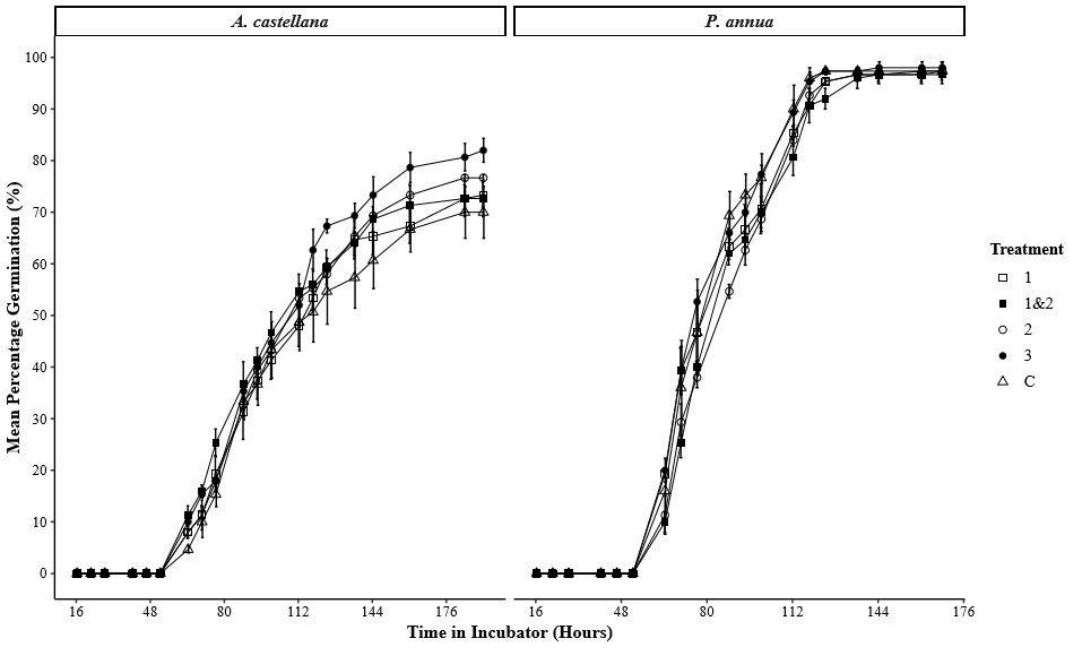

Figure 3. *Mean percentage germination over time for A. castellana and P. annua. Seeds were sown onto filter paper that was treated with water (C) and wetting agents 1, 1&2, 2 and 3.*

3.2 - Turf Assessments

3.2.1 - Water Infiltration

There was no significant difference ($p = 0.286$) in the water infiltration time between the two watering regimes (Figure 4). However, there was a significant interaction effect ($p < 0.05$) between treatment and regime, which suggests the relationship between treatment and infiltration time depends on whether soils are well-watered or droughted. This analysis was performed after the infiltration time data was logarithmically transformed in order to make the residuals of the model follow an approximately normal distribution. All wetting agent treatments gave a significantly faster ($p < 0.05$) infiltration time than the untreated soil (Figure 4). There was no significant difference between wetting agents 1&2 and 1, wetting agents 3 and 1 and wetting agents 3 and 1&2 ($p = 0.999$, $p = 0.985$ and $p = 0.991$, respectively). Wetting agents 1, 1&2 and 3 all gave a significantly faster infiltration time ($p < 0.05$ in all cases) than wetting agent 2 (Figure 4).

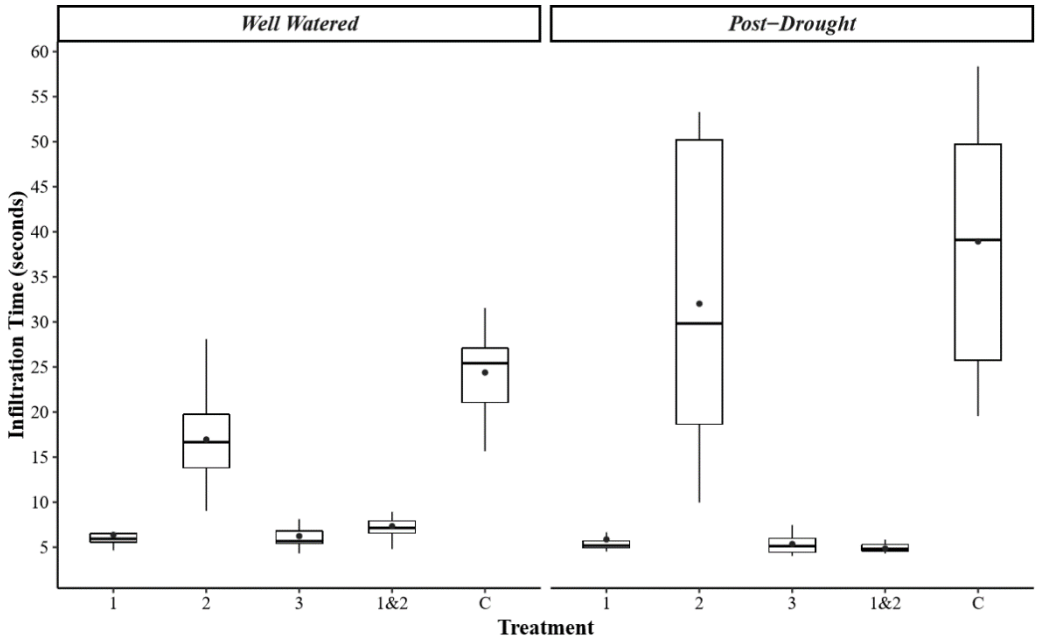

Figure 4. *The durations for 40 ml of water to fully infiltrate into the soil for both the well-watered and post-drought phase of the experiment. Species were combined by treatment due to there being no significant differences between the species within treatment groups; p = 0.459 and p = 0.645 for well-watered and drought treatments respectively. The ANOVA performed on the drought data used logarithmically transformed values, as the residuals of the model did not follow a normal distribution using the raw data.*

3.2.2 - Growth Rate

The decision was made to split the well-watered period into two phases for data analysis. This is due to the rapid decrease in growth rate after the initial stimulation of growth that was observed after the first time the grasses were cut (phase 1 on Figure 5). The first cut was less vigorous than subsequent cuts, so it is important to make this distinction for analysis of the data. For all subsequent cuts, the growth rate remained approximately constant throughout the rest of the well-watered period (phase 2 on Figure 5). As such, a mean growth rate for each replicate within each treatment was calculated using the measurements taken across the five dates, i.e. each date was treated as a technical replicate. This provided five functional replicates for each treatment, which were then used to produce Figure 6 and carry out statistical analysis.

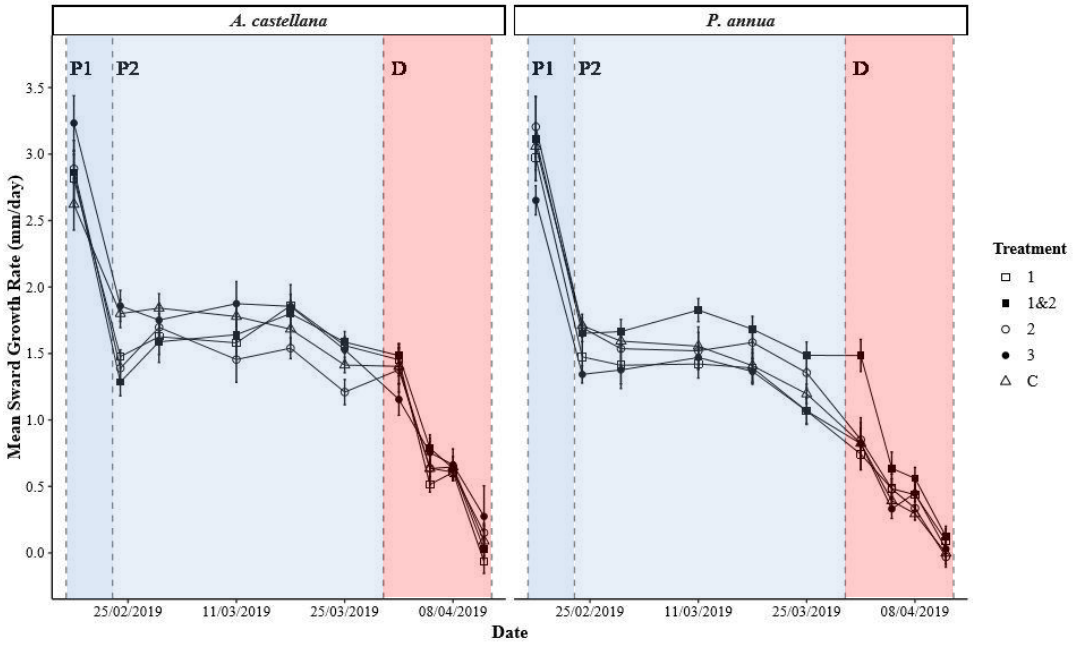

Figure 5. *Mean growth rate over time for each treatment and for both species. Well-watered phase 1 (P1), well-watered phase 2 (P2) and the onset of the drought phase (D) are indicated by the dashed vertical lines and the dark blue, light blue and red highlighting respectively.*

Well-watered phase 1

No significant difference (p = 0.427) in growth rate was present between treatments for *P. annua* but for *A. castellana* the growth rate was significantly higher (p = 0.0363) for turfs growing under treatment 3 compared to the control turfs (Figure 6). There was no significant difference (p = 0.375) between the growth rate of the two species across treatments (Figure 6).

Well-watered phase 2

For *P. annua* a significant difference in growth rate (p = 0.0281) was present between treatments. These differences were between treatments 1 and 1&2 (p = 0.00876) and 3 and 1&2 (p = 0.00462), with treatment 1&2 resulting in a significantly higher growth rate in both comparisons (Figure 6). However, the test showed no significant difference between the untreated control and any of the wetting agent treatments. For *A. castellana* growth rate under treatment 2 was significantly lower than the control group (p = 0.0373) and treatment 3 (p = 0.00902). There was a significant difference in the growth rate (p = 0.00310) between the two species and a significant interaction effect (p = 0.00395) between treatment and species (Figure 6). A Tukey post-hoc test showed that the growth rate was significantly higher (p = 0.00572) for *A. castellana* compared to *P. annua* under treatment 3 (Figure 6).

Drought Phase

As would be expected during the drought phase, for both species there was a significant effect ($p < 0.05$) of soil water content on growth rate (Figure 7). No significant treatment effect on sward growth rate ($p = 0.665$) or interaction effect between treatment and soil water content ($p = 0.295$) was present for *A. castellana*. For *P. annua*, there was a significant treatment effect on growth rate ($p < 0.05$) and a significant interaction effect ($p < 0.05$) between treatment and soil water content (Figure 7). We can determine where these treatment differences are likely to be by looking at the intercepts and slopes of the regression lines plotted for each treatment (Table 4). It is also worth mentioning that when the first measurements during the drought phase were taken, a significantly higher growth rate ($p = 0.00256$) was found for *P. annua* under treatment 1&2 compared to the control (Figure 7). The significant interaction suggests the relationship between growth rate and soil water content may depend on the treatment for *P. annua*. The decrease in growth rate was significantly faster for treatment 1 ($p = 0.00432$) in *A. castellana* compared to *P. annua* (Figure 7).

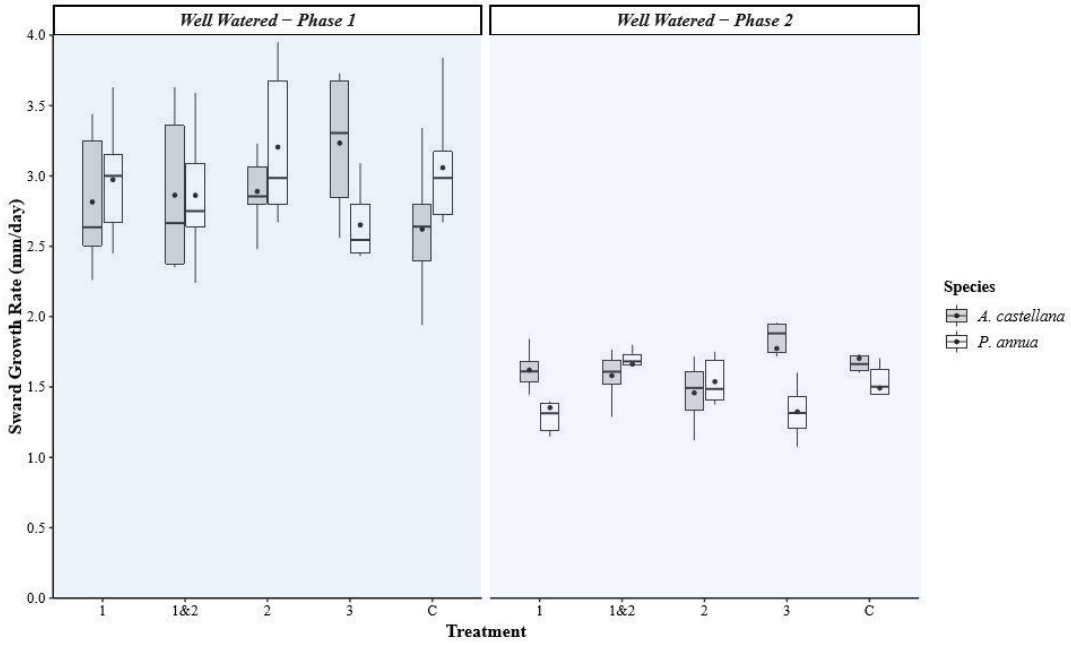

Figure 6*. Sward growth rate expressed in millimetres of growth per day for well-watered phase 1 and 2. Well-watered phase 1 is the initial stimulation of growth that took place after the first time the turfs were cut. Phase 2 includes the growth data that was gathered after all subsequent cuts before the drought period.*

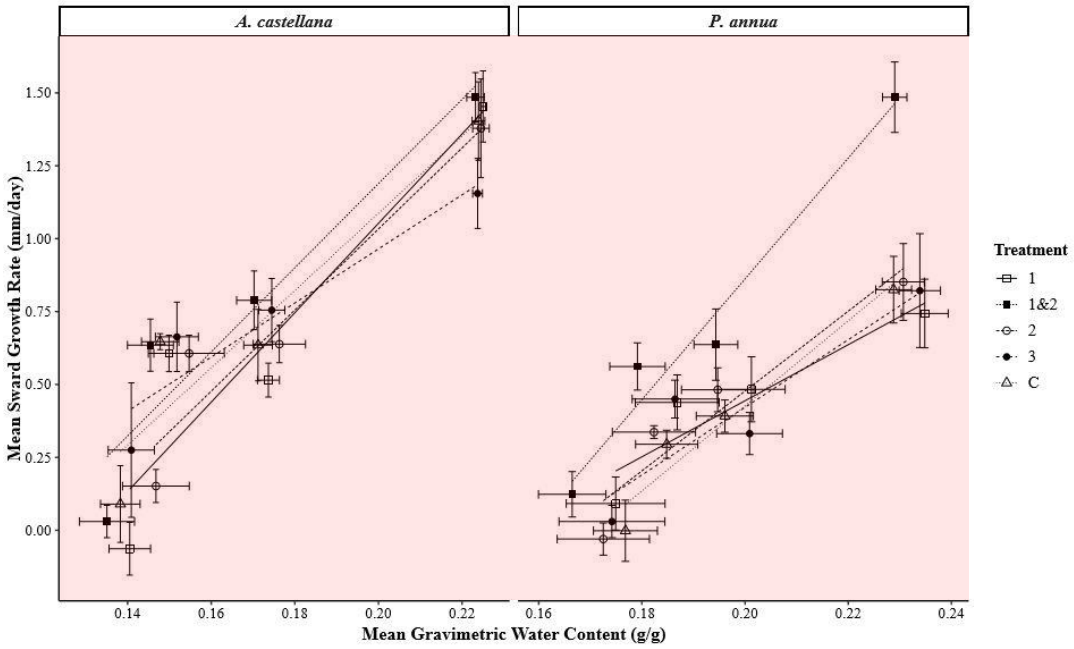

Figure 7. *Mean growth rate during the drought phase of the experiment plotted against mean soil gravimetric water content (expressed in grams of water per gram of dry soil). A linear regression model has been fitted for each treatment. Intercept, slope, P-values and R^2-values for each regression line are shown in Table 4.*

Table 4: The r-squared value and associated coefficients of each of the regression lines fitted to the data shown in Figure 7. The much steeper slope for *P. annua* under treatment 1&2 suggests these turfs had the fastest decrease in growth rate. Asterix' denote a significant p-value using a significance threshold of $p < 0.05$.

