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Mycorrhizal colonisation in roots of *Holcus lanatus* (Yorkshire Fog) in a permanent pasture under conditions of reduced precipitation

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Abstract

The UK climate is projected to become warmer, with more frequent hotter, drier summers. Many governments and international organisations are concerned about how climate change will affect food production and security. Mycorrhizal fungi are an essential part of agricultural systems and yet little is known about how climate change will affect mycorrhizal fungi.

We investigated the effect of reduced precipitation on levels of arbuscular mycorrhizal (AM) colonisation in the top 10 cm of soil in the grass *Holcus lanatus* L. (Yorkshire Fog) in a permanent pasture in South Gloucestershire, UK. Incident rainfall was reduced, by approximately 50 %, using clear gutters supported on steel frames. Over three growing seasons we observed little difference in levels of AM colonisation and numbers of intra-root fungal structures between the roots of *H. lanatus* grown with reduced or full incident rainfall. Time of year when water stress occurred had a stronger effect on levels of colonisation than the absolute amount of precipitation received**.**

In *H. lanatus,* growing in a permanent pasture, levels of AM colonisation were around 40 - 50%, across a range of precipitation, from 18% above to 36% below the long-term average. The results highlighted the complex relationship between mycorrhizal fungi, host plant and abiotic stress.

Key words

Arbuscular mycorrhizal fungi, climate change, drought, *Holcus lanatus*, reduced precipitation, water stress

Introduction

Recent studies of the UK climate (Met Office 2018) suggested that, in the UK, average and maximum temperatures will rise and summers will become warmer and drier. The ten hottest years on record in UK have all occurred since 2002 (Kendon et al. 2019). Average summer temperatures for 2009-2018 were 0.8 °C warmer than the 1961-1990 average and the 2001-2018 average summer temperature is the highest since records began in 1659 (Kendon et al. 2019). In other parts of the World, such as Australia, periods of extreme hot and dry weather are becoming more frequent and are predicted to become more common (CSIRO 2019).

Concerns have been raised by governments and international organisations about how cropping systems, agricultural production and food security might be affected by climate change (Rivington and Koo 2010; Elliott et al. 2014). The importance of soil microorganisms, particularly in permanent grasslands where a large proportion of the total phosphorus is within the microbial biomass, has been emphasised by Leibisch et al. (2014). If we are to protect crop production and prepare for changing climatic conditions it is important that we understand the impact climate change may have, not only on above ground plant material, but also on soil microorganisms, such as mycorrhizal fungi, either directly, or as a result of host plant response to changes in environment.

Arbuscular mycorrhizal fungi (AMF) form intimate mutualistic symbiotic relationships with the roots of autotrophic plants, mycorrhiza. The symbiosis involves the bidirectional transfer of nutrients; the fungus receives organic carbon from the plant and the plant receives soil-derived nutrients via the fungus. AMF have been found in almost every family of plants and every terrestrial ecosystem (Brundrett and Tendersoo 2018), and colonisation by AMF is now accepted as important for plant growth. AMF research has primarily focused on the