Species	**Treatment**	**Intercept**	**Slope (with p-value)**	**R^2**
A. castellana	C	-1.56	13.2 (0.0591)	0.885
	1	-2.013	15.3 (0.0724)	0.8604
	1&2	-1.70	14.4 (0.0506)	0.9013
	2	-1.75	13.9 (0.0434 *)	0.915
	3	-0.889	9.27 (0.0563)	0.891
P. annua	C	-2.49	14.5 (0.0248 *)	0.951
	1	-1.48	9.61 (0.0698)	0.865
	1&2	-3.28	20.7 (0.0163 *)	0.968
	2	-2.26	13.7 (0.0454 *)	0.911
	3	-1.90	11.6 (0.0864)	0.835

3.2.3 - Plant Tissue Nutrients

For both species, no significant differences ($p > 0.05$ in all cases) in nutrient content between treatments were present for any of the macro- or micro-nutrients analysed (see Tables 5 and 6). For *A. castellana*, two replicates were consistently skewing the results for treatments 1 and 2 and the decision was made to remove them, as they lay well outside two standard deviations of the mean for each treatment. This was also the case for one of the treatment 3 replicates for *P. annua*. As a result, conventional ANOVA's could not be used due to the now unequal number of observations for each treatment. Instead, a type-III sums of squares ANOVA was used for each comparison. For the same reason, this type of analysis was also used to compare differences between the two species.

For macro-nutrients K, Mg and P no significant differences were present across all treatments between the two species ($p > 0.05$ in all cases). This was also true for micro-nutrients Fe, B, Mn, Cu and Mo ($p > 0.05$ in all cases). For macro-nutrients Ca and S, *P. annua* had significantly higher quantities than *A. castellana* across treatments ($p < 0.005$ in both cases). However, for within treatment comparisons this was the case for all treatments except treatment 1&2 ($p < 0.05$) for Ca and only for treatment 3 ($p = 0.0268$) for S. It is also worth mentioning that the control treatment was on the threshold for significant difference for S ($p = 0.0516$). For micro-nutrient Zn, although no significant differences across treatments were present ($p = 0.0528$), for within treatment comparisons there were significantly higher quantities under treatments 2 and 3 in *P. annua* than *A. castellana* ($p < 0.05$ in both cases).

Table 5: Macro-nutrients contained within the plant tissue that were analysed using ICP-OES. Quantities are expressed as milligrams of the nutrient per gram of dry plant matter. The p-value associated with each nutrient for each species is the result of a comparison between treatments using one-way ANOVA's.

Element	**Species**	**Nutrient Content split by Treatment (mean mg/g ± standard error)**					**p**
		1	**1&2**	**2**	**3**	**C**	
K	*A. castellana*	37.2 ± 1.28	40.6 ± 3.38	31.1 ± 5.13	32.9 ± 3.87	33.6 ± 2.60	0.338
	P. annua	31.3 ± 3.043	41.8 ± 5.82	32.0 ± 3.84	39.2 ± 7.56	42.0 ± 3.20	0.320
Ca	*A. castellana*	3.44 ± 0.175	4.069 ± 0.4067	3.39 ± 0.08041	3.53 ± 0.252	3.93 ± 0.350	0.382
	P. annua	6.58 ± 0.501	5.84 ± 0.443	6.24 ± 0.767	6.92 ± 0.5091	6.60 ± 0.524	0.726
Mg	*A. castellana*	4.14 ± 0.0797	4.19 ± 0.196	3.51 ± 0.372	3.70 ± 0.455	4.00 ± 0.354	0.562
	P. annua	3.051 ± 0.211	4.033 ± 0.667	3.41 ± 0.535	4.44 ± 0.853	4.14 ± 0.445	0.423
P	*A. castellana*	3.15 ± 0.0777	3.53 ± 0.276	2.79 ± 0.513	2.71 ± 0.274	2.88 ± 0.228	0.295
	P. annua	2.78 ± 0.231	3.32 ± 0.453	2.803 ± 0.317	3.6048 ± 0.758	3.74 ± 0.326	0.386
S	*A. castellana*	3.51 ± 0.0680	4.00 ± 0.370	3.25 ± 0.376	3.19 ± 0.239	3.40 ± 0.244	0.282
	P. annua	4.11 ± 0.330	4.60 ± 0.422	4.22 ± 0.463	5.049 ± 0.483	5.053 ± 0.405	0.375

Table 6: Micro-nutrients contained within the plant tissue that were analysed using ICP-OES. Quantities are expressed as milligrams of the nutrient per gram of dry plant matter. The p-value associated with each nutrient for each species is the result of a comparison between treatments using one-way ANOVA's.

Element	**Species**	**Nutrient Content split by Treatment (mean mg/g ± standard error)**					**p**
		1	**1&2**	**2**	**3**	**C**	
Fe	*A. castellana*	2.56 ± 0.723	1.77 ± 0.272	3.47 ± 0.760	2.38 ± 0.279	3.089 ± 0.859	0.365
	P. annua	1.90 ± 0.386	2.90 ± 0.485	3.13 ± 1.41	4.22 ± 0.414	5.053 ± 1.82	0.332
B	*A. castellana*	0.0838 ± 0.00174	0.0860 ± 0.00640	0.0647 ± 0.00594	0.0742 ± 0.00734	0.0892 ± 0.00974	0.139
	P. annua	0.0841 ± 0.00994	0.0968 ± 0.0116	0.0896 ± 0.0125	0.10097 ± 0.0181	0.0904 ± 0.0117	0.901 4
Mn	*A. castellana*	0.587 ± 0.0114	0.472 ± 0.0643	0.423 ± 0.0758	0.479 ± 0.0860	0.533 ± 0.0833	0.605 7
	P. annua	0.2060 ± 0.0174	0.361 ± 0.1037	0.244 ± 0.0463	0.4066 ± 0.1401	0.342 ± 0.116	0.535
Zn	*A. castellana*	0.0944 ± 0.0311	0.0832 ± 0.00710	0.0716 ± 0.00787	0.0659 ± 0.00783	0.0756 ± 0.00727	0.673
	P. annua	0.1303 ± 0.00955	0.1068 ± 0.00818	0.147 ± 0.0181	0.149 ± 0.0132	0.113 ± 0.0159	0.133
Cu	*A. castellana*	0.0318 ± 0.000970	0.0421 ± 0.00248	0.0366 ± 0.00215	0.0344 ± 0.00277	0.0426 ± 0.00729	0.270
	P. annua	0.0685 ± 0.00725	0.0516 ± 0.00605	0.0639 ± 0.00148	0.0782 ± 0.00465	0.0584 ± 0.00688	0.335
Mo	*A. castellana*	0.0533 ± 0.00480	0.0586 ± 0.00489	0.0474 ± 0.00848	0.0565 ± 0.00977	0.0522 ± 0.00399	0.815
	P. annua	0.0589 ± 0.00786	0.0776 ± 0.0152	0.0849 ± 0.0202	0.08076 ± 0.00745	0.0745 ± 0.00841	0.678

3.3 - Single Plant Assessments

3.3.1 - Growth Rate

Well-watered

For both species, there was no significant difference in plant growth rate between treatments ($p = 0.828$ and $p = 0.616$, for *P. annua* and *A. castellana* respectively). However, there was a significant difference in the plant growth rate between the two species ($p < 0.05$), with growth rate being significantly higher ($p = 0.0199$) for *A. castellana* compared to *P. annua* under treatment 3 (Figure 8).

Deficit irrigation

For both species, there was no significant difference in plant growth rate between treatments ($p = 0.2012$ and $p = 0.955$, for *P. annua* and *A. castellana* respectively). Again, there was a significant difference in the growth rate between species with *A. castellana* having the higher growth rate ($p = 0.0249$), but there were no differences between the two species when within treatment comparisons were made (Figure 8). The target gravimetric water contents for each of the two watering regimes used throughout the experiment are shown in Table 7.

Table 7: The target gravimetric water content (expressed as grams of water per gram of dry soil) for each of the two watering regimes used in the experiment. The well-watered value is based on the drained field capacity of the soil and the deficit irrigation value is based on that which corresponds to approximately a forty percent decrease in stomatal conductance (see Appendix 2).

Watering Regime	**Mean Gravimetric Water Content (g/g)**
Well-watered	0.214
Deficit Irrigation	0.05033

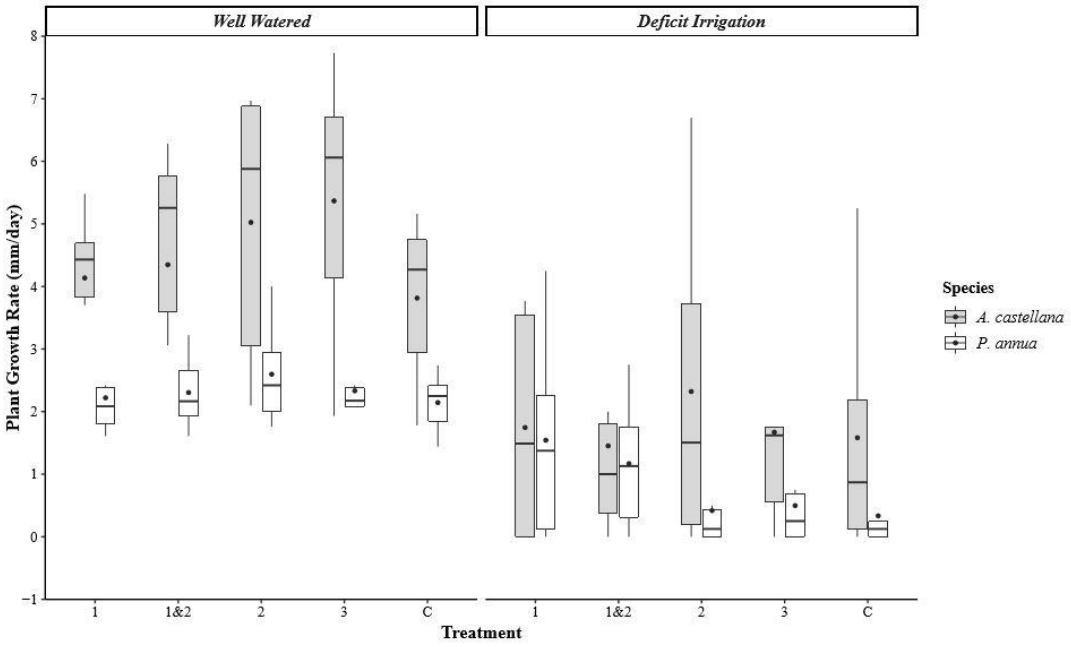

Figure 8. *Plant growth rate expressed in millimetres of growth per day for plants under well-watered and deficit irrigation regimes. The well-watered phase includes all growth that took place between 26/06/19 and 02/07/19. The deficit irrigation phase includes all growth that took place between 28/06/19 and 02/07/19, as prior to 28/06/19 the water content of the soil had not yet sufficiently decreased.*

Assessing the interaction between watering regime and species found that *A. castellana* had a significantly higher growth rate across treatments when well-watered ($p < 0.005$), but the two species' growth rates were not significantly different from one another under deficit irrigation ($p = 0.0648$). Both had lower growth rates under deficit irrigation as expected, but that of *P. annua* was more similar between the two regimes. This is reflected by the greater difference in the mean growth rate between the two regimes for *A. castellana* than *P. annua* (Figure 9).

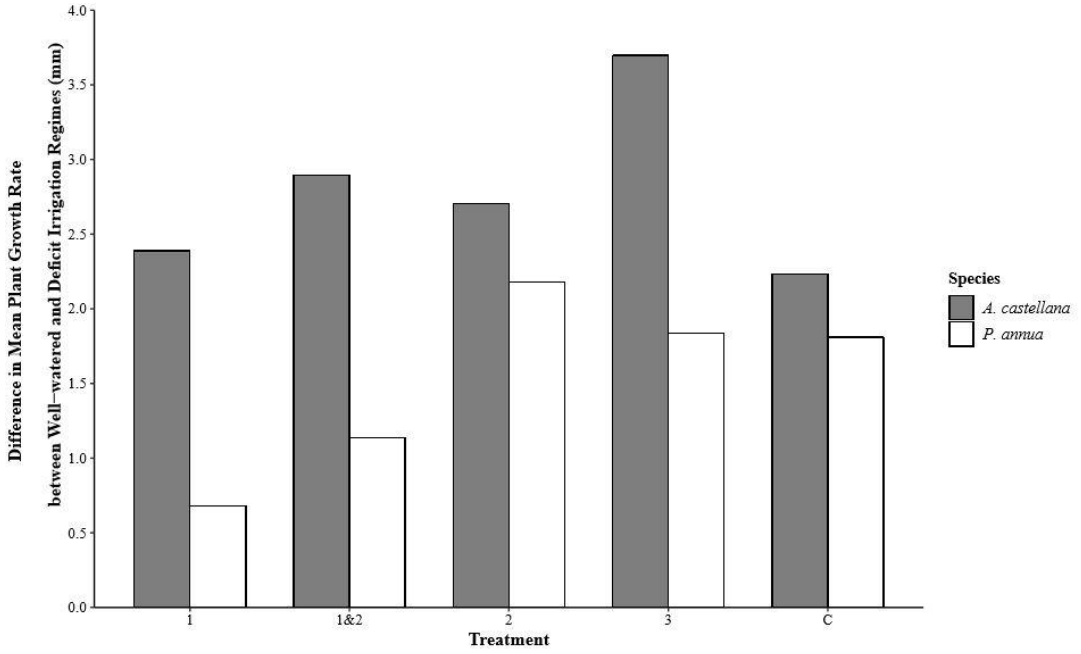

Figure 9*. Mean difference in growth rate for the two species for each treatment between the well-watered and deficit irrigation regimes.*

3.3.2 - Above-ground Biomass

Well-watered

No significant differences in above-ground biomass between any of the treatments and the control were present for either *P. annua* or *A. castellana* ($p = 0.566$ and $p = 0.134$, respectively). The above-ground biomass was significantly higher for *P. annua* than *A. castellana* across treatments ($p < 0.05$), but there were no differences between the two species when within treatment comparisons were made (Figure 10). The above-ground biomass was statistically identical for both species under treatment 3 ($p = 1.00$) but under all other treatments *P. annua* had a higher mean (Figure 10).

Deficit irrigation

For *P. annua*, above-ground biomass was significantly higher ($p < 0.005$) under treatment 1&2 when compared to the control treatment (Figure 10). A significant difference was found in the above-ground biomass between treatments in *A. castellana* ($p = 0.0112$), but there were no significant differences when any of the wetting agent treatments were compared to the control treatment (Figure 10). There was a significant difference in the above-ground biomass between the two species ($p < 0.05$), with *P. annua* being significantly higher ($p < 0.05$) than *A. castellana* under treatment 1&2 (Figure 10). There was also a significant interaction between treatment and species ($p < 0.05$).

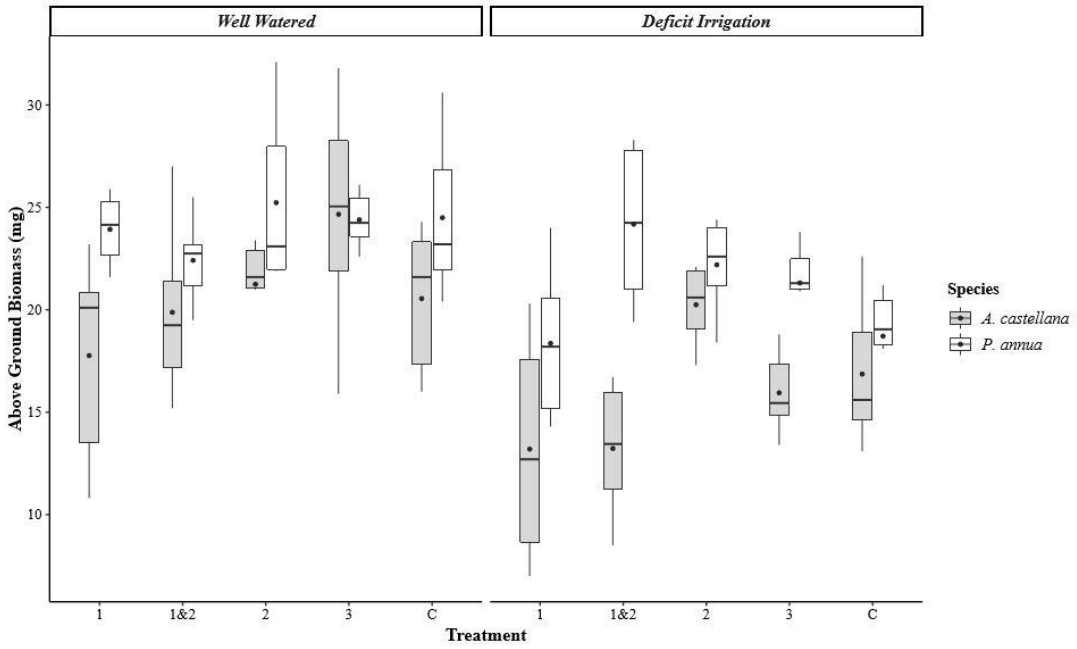

Figure 10*. Above-ground biomass expressed in milligrams of dry matter for both species under each treatment for both well-watered and deficit irrigation regimes.*

3.3.3 - Shoot Relative Water Content

Well-watered

For *P. annua*, plants under treatments 1&2 and 2 had a significantly lower RWC ($p < 0.05$ in both cases) than the control plants, but there were no significant differences between treatments ($p = 0.1019$) for *A. castellana* (Figure 11). There was a significant difference in the RWC between the two species, with that of *A. castellana* being significantly higher than *P. annua* for treatments 1, 1&2 and 3 ($p < 0.05$ in all cases). However, there was no significant difference between the two species for the control plants ($p = 0.395$) or those under treatment 2 ($p = 0.0686$).

Deficit irrigation

For *P. annua*, plants under treatments 1&2, 2 and 3 had a significantly higher ($p < 0.05$ in all cases) RWC than the control plants, while treatment 1 produced plants with a significantly lower RWC ($p < 0.05$) than the control plants for *A. castellana* (Figure 11). *A. castellana* had a significantly higher RWC than *P. annua* across treatments, but this was only the case for control plants when within treatment comparisons were made ($p < 0.05$).

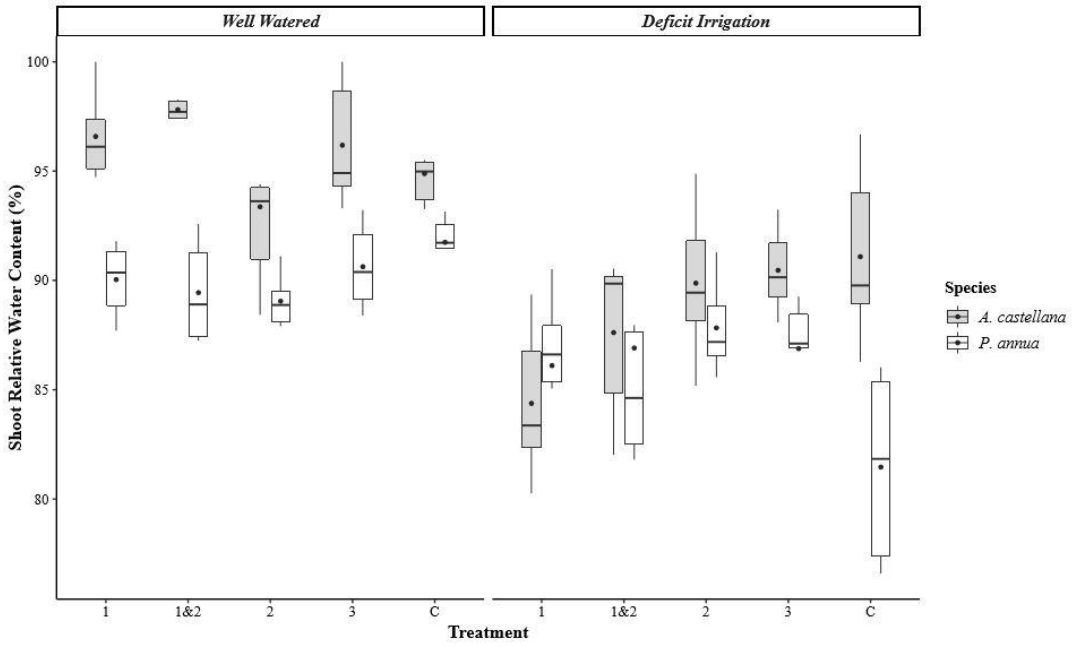

***Figure 11**. Shoot relative water content (RWC) expressed as a percentage for both species under each treatment for both well-watered and deficit irrigation regimes.*

3.3.4 - Stomatal Conductance

Well-watered

Plants under treatments 1, 1&2 and 3 had significantly higher stomatal conductance ($p < 0.05$ in all cases) than the control plants for *P. annua* (Figure 12). There were no significant differences in stomatal conductance between plants under any of the treatments for *A. castellana* ($p = 0.984$). *P. annua* had significantly higher stomatal conductance than *A. castellana* across treatments ($p < 0.05$), but there were no differences between the two species when within treatment comparisons were made (Figure 12).

Deficit Irrigation

Again, plants under treatments 1, 1&2 and 3 had significantly higher stomatal conductance ($p < 0.05$ in all cases) than the control plants for *P. annua* (Figure 12). There were no significant differences in stomatal conductance between plants under any of the treatments for *A. castellana* ($p = 0.3012$). There was no significant difference in the stomatal conductance between the two species across treatments ($p = 0.644$), but there was a significant interaction effect between treatment and species ($p = 0.0235$).

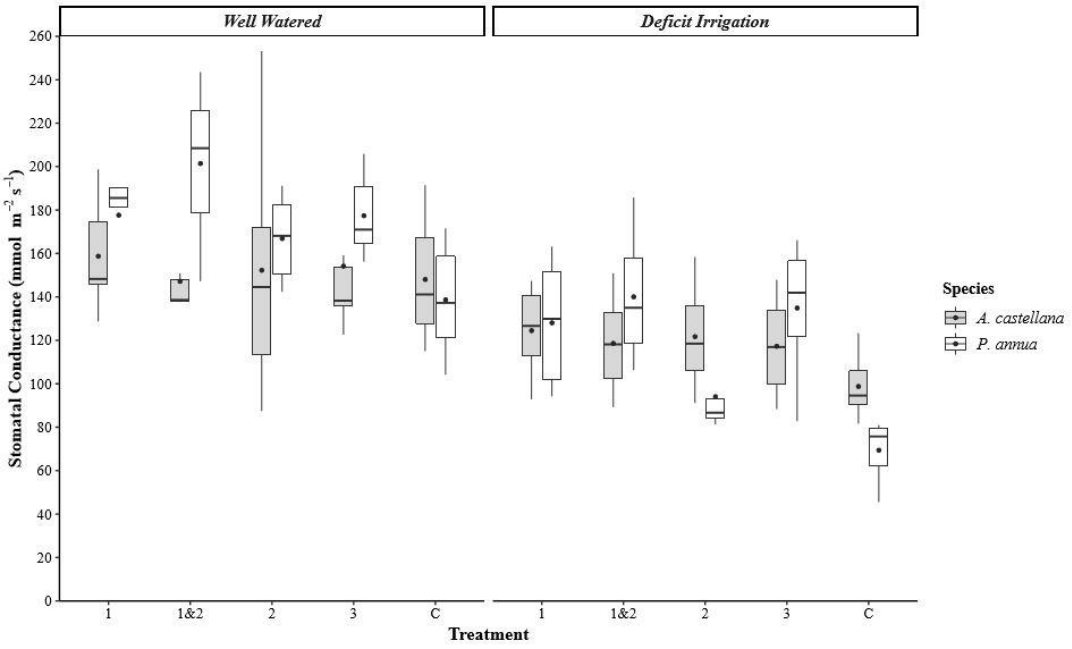

Figure 12*. Stomatal conductance taken from the oldest living leaf of each plant for both species under each treatment for both well-watered and deficit irrigation regimes. The values for stomatal conductance were adjusted to reflect differences in leaf area – the process for this is outlined in methods section 2.3.4.*

3.3.5 - Rhizosheath and Soil Water Content

Well-watered – Rhizosheath Weight

Normalised rhizosheath weight was significantly higher than the control group for treatments 1&2 ($p < 0.05$) and 3 ($p = 0.0215$) for *P. annua* (Figure 13). For *A. castellana*, normalised rhizosheath weight was significantly higher for treatment 3 ($p = 0.0132$) compared to the control group (Figure 13). There was no significant difference in normalised rhizosheath weight between species ($p = 0.0769$).

Deficit Irrigation – Rhizosheath Weight

Normalised rhizosheath weight was significantly higher for treatments 1 ($p = 0.0218$), 1&2 ($p = 0.0411$) and 2 ($p = 0.0299$) when compared with the control treatment for *P. annua* (Figure 13). For *A. castellana*, normalised rhizosheath weight was found to be significantly higher for treatments 2 ($p = 0.00973$) and 3 ($p = 0.0258$) when compared to the control treatment (Figure 13). There was no significant difference in normalised rhizosheath weight between species ($p = 0.1603$).

Well-watered – Rhizosheath versus Bulk Soil Water Content

For both species, the ratio between rhizosheath water content and bulk soil water content was significantly higher for all wetting agent treatments ($p < 0.05$ in all cases) compared to the

control treatment (Figure 14). No significant difference between species was present ($p = 0.0525$).

Deficit Irrigation – Rhizosheath versus Bulk Soil Water Content

For *P. annua*, again the ratio between rhizosheath water content and bulk soil water content was significantly higher for all wetting agent treatments ($p < 0.05$ in all cases) compared to the control treatment (Figure 14). However, there were no significant differences ($p = 0.458$) between any of the wetting agent treatments compared to the control treatment for *A. castellana*. There was a significantly higher water content ratio across wetting agent treatments for *P. annua* compared to *A. castellana* ($p < 0.05$), but not for the control plants ($p = 0.647$). There was a significant interaction effect ($p < 0.05$) between treatment and species. Rhizosheath to bulk soil water content ratio was significantly higher in deficit irrigation plants compared well-watered plants across treatments ($p < 0.05$) and there was a significant interaction effect ($p = 0.0244$) between watering regime and treatment (Figure 14).

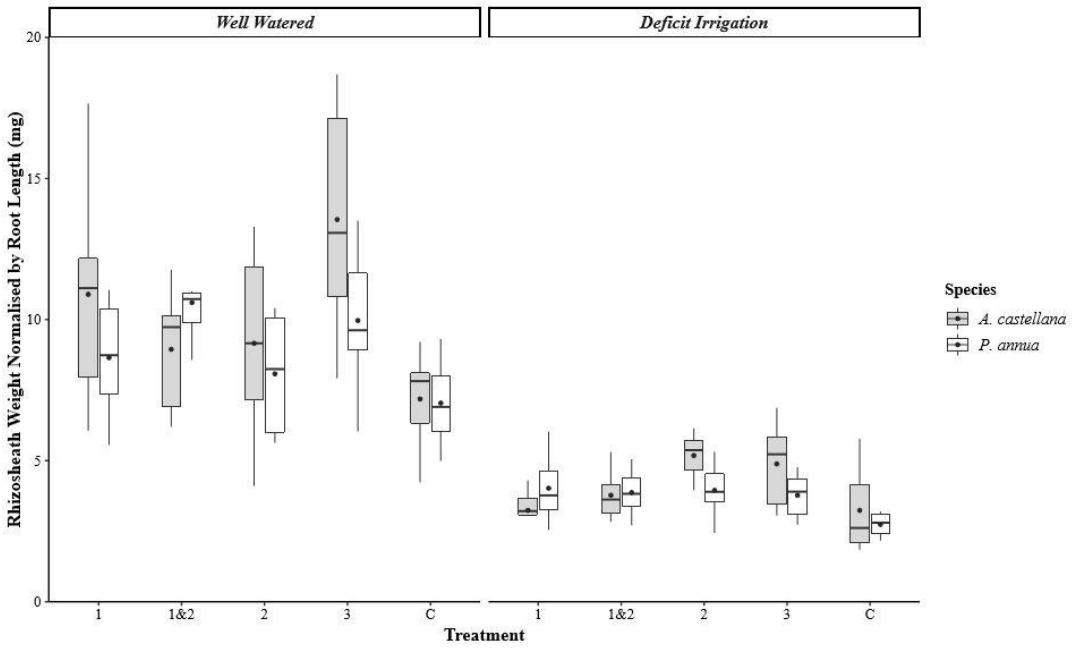

Figure 13. *Dry rhizosheath weight expressed in milligrams, normalised by root length. Data is shown for both species for each of the treatments under well-watered and deficit irrigation regimes.*

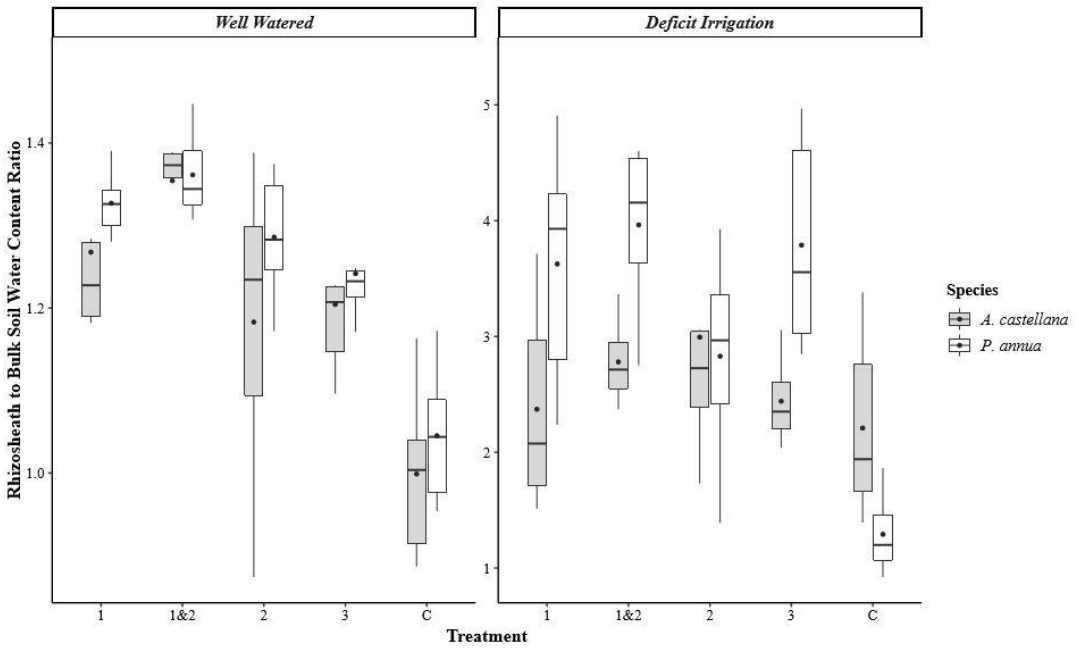

Figure 14. *The ratio between the gravimetric water content of the rhizosheath and the gravimetric water content of the surrounding bulk soil for both species under each treatment for well-watered and deficit irrigation regimes. Each quantity was expressed on a grams of water per gram of dry soil basis before the ratio was calculated.*

3.3.6 - Roots

Below-ground Biomass – Well-watered

No significant differences in below-ground biomass were present between any of the treatments for either species ($p = 0.7708$ and $p = 0.569$ for *P. annua* and *A. castellana*, respectively). *P. annua* had significantly higher below-ground biomass than *A. castellana* across treatments ($p < 0.05$), but there were no differences between the two species when within treatment comparisons were made (Figure 15).

Below-ground Biomass – Deficit irrigation

Under treatments 1&2, 2 and 3 below-ground biomass was significantly higher ($p < 0.05$ in all cases) than under the control treatment for *P. annua* (Figure 15). There was a significant difference in below-ground biomass between treatments for *A. castellana* ($p = 0.0177$), however there was no difference between any of the wetting agent treatments and the control treatment (Figure 15). There was a significant difference in below-ground biomass ($p < 0.05$) between the two species and that of *P. annua* was significantly higher than that of *A. castellana* ($p < 0.05$) for treatment 1&2 (Figure 15). There was also a significant interaction between treatment and species ($p < 0.05$).

Root Length – Well-watered

No significant differences in root length were present between any of the treatments for either species ($p = 0.280$ and $p = 0.2803$ for *P. annua* and *A. castellana*, respectively). *P. annua* had significantly longer roots than *A. castellana* across treatments ($p < 0.05$), but there were no differences between the two species when within treatment comparisons were made (Figure 16).

Root Length – Deficit irrigation

For *P. annua*, plants under treatment 3 had significantly shorter roots ($p < 0.05$) than those under the control treatment (Figure 16). There were no significant differences in root length between any of the treatments for *A. castellana* ($p = 0.173$). Again, *P. annua* had significantly longer roots than *A. castellana* across treatments ($p < 0.05$), but there were no differences between the two species when within treatment comparisons were made (Figure 16).

Average Root Diameter – Well-watered

For *P. annua*, roots under all wetting agent treatments had a significantly smaller average diameter ($p < 0.05$ in all cases) than the control treatment (Figure 17). No significant differences in root average diameter were present between treatments for *A. castellana* ($p = 0.860$). *A. castellana* plants were significantly larger in average root diameter than *P. annua* across treatments ($p < 0.05$), however for within treatment comparisons this was only the case for treatment 3 ($p < 0.05$).

Average Root Diameter – Deficit irrigation

For *P. annua*, only treatment 1&2 and treatment 3 were significantly smaller in average root diameter ($p < 0.05$ in both cases) than the control treatment (Figure 17). Again, there were no significant differences in average root diameter between treatments for *A. castellana* ($p = 0.0756$). There was no significant difference in average root diameter across treatments between the two species ($p = 0.439$), but there was a significant interaction ($p = 0.0471$) between treatment and species (Figure 17).

Analyses of additional root traits can be viewed in Appendix 5.

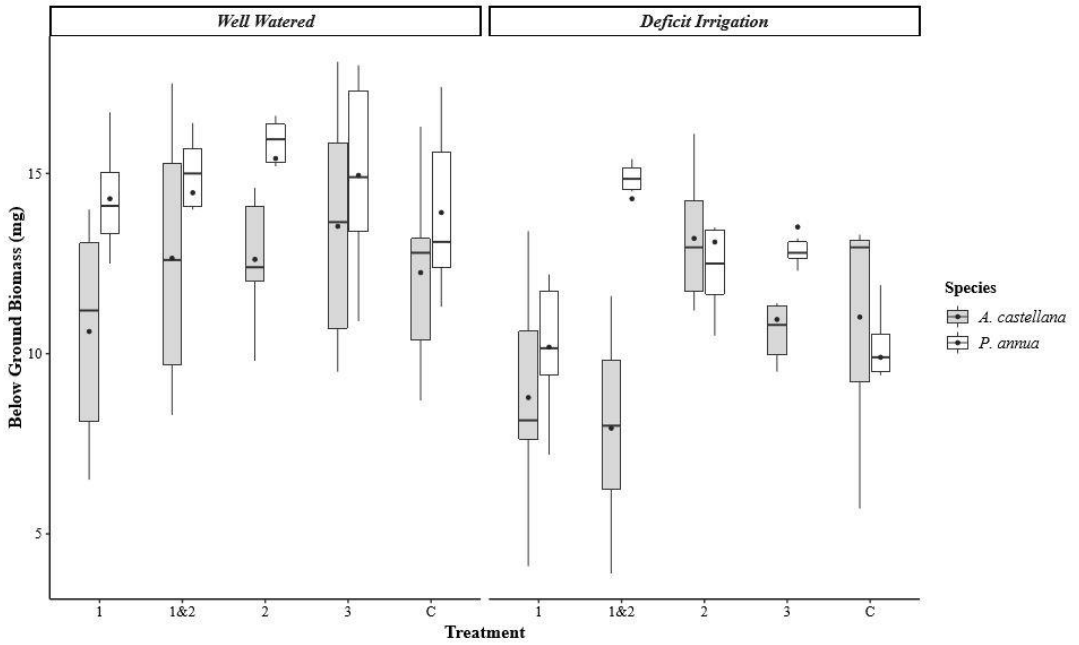

Figure 15. *Below-ground biomass expressed in milligrams of dry matter for both species under each treatment for both well-watered and deficit irrigation regimes.*

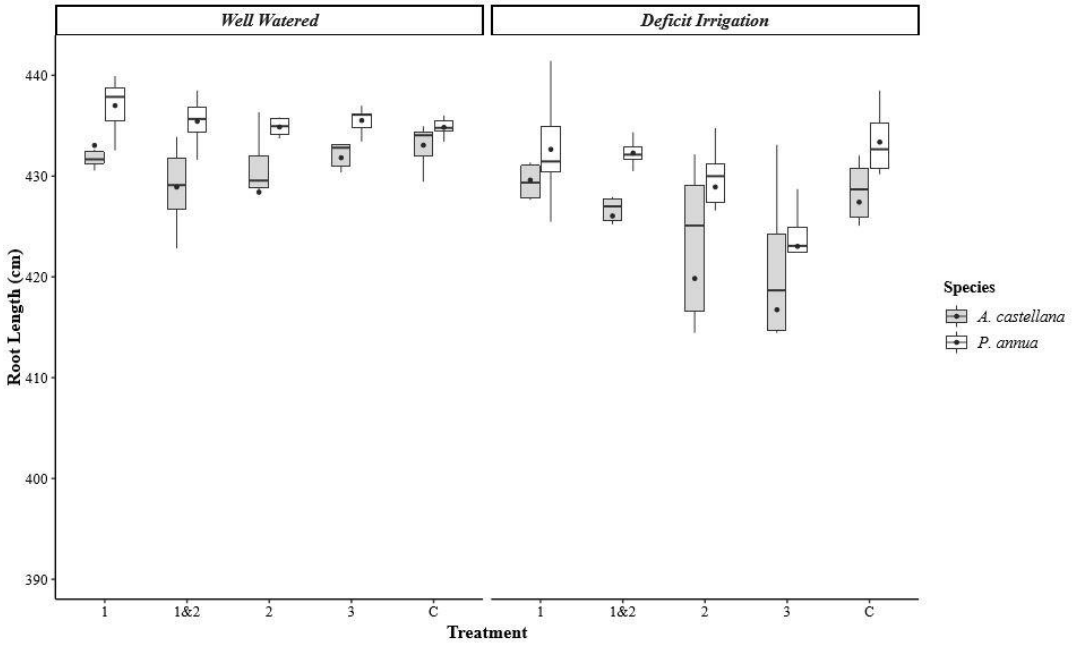

Figure 16. *Root length expressed in centimetres for both species under each treatment for both well-watered and deficit irrigation regimes.*

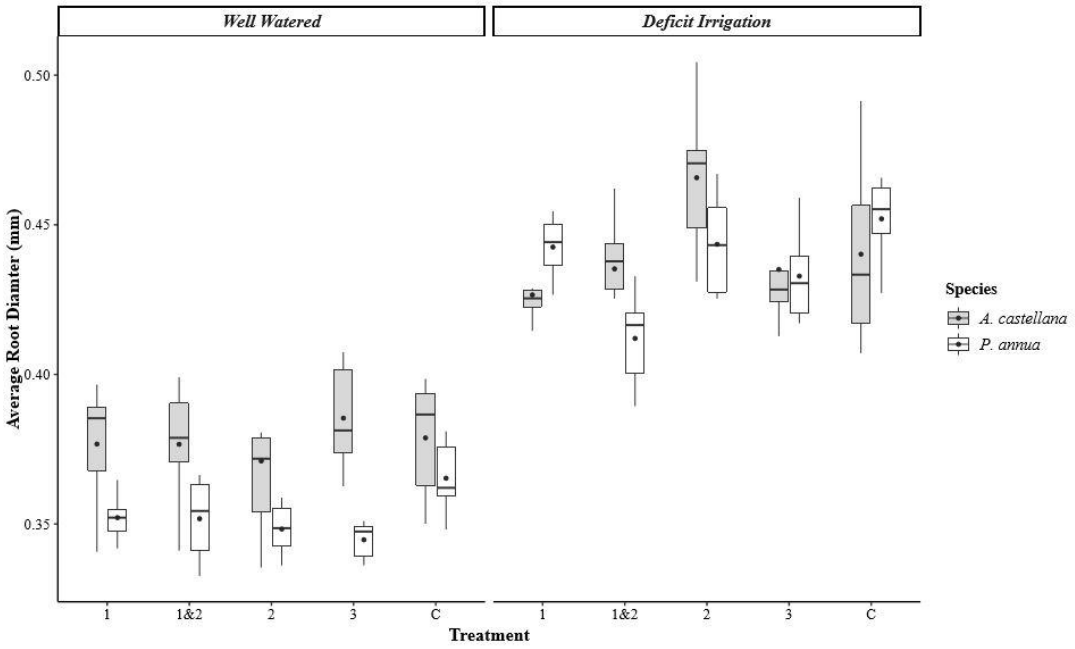

Figure 17*. Average root diameter expressed in millimetres for both species under each treatment for both well-watered and deficit irrigation regimes.*

3.3.7 - Root to Shoot Ratio

Well-watered

There was no significant difference in the root to shoot ratio between any of the treatments for either species ($p = 0.310$ and $p = 0.622$ for *P. annua* and *A. castellana*, respectively). There was also no significant difference in root to shoot ratio ($p = 0.672$) across treatments between the two species (Figure 18).

Deficit irrigation

For *P. annua*, the root to shoot ratio was significantly higher for plants under Treatment 3 ($p < 0.05$) compared to the control treatment (Figure 18). Again, there was no significant difference in the root to shoot ratio between any of the treatments for *A. castellana* ($p = 0.756$). *A. castellana* had a significantly higher root to shoot ratio than *P. annua* across treatments ($p < 0.05$), but there were no differences between the two species when within treatment comparisons were made (Figure 18).

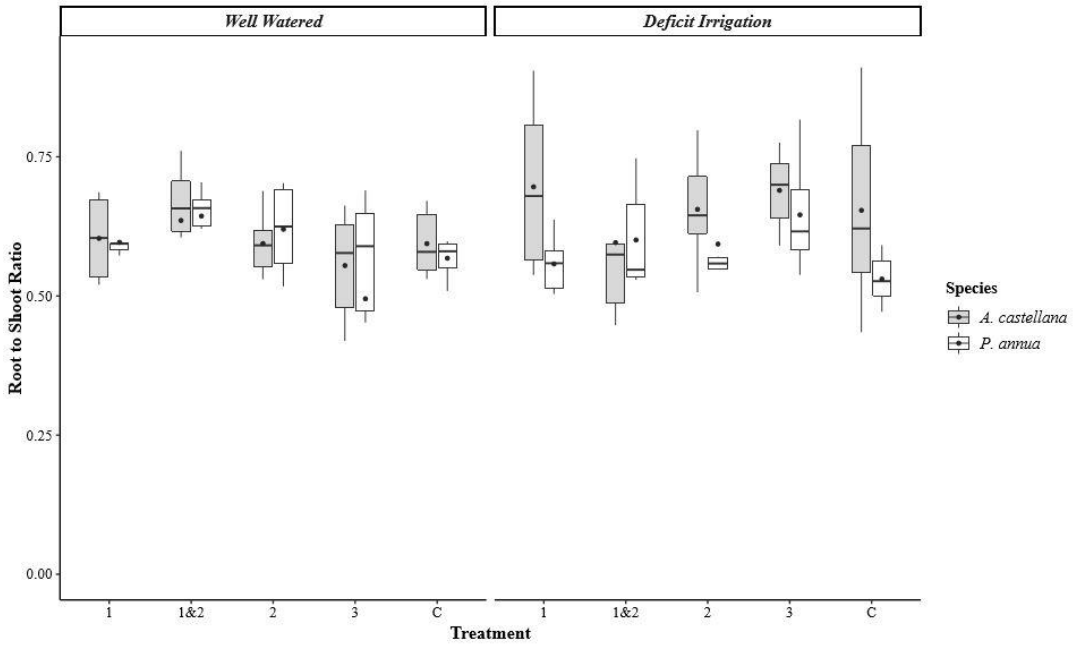

Figure 18*. The root to shoot ratio for both species under each treatment for both well-watered and deficit irrigation regimes.*

Discussion

In this study, the impacts of wetting agents on soil properties, plant physiology and plant-soil-water interactions often differed between well-watered and drought/deficit-irrigation conditions. The impacts also varied depending on which wetting agent was in use, which is consistent with other studies that compared different products (Henie *et al*., 2007). In terms of their traditional uses, wetting agents did improve water infiltration into the soil, with the effectiveness of this again varying depending on the type of wetting agent used (Figure 4). They also worked effectively on both well-watered and droughted soils (Figure 4). This improvement in infiltration has the potential to improve water conservation through reductions in runoff and improved irrigation efficiency (Dekker *et al*., 2005; Moore *et al*., 2010).

4.1 - Germination

Wetting agents may have an impact upon germination success, at least in *A. castellana*. There are grounds to accept hypothesis 1 as treatment 3 did improve overall germination success compared to untreated seeds in *A. castellana*, but none of the treatments significantly altered success in *P. annua* (Figure 3). Although stimulation of germination has been shown to occur in seeds of other species with surfactant application (Hurtt and Hodgson, 1987; Madsen *et al*., 2016), there is little explanation as to why this may be the case - other than the general idea that the treatment may be acting on cellular membranes to quicken the imbibing process (Endo *et al*., 1969). It is not clear why this effect could only be seen in treatment 3. This result is supported by the findings from the master's project this study is based on, in which treatment 3 was the only wetting agent to significantly increase *A. castellana* seedling establishment in a field trail (Baldwin, 2019). Should wetting agent treatment 3 cause the germination success rate of *A. castellana* seed to be closer to that of *P. annua* in the field, the natural competitive dynamic between the two species in a sward could be altered.

4.2 - Plant Growth Rate

Plant growth rate was affected by wetting agent use in both undisturbed plants and turfs that were cut regularly. In well-watered conditions, treatment 3 may potentially alter the competitive dynamic between *A. castellana* and *P. annua* by increasing growth rate in *A. castellana* and affecting the relative difference in growth rate between the two species – *A. castellana* had a significantly higher growth rate than *P. annua* when both species were under treatment 3 (Figures 6 and 8). This may translate in the field to *A. castellana* becoming more dominant in the sward than it would under natural conditions, as has been shown in the field trails that this study was based on (Baldwin, 2019). The fact that growth rate was higher in *A. castellana* than *P. annua* under treatment 3 in both the turf and single-plant assessments

means we can be confident that the treatment is affecting the two species differently, irrespective of factors such as intraspecific competition.

This effect is not consistent between different types of wetting agent with the other treatments not favouring either species and with treatment 2 potentially resulting in some negative effects on growth rate in *A. castellana* (Figure 6). This is mirrored by the fact that treatment 2 had no effect on the sward composition in the field compared to control plots – there was no apparent competitive advantage gained by *A. castellana* (Baldwin, 2019). Non-ionic surfactants have been documented as having negative effects on plant growth in several studies (Singh and Orsenigo, 1984)., which could be attributed to a slight phytotoxic effect of treatment 2 on *A. castellana*. This result demonstrates the marked difference in the effects different wetting agent chemistries can have on plant growth. The effects are also not consistent between well-watered and drought conditions, with a potential improvement in drought tolerance for *P. annua* under wetting agent treatments, specifically treatment 1&2, but not for *A. castellana* (Figure 7). Any improvements in drought tolerance induced by wetting agents may be expected to be more pronounced in *P. annua* due to it naturally having lower drought tolerance (Appendix 2). The interspecific differences also change, with treatment 3 no longer resulting in any differences between the two species in their growth rate response to drought (Figure 7).

No differences in growth rate were observed between treatments in either species under deficit irrigation scenarios (Figure 8). This suggests that the additional benefits wetting agents may have in terms of sward species composition may be most noticeable when water is readily available in the soil. However, as there was no significant difference in the rate of growth decrease between *P. annua* and *A. castellana* under treatment 3 when drought was imposed (Figure 7), it is likely that any competitive dominance gained by *A. castellana* during well-watered periods may sustain into periods of stress.

The growth rate aspect of Hypothesis 2 can only be partially accepted, as although a higher growth rate was observed in *A. castellana* than *P. annua* with treatment 3 under well-watered scenarios, it appears that some wetting agents may favour *P. annua* when under drought stress or deficit irrigation (particularly treatment 1&2). There is little evidence that growth rate can be sustained by any surfactant treatment when plants are under moderate-severe drought stress (Figure 7). The growth rate of *P. annua* was more similar between well-watered and deficit irrigation regimes than that of *A. castellana* (Figure 9), however this can likely be attributed to the fact that *P. annua* reaches a sTable phase of growth faster than *A. castellana*, so its growth rate had already slowed down to a greater extent than *A. castellana* before the onset of deficit irrigation.

The results highlight the need for those in the field to conduct trials of different wetting agents before applying them to large areas, as causing a negative effect on growth (treatment 2, well-watered phase 2, Figure 6) would be undesirable. Negative impacts were only observed under treatment 2, which also performed the worst out of the wetting agents in terms of improving water infiltration into the soil (Figure 4). This is the opposite of what has been seen in other studies, in which products that cause short-term damage to grasses and produce a lower quality turf colour have been those that are most effective in reducing water drop penetration time (Panina, 2010).

4.3 - Above-ground Biomass

There is evidence provided by the data for above-ground biomass accumulation to support the suggestion that the competitive dynamic between the two species may be altered by wetting agent treatment 3. We would expect under controlled conditions *P. annua* to have higher above-ground biomass than *A. castellana* due to having a thicker stem base and larger leaves. This was indeed the case under well-watered conditions for control plants and treatments 1, 1&2 and 2 (Figure 10). However, the above-ground biomass accumulation was statistically identical in the two species when under treatment 3 (Figure 10). This is potentially very promising for use of the product in the amenity turf industry as a sward that contains plants with comparable above-ground biomass will provide a more uniform surface, improving playability. However, again this observation was only true under well-watered conditions and effects changed under deficit irrigation, with treatment 1&2 resulting in significantly higher biomass for *P. annua* when compared to control treatments and *A. castellana* (Figure 10). This is consistent with the treatment appearing to improve drought tolerance in *P. annua* in terms of maintaining growth when water is limited (Figure 7).

In the field, it is likely that a more similar level of biomass production, as is the case with treatment 3 under well-watered conditions, would alter the competition intensity between the two species which could result in lower biomass production than that which is observed in monoculture (Reader *et al*., 1994). This was the case in a study conducted by Reader *et al.* (1994) who found that the growth of another member of the Poaceae family (*Poa pratensis*) could be reduced by increasing competition intensity in some plant communities. More comparable biomass between plants grown under treatment 3 is another factor that could help explain *A. castellana's* dominance in the sward when the two species are growing with the treatment in the field (Baldwin, 2019). It is likely that the larger difference in growth rate between the two species under treatment 3 (Figures 6 and 8) explains the greater similarity in above-ground biomass accumulation in well-watered conditions.

The above-ground biomass aspect of hypothesis 2 can again only be partially accepted, as although there was no difference in above-ground biomass between the two species under treatment 3 in well-watered conditions, the extent to which *P. annua* was heavier than *A. castellana* under the control treatment was not significant (Figure 10). It is also true that wetting agent effects (particularly in the case of treatment 1&2) seem to be more pronounced in *P. annua* than *A. castellana* when under deficit irrigation conditions (Figure 10).

4.4 – Shoot Relative Water Content and Stomatal Conductance

Shoot relative water content (RWC), which is a commonly used indicator of plant water status (Yamasaki and Dillenburg, 1999), may also be affected by wetting agent treatments. Under well-watered conditions, treatments 1, 1&2 and 3 appeared to increase the difference in RWC between *A. castellana* and *P. annua*, with that of *A. castellana* being significantly higher (Figure 11). These observations are somewhat supported by the stomatal conductance data, in that *P. annua* had significantly higher stomatal conductance than *A. castellana*, indicating that it was transpiring at a higher rate than *A. castellana* under well-watered conditions (Farquhar and Sharkey, 1982), particularly under treatments 1, 1&2 and 3 (Figure 12). Consideration must be given to the fact that when well-watered plants were harvested water had been withheld from them for one day to facilitate their removal from the tubes. This may be a factor in explaining why values were never 100% and why *P. annua* had lower values than *A. castellana* as perhaps the plants were experiencing a mild drought stress, to which *P. annua* is more susceptible (see Appendix 2). However, these lower values could also be attributed to possible supersaturation when fully-turgid measurements were taken (Maxwell and Redmann, 1978). Stomatal conductance values also had to be adjusted to reflect differences in leaf area, which could limit the reliability of the measurements.

Again, the observed effects under well-watered conditions disappear under deficit irrigation conditions, with *P. annua* matching *A. castellana* in RWC under all wetting agent treatments but not in control plants, suggesting again that drought tolerance may be improved by wetting agents in *P. annua* but not in *A. castellana* (Figure 11). This is again supported by the stomatal conductance data, with no differences between the two species but a significantly higher stomatal conductance detected for *P. annua* when under treatments 1, 1&2 and 3 (Figure 12). Stomatal conductance is expected to decrease rapidly as plants become water stressed (Miyashita *et al*., 2005), thus the fact that some treated plants had higher stomatal conductance than the control plants for *P. annua* suggests an improvement in drought tolerance. This is consistent, at least for treatment 1&2, with the drought tolerance observations made in the turf experiment (Figure 7). Interestingly, treatment 1 produced plants with a significantly lower RWC than the control plants for *A. castellana*, which was not true for any of the other wetting agent treatments (Figure 11). It is possible that as treatment 1

is known to have its greatest effect on the surface layers of the soil, this was having a negative effect on plant water status in *A. castellana* when water was limited. However, it is worth remembering that these measurements were only taken during the single-plant assessments and it is unknown how these values might differ with the influence of inter- and intraspecific competition in the field – stomatal conductance has been shown to be reduced by competition (Vysotskaya *et al*., 2011).

There was also a significant interaction between treatment and species under deficit irrigation conditions – mean stomatal conductance was higher in *P. annua* than *A. castellana* under treatments 1, 1&2 and 3, but this trend was reversed for treatment 2 and the control plants (Figure 12). This suggests that the treatments that result in the fastest water infiltration times (Figure 4) have the greatest potential for improving drought tolerance in species that are more susceptible to drought stress. This may not have a great impact in the amenity turf industry due to generally not wanting to promote easily stressed species such as *P. annua*, but is promising for potential uses of surfactants in the agricultural and horticultural context.

There is some support for hypothesis 4, as treatment effects on RWC and stomatal conductance were observed. However, no significant differences in evapotranspiration rate could be attributed to any of the wetting agent treatments for either species (Appendix 6) and there is little evidence to suggest that wetting agents can affect this (Hallett and Gaskin, 2007). The RWC measurements do not really help to explain the observed differences in growth rate between the two species under treatment 3 for well-watered conditions, as RWC was higher for *A. castellana* for treatments 1, 1&2 and 3 and no differences in growth rate were observed under treatments 1 and 1&2. This is also the case for deficit irrigation scenarios as all wetting agent treatments resulted in a higher RWC than control plants for *P. annua* but there were no significant differences in plant growth rate observed (Figure 8).

It must be acknowledged that certain limitations exist which reduce the reliability of the RWC measurements. An imbibition curve was not produced before the study was carried out – the timeframe used for plants to reach maximum cell turgidity was simply based on other studies, but as these studies did not use the same species it is possible that some of the mass changes recorded were due to cell expansion and not water uptake needed to compensate the water deficit (Yamasaki and Dillenburg, 1999). There is also potential that changes in tissue dry mass may have occurred during the water absorption period - as the photosynthetic light compensation point of the plant tissue was not known, photosynthetic gains or respiratory losses may have occurred (Barrs and Weatherley, 1962; Barrs, 1968). These limitations must be considered when interpreting the results presented for RWC (Figure 11).

4.5 - Plant Nutrient Uptake

The impact of wetting agents on plant nutrient uptake appears to be limited (at least in well-watered conditions), with no intraspecific variation and interspecific variation being confined to a small number of nutrients (Tables 5 and 6). As a result, we can reject hypothesis 4. There is little reason to suspect that the differences observed in the levels of Zn, Ca and S between the two species are a result of the wetting agents themselves, as the manufacturer confirmed that none of these elements are present in any of the products and contamination during manufacture is highly unlikely. There is potential that some of the tissue samples may have been contaminated with small amounts of sand/soil which may have been present on the leaves during harvesting, which could have led to these observations. Some studies have shown that surfactants can decrease plant uptake of nutrients such as P, K, Ca, S, Cu and Zn (Banks *et al*., 2015), while others have documented increased uptake of N, P, K and some micronutrients (Baratella and Trinchera, 2018). As we have little support for observations in either direction, we can conclude that differential nutrient uptake is unlikely to be responsible for the growth rate differences observed under well-watered conditions (Figures 6 and 8).

However, it is worth mentioning that we were unable to evaluate the uptake of N due to limitations in the method, which could well have impacted growth rate, as N is known to increase biomass production in turfgrasses and its availability has important implications for growth (Richardson *et al*., 2009; López-Bellido *et al*., 2010). Nutrient uptake was also only evaluated in plants that had been growing under well-watered conditions, so we cannot say if any impacts of surfactant treatments on nutrient uptake are present under drought/deficit-irrigation scenarios.

4.6 - Below-ground Interactions

Perhaps the most informative results of the study are provided by the data for the roots and rhizosheath. Under well-watered conditions, rhizosheath weight normalised by root length was significantly increased by wetting agents 1&2 and 3 for *P. annua* and treatment 3 for *A. castellana* (Figure 13). The fact that this is only the case for treatment 3 in *A. castellana* could help explain some of the observations made in terms of growth rate (Figures 6 and 8) and biomass accumulation (Figure 10). Rhizosheath's have been shown to assist plant survival in harsh environments and therefore may also help to maximise plant health in favourable conditions (Pang *et al*., 2017).

Studies have suggested that many root derived compounds can increase water retention in the rhizosphere (Naveed *et al*., 2019), the effect of which could be being mimicked by the wetting agent treatments in the rhizosheath. All wetting agents increased the water content of the rhizosheath relative to that of the bulk soil in both species, which occurred to a greater extent

in deficit irrigation conditions (Figure 14). In drying soil, it has often been observed that the moisture content of the rhizosheath is higher than that of the bulk soil (Young, 1995; North and Nobel, 1997; Pang *et al*., 2017). There is potential that as the wetting agents appeared to be having this effect in well-watered conditions, they were concentrating the soil moisture into the rhizosheath/rhizosphere and the surrounding bulk soil was drier than it naturally would be under well-watered scenarios (Figure 14). This could have positive implications in terms of water uptake and be partly responsible for the observed growth rate differences (Figures 6 and 8).

Plant root exudates and mucilage can form polymeric gels that can absorb large volumes of water, keeping the rhizosheath hydrated (Naveed *et al*., 2019). The root exudate lethicin (which is also an ingredient present in treatment 3) has been shown to increase the amount of phosphate in solution and increase uptake by the roots, which could potentially affect growth rate (Read *et al*., 2003; Dunbabin *et al*., 2006). Unfortunately, we were unable to perform nutrient analysis on the plants from the single-plant assessments to determine if this was the case. As the nutrient analysis was performed on the turf systems (Tables 5 and 6), it is possible that the values obtained would differ should the same analysis be performed on the plants grown in isolation, due to the lack of cutting and intraspecific competition (Aerts, 1999).

Under deficit-irrigation, plants under treatment 3 still showed a higher rhizosheath weight than control plants for *A. castellana*, but not for *P. annua* (Figure 13) and although the trends for water-content ratio are maintained for *P. annua*, there were no longer any differences for *A. castellana* (Figure 14). These trends in the rhizosheath to bulk soil water content ratio data for deficit irrigation conditions support the observations made for RWC (Figure 11), with *P. annua* having a higher ratio than *A. castellana* across all treatments except for the control (Figure 14). This may help explain why the *P. annua* control plants were the only group to not match *A. castellana* in terms of RWC under deficit irrigation, as their water uptake may have been compromised by a reduced amount present in the rhizosheath relative to that in the bulk soil. It is also apparent that all wetting agent treatments increased the rhizosheath to bulk soil water content ratio in deficit irrigation conditions compared to well-watered conditions to a higher extent than control plants (Figure 14). This could be providing benefits in terms of water uptake and for nutrients that are largely acquired via mass flow, such as N (Pang *et al*., 2017) but further work is needed to confirm if this is the case.

In terms of below-ground biomass and root length, differences between treatments only became significant under deficit irrigation conditions. Again, the effects were more pronounced in *P. annua* with wetting agents 1&2, 2 and 3 resulting in significantly higher

biomass than control plants and when comparing the two species, treatment 1&2 again appeared to be having a greater impact on *P. annua* than *A. castellana* (Figure 15). Curiously, plants under treatment 3 had significantly shorter roots (Figure 16) and a higher root to shoot ratio (Figure 18) for *P. annua* when compared to the control plants under deficit irrigation, but there were no differences in *A. castellana* or between the two species when within treatment comparisons were made (Figure 16). Again, this suggests treatment 3 is having a different effect on the two species, which is consistent with the observations made in the field trails (Baldwin, 2019).

The root average diameter data showed treatment differences under well-watered conditions. Wetting agents appear to decrease root diameter in *P. annua* but not *A. castellana* (Figure 17). Interestingly, *A. castellana* roots were only significantly larger in diameter when both species were under treatment 3 (Figure 17). This could be a result of a relative increase in root hair length or quantity and could help explain a larger rhizosheath in *A. castellana* (Figure 13) and the relative differences in growth rate between the two species (Figures 6 and 8) when under treatment 3. Several studies have shown a positive correlation between rhizosheath size and root hair length (Haling *et al*., 2010; Delhaize *et al*., 2015; James *et al*., 2016) and root hairs appear to be integral to the formation of the rhizosheath (Wen and Schnable, 1994; Pang *et al*., 2017).

The fact that all wetting agent treatments produced plants with a smaller average root diameter than control plants in well-watered conditions in *P. annua* but did not have any significant effect on *A. castellana* (Figure 17) is perhaps an indication that any adverse effects of wetting agents will be more pronounced in less stress tolerant species. As wetting agents were applied at a high application rate for the single plant assessments (an equivalent of six doses in a single application) we might expect any negative impacts of the wetting agents on root development to be more pronounced than under field conditions. However, it is difficult to draw conclusions based on this observation as this trend was not consistent across wetting agent treatments under deficit-irrigation conditions or for the other root traits analysed (Figures 15, 16 and 17).

Root hairs facilitate water uptake and greatly increase the contact area between the plant and the soil, enmeshing soil particles around the roots and providing a physical framework for rhizosheath extension (Carminati *et al*., 2017; Pang *et al*., 2017). Although we cannot say for certain that the differences in root diameter detected using the WinRhizo© software (Regent Instruments Inc., Québec, Canada) were a result of changes to root hairs, it is an interesting hypothesis that could help explain the differences observed in rhizosheath size under treatment 3 (Figure 13). Analysis of root hair development should be conducted in the future

when analysing the effects of wetting agents on plant physiology. Under deficit irrigation, average root diameter was larger than under well-watered conditions for plants under all treatments (Figure 17), but only treatments 1&2 and 3 produced plants with a smaller root diameter than the control plants in *P. annua*.

There is evidence to suggest we can accept hypothesis 3, as the distribution of water resources in the soil was affected in both species under both well-watered and deficit irrigation regimes by wetting agent application. This effect resulted in a higher water content in the rhizosheath relative to that of the bulk soil. Although it focused on the rhizosphere rather than the rhizosheath, this is consistent with another wetting agent study that found the wettability of the rhizosphere could be increased by surfactant application (Ahmadi *et al*., 2017). There was also a positive impact of the wetting agent treatments on rhizosheath size and this impact was more pronounced under treatment 3 for *A. castellana* than *P. annua*, at least under well-watered conditions.

4.7 - Conclusions

The observations made in the field suggest that wetting agent treatment 3 has the potential to alter the competitive dynamic between *A. castellana* and *P. annua*, increasing the proportion of *A. castellana* in the sward compared to that which would be expected under natural conditions (Baldwin, 2019). We have found evidence to suggest that this could be due to an increase in the growth rate of *A. castellana* and an increase in the growth rate difference between *A. castellana* and *P. annua* under the treatment compared to control conditions. The most likely mode of action for this increase based on our data is due to changing below-ground conditions in a way that is more favourable for *A. castellana*, namely the properties of the rhizosheath and root diameter, which we suggest could be due to effects on root hair development. Growth rate differences could then be explained by increased water and N uptake, but further research is needed in this area to prove this. There is also a need to formally assess the effects of long-term use of wetting agents on soil microbial communities. The observations made in this study have positive environmental implications, as correct use of treatment 3 in the field may reduce the need to use herbicides to control the growth of *P. annua* on golf greens.

With regards to wetting agents improving plant health under drought conditions, there is evidence to suggest this is true for *P. annua* but there is little effect on *A. castellana*. This positive effect on *P. annua* was most pronounced when treatments 1 and 2 were used in combination with one another. As the same effects on *P. annua* were not observed with treatment 3, this could be attributed to the combined treatment having its greatest effect in the surface layers of the soil and *P. annua* being a naturally shallower rooting species than *A.*

castellana. However, we cannot explain why treatments 1 and 2 did not seem to have the same effects when used in isolation without further knowledge of the chemistry of each of the formulations. It is also likely that these effects would be less pronounced in the field when the effects of interspecific competition become significant. Based on this study, the suggestion is that a combination of treatments 1 and 2 will provide the most benefits to drought intolerant species, treatment 3 will provide the most benefits in terms of achieving a desirable sward composition in amenity turf and treatment 2 offers little value in the field due to less improvement of water infiltration compared to the other treatments and limited positive effects on plant development. The results are promising in terms of using appropriate wetting agents outside the amenity turf context to aid the growth of drought intolerant species when water is limiting. This potential, coupled with the positive effects wetting agents can have on water infiltration into the soil and soil water distribution, provides grounds for optimism in terms of improving water conservation and plant health in future climate scenarios.

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Appendices

Appendix 1

Table 1. The recommended field application rate of each wetting agent as provided by the manufacturer. A suiTable amount to add to the quantities of soil used in the experiments could be calculated from these Figures.

Wetting Agent	**Recommended Field Application Rate**
1	20 l in 700 l water per hectare
2	1 l in 700 l water per hectare
3	20 l in 700 l water per hectare
1&2	19 l of 1, 1 l of 2 in 700 l water per hectare

Soil area in pots (experiment 1) = 0.011 m^2

Soil area in tubes (experiment 2) = 0.000573 m^2

Appendix 2

There is a suggestion from the data shown in Figure 1 that *A. castellana* has a higher drought tolerance than *P. annua* due to the shallower gradient (m) of the fitted regression line (m = 169 and m = 259, respectively). However, further tests would be required in this area to confirm this due to the low r^2 values of the regression models. The purpose of the pilot study was only to inform the selection of a deficit irrigation target value and so no formal analysis of the data needed to be undertaken. As the same level of deficit irrigation was needed for both species in order to easily compare any wetting agent treatment effects, the data for the two species was combined (Figure 2). The equation generated by the regression model fitted to the combined data ($y = 214x + 32.3$) could then be used to predict stomatal conductance values (y) at different gravimetric water contents (x).

The mean stomatal conductance was calculated for all readings for which the gravimetric water content was at 0.21 g/g (field capacity) to give a value for 'maximum' stomatal conductance. This value could then be multiplied by 0.6 to give an approximation for a 40% reduction in stomatal conductance. The resulting value could then by substituted into the regression line equation to find the gravimetric water content which corresponds to this 40% reduction. The gravimetric water content that resulted in a 40% reduction in stomatal conductance was selected as the deficit irrigation target as at this level no zero readings for stomatal conductance were recorded. The values discussed in this section can be viewed in Table 2.

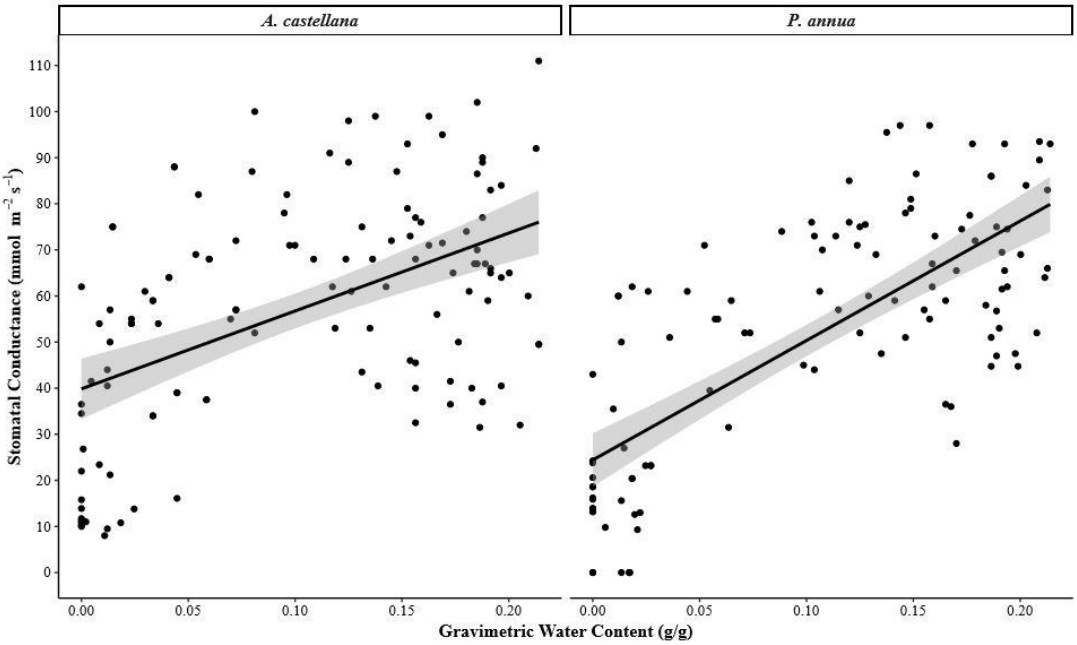

Figure 1. *Stomatal conductance readings plotted against soil gravimetric water content; which is expressed as grams of water per gram of dry soil. The graph has been faceted by species - on the left is the data for A. castellana and on the right is the data for P. annua. A linear regression model has been fitted to each set of data with the shaded region around the line representing the 95% confidence interval. The adjusted r^2 values for the A. castellana and P. annua models are 0.246 and 0.533 respectively.*

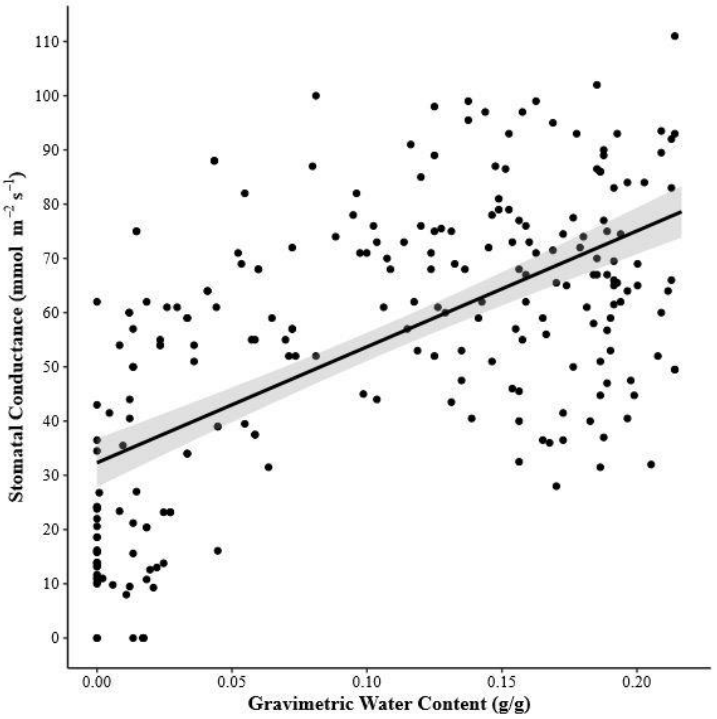

Figure 2. *Stomatal conductance readings for both species plotted against soil gravimetric water content; which is expressed as grams of water per gram of dry soil. A linear regression model has been fitted (adjusted $r^2 = 0.376$) and the shaded region around the line represents the 95% confidence interval.*

Table 2. The values derived from the procedure discussed in the above paragraph. The deficit irrigation target corresponds to a 'tube weight' of 91 g which would be maintained by weighing the tubes daily and adding any water that was required.

'Maximum' Stomatal Conductance (mmol m^{-2} S^{-2})	70
40% Reduction in Stomatal Conductance (mmol m^{-2} S^{-2})	42
Deficit Irrigation target (g/g)	0.05
Field Capacity Tube Weight (g)	103
Deficit Irrigation Tube Weight (g)	91

Appendix 3

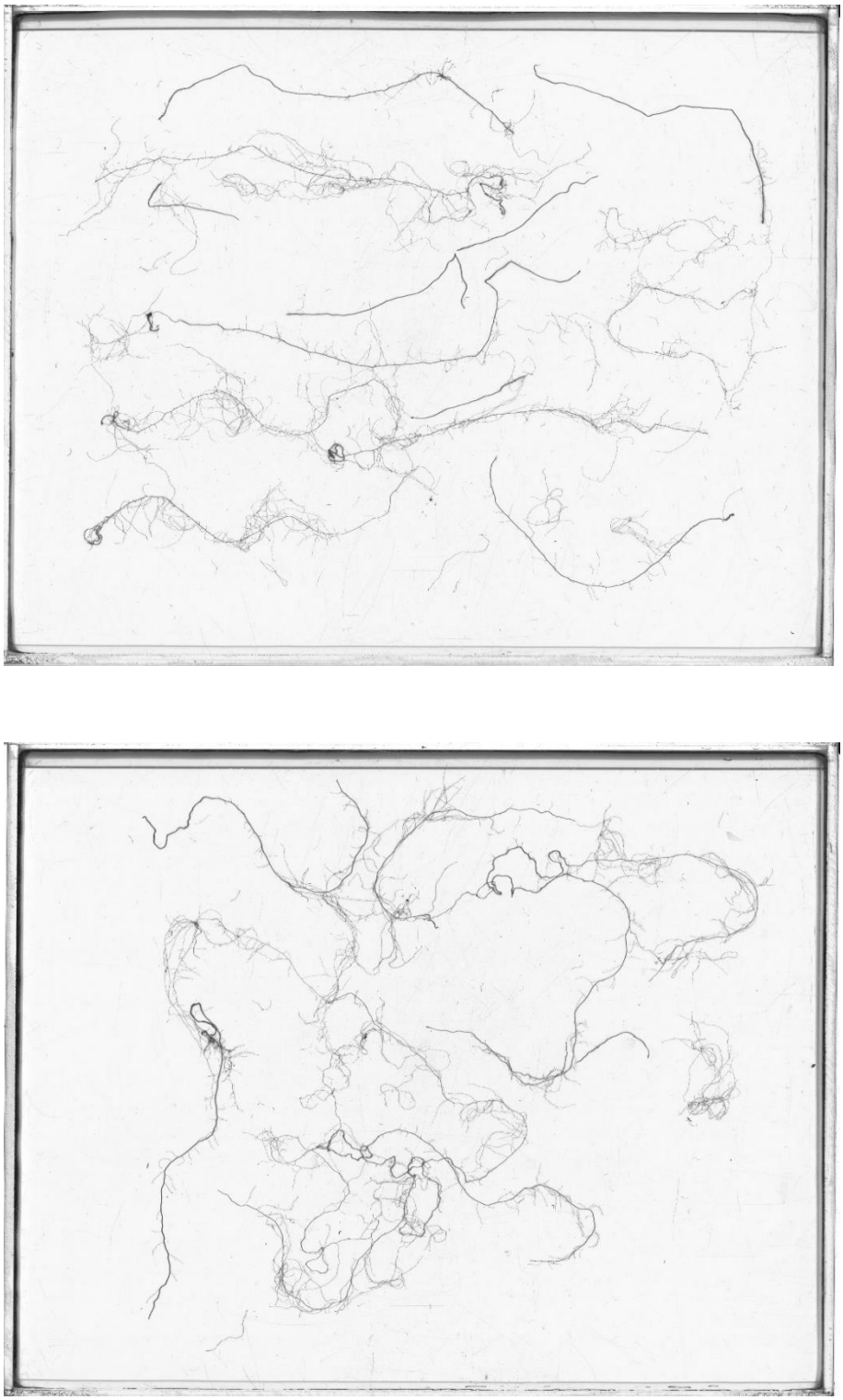

Figure 3*. Two examples of the scanned images analysed using WinRhizo© software (Regent Instruments Inc., Québec, Canada). From top to bottom, the examples are A. castellana under well-watered treatment 3 and P. annua under deficit irrigation treatment 3.*

Appendix 4

Correlation analysis was carried out on data obtained from the single-plant assessments using the Pearson method for variables for which a relationship might be expected. These variables were as follows:

- Rhizosheath weight normalised by root length (NRW), measured in milligrams.
- Rhizosheath gravimetric water content (GWC (R)), measured in grams of water per gram of dry soil.
- Leaf Relative water content (RWC), measured as a percentage.
- Stomatal conductance (SC), measured in mmol m^{-2} s^{-1}.

The scatter graphs for each of these variables plotted against one another and each associated Pearson's product moment correlation co-efficient (PMCC) value are shown for well-watered plants (Figure 9) and deficit irrigation plants (Figure 10).

Further correlation analysis was carried out to assess any relationships between root diameter and the rhizosheath (Figure 11) in well-watered plants for the following variables:

- Average root diameter (D), measured in millimetres.
- Rhizosheath weight normalised by root length (NRW), measured in milligrams.
- The ratio between rhizosheath gravimetric water content and bulk soil gravimetric water content (R:B).

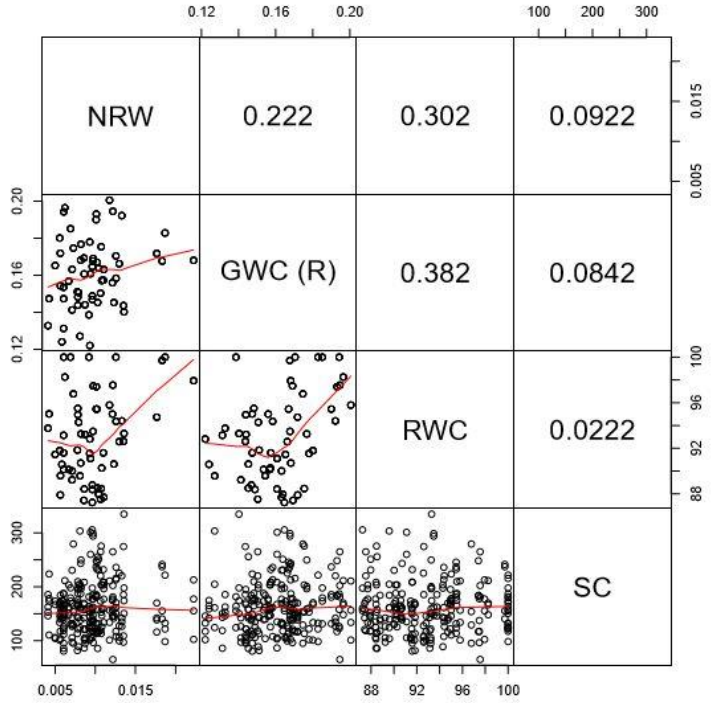

Figure 9. *Scatter plots showing the relationships between rhizosheath weight normalised by root length (NRW), rhizosheath gravimetric water content (GWC (R)), leaf Relative water content (RWC) and stomatal conductance (SC) for well-watered plants. Associated PMCC values for each relationship are shown.*

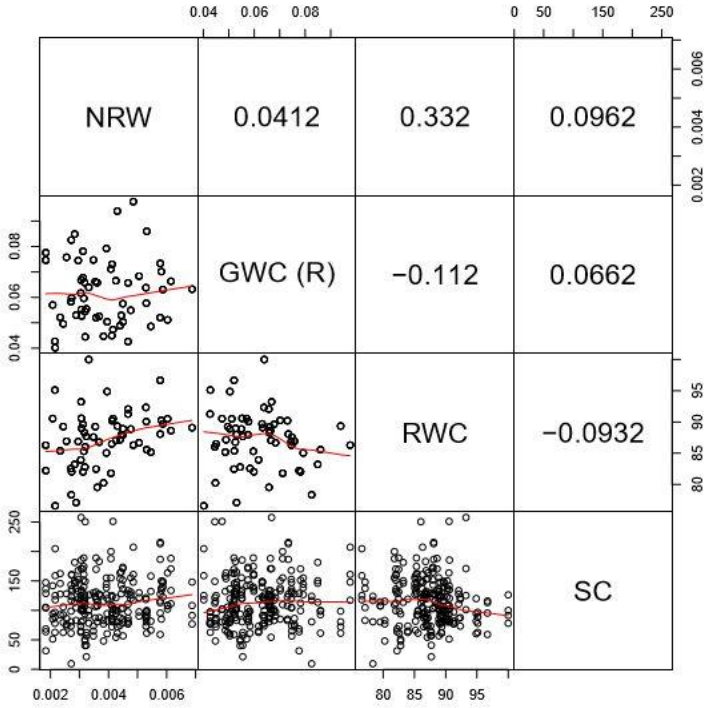

Figure 10. *Scatter plots showing the relationships between NRW, GWC (R), RWC and SC for plants under deficit irrigation. Associated PMCC values for each relationship are shown.*

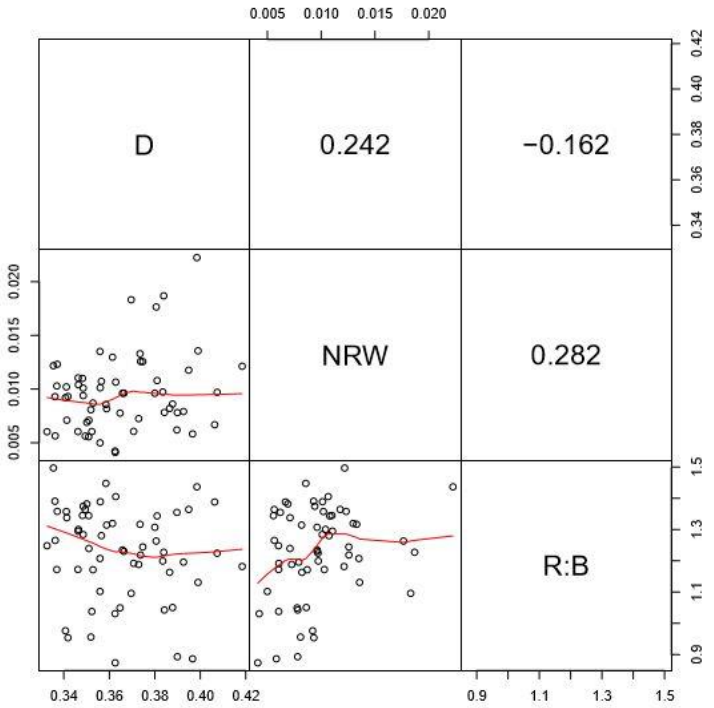

Figure 11. *Scatter plots showing the relationships between average root diameter (D), rhizosheath weight normalised by root length (NRW) and the ratio between rhizosheath gravimetric water content and bulk soil gravimetric water content (R:B) for well-watered plants. Associated PMCC values for each relationship are shown.*

Appendix 5

Additional Analysis of Roots

Root Surface Area – Well-watered

For *P. annua*, no significant differences in root surface area were present between treatments ($p > 0.05$). For *A. castellana*, plants under treatment 1&2 had a significantly lower root surface area than control plants ($p = 0.0206$). *P. annua* had a significantly higher root surface area across treatments ($p < 0.05$), but there were no differences between the two species when within treatment comparisons were made.

Root Surface Area – Deficit irrigation

For *P. annua*, root surface area for plants under treatment 3 was significantly lower than in control plants ($p < 0.05$). For *A. castellana*, no significant differences in root surface area were present between treatments ($p > 0.05$). *P. annua* had a significantly higher root surface area across treatments ($p < 0.05$), but there were no differences between the two species when within treatment comparisons were made.

Root Volume – Well-watered

No significant differences in root volume were present between any of the treatments for either species ($p > 0.05$ in both cases). There were also no significant differences in root volume between the two species across all treatments ($p > 0.05$).

Root Volume – Deficit irrigation

For *P. annua*, root volume for plants under treatments 1&2, 2 and 3 was significantly higher than in control plants ($p < 0.05$). For *A. castellana*, root volume for plants under treatment 2 was significantly higher than in control plants ($p < 0.05$). *P. annua* had a significantly higher root volume across treatments ($p < 0.05$) and for within treatment comparisons, root volume was significantly higher in *P. annua* than *A. castellana* under treatment 1&2 ($p < 0.05$).

Root Tips – Well-watered

No significant differences in the number of root tips were present between any of the treatments for either species ($p > 0.05$ in both cases). *P. annua* had a significantly greater number of root tips across treatments ($p < 0.05$), but there were no differences between the two species when within treatment comparisons were made. There was a significant interaction between treatment and species ($p< 0.05$).

Root Tips – Deficit irrigation

For *P. annua*, no significant differences in the number of root tips were present between treatments ($p > 0.05$). For *A. castellana*, there was a significantly greater number of root tips present for plants under treatment 2 than in control plants ($p = 0.0336$). *P. annua* had a significantly greater number of root tips across treatments ($p < 0.05$) and for within treatment comparisons, the number of root tips was significantly greater in *P. annua* than *A. castellana* under treatment 1&2 ($p < 0.05$).

Root Forks – Well-watered

No significant differences in the number of root forks were present between any of the treatments for either species ($p > 0.05$ in both cases). There were also no significant differences in the number of root forks between the two species across all treatments ($p > 0.05$).

Root Forks – Deficit irrigation

For *P. annua*, plants under all wetting agent treatments had a significantly greater number of root forks than control plants ($p > 0.05$). For *A. castellana*, there was a significantly greater number of root forks present for plants under treatments 2 and 3 than for control plants ($p < 0.05$ in both cases). There was no significant difference in the number of root forks between the two species across treatments ($p = 0.0864$) but for within treatment comparisons, the number of root forks was significantly greater in *P. annua* than *A. castellana* under treatment 1&2 ($p < 0.05$). There was a significant interaction between treatment and species ($p < 0.05$).

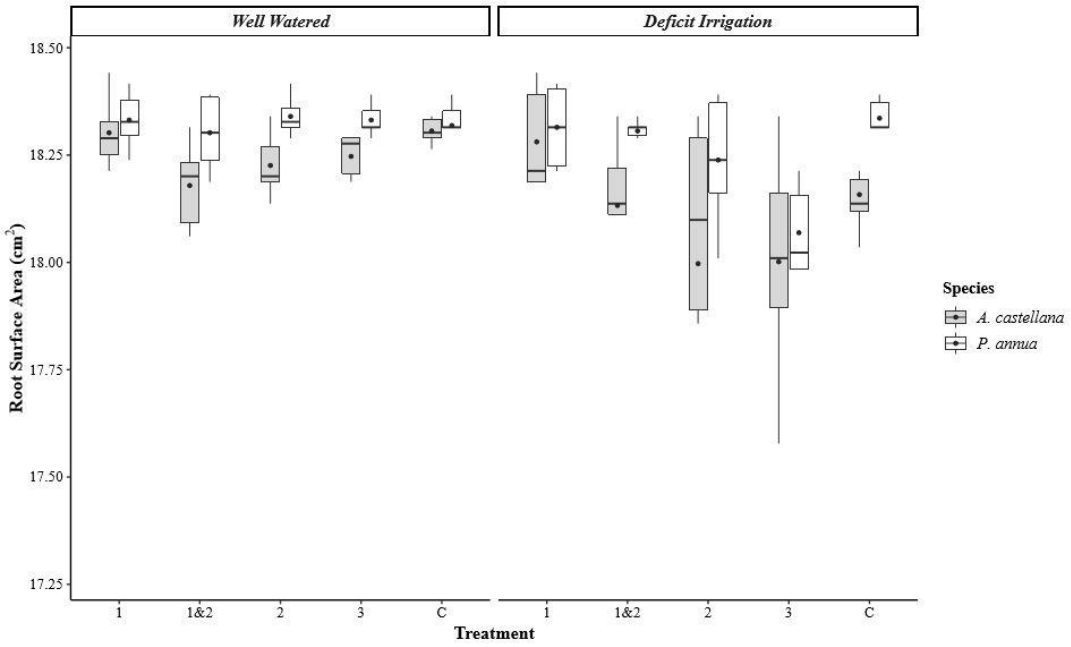

Figure 5. *Root surface area expressed in square centimetres for both species under each treatment for both well-watered and deficit irrigation regimes.*

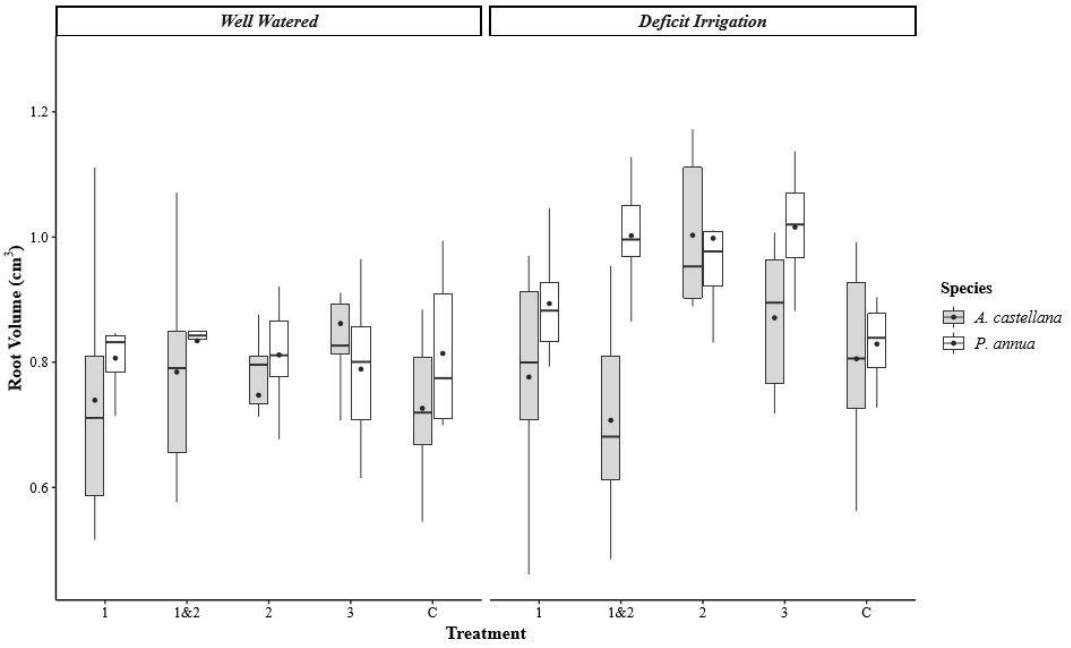

Figure 6. *Root volume expressed in cubic centimetres for both species under each treatment for both well-watered and deficit irrigation regimes.*

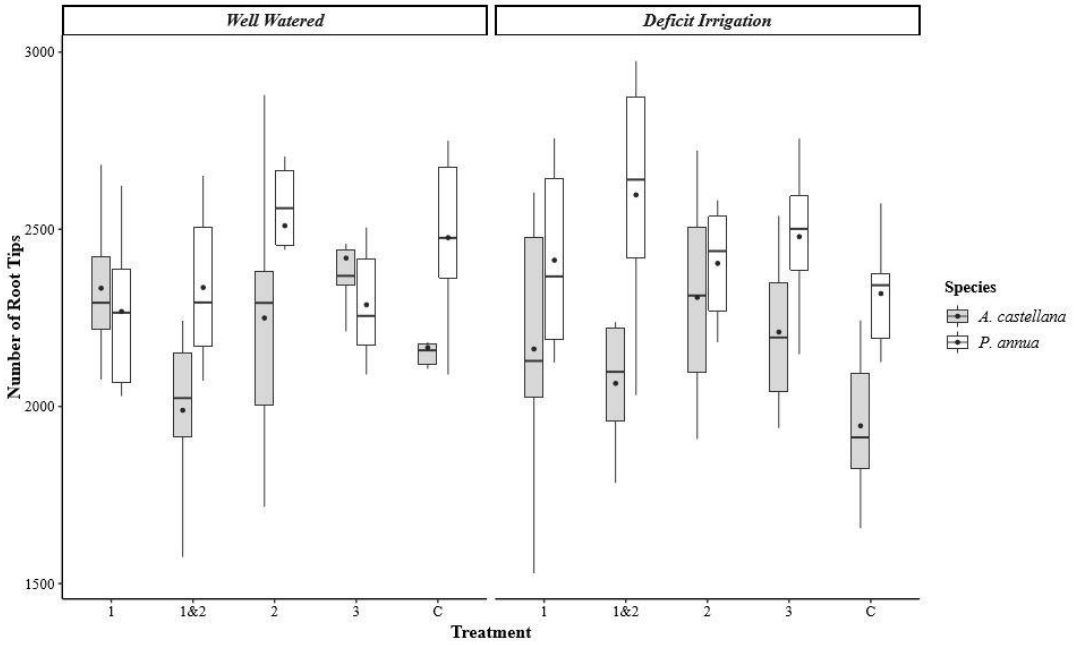

Figure 7. *Number of root tips for both species under each treatment for both well-watered and deficit irrigation regimes.*

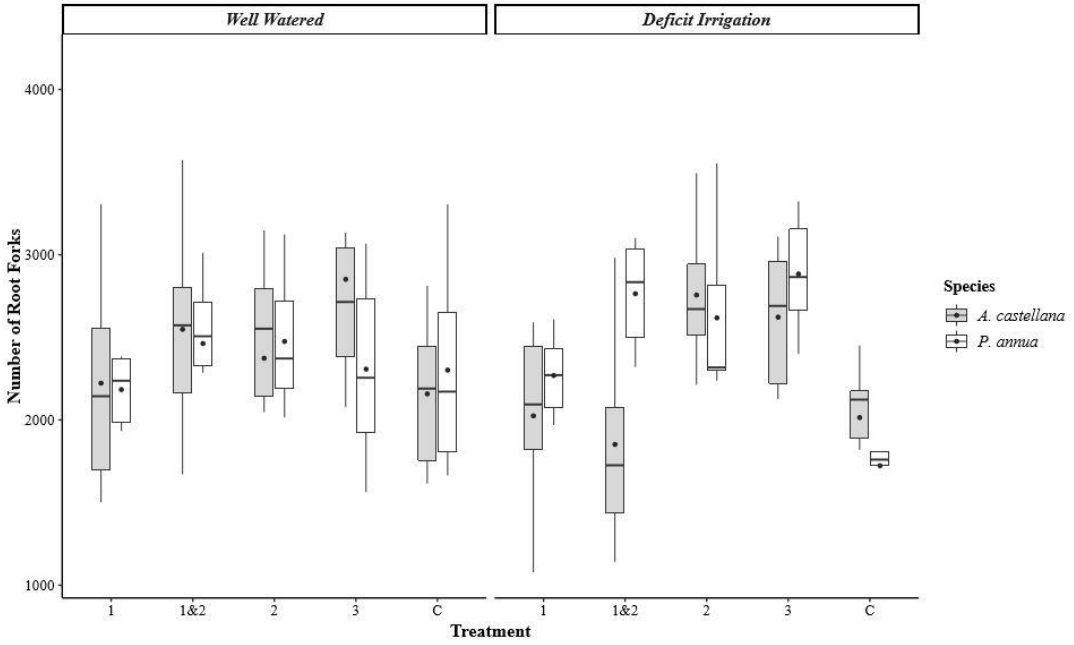

Figure 8. *Number of root forks for both species under each treatment for both well-watered and deficit irrigation regimes.*

Appendix 6

Evapotranspiration

For *A. castellana*, no significant difference was found between the treatments ($p = 0.186$) and no significant interaction between treatment and time was found ($p = 0.727$). These results were mirrored by the data for *P. annua* with no significant differences found between treatments ($p = 0.538$) and no significant interaction ($p = 0.953$). However, there is a significant difference between species in terms of evapotranspiration rate over time ($p < 0.05$) and a significant interaction between species and time was found ($p < 0.05$). This can likely be attributed to the different growth habits of the species, with A. castellana having a much denser sward.

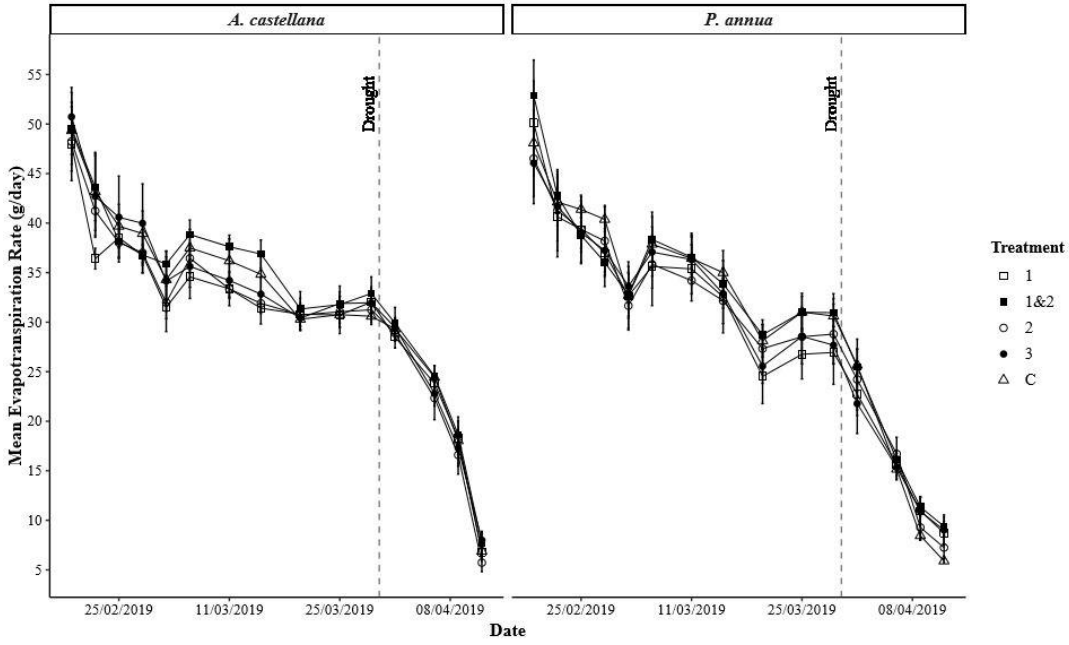

Figure 4. *Mean evapotranspiration rate over time for each treatment and for both species. The onset of the drought phase is indicated by the dashed vertical lines.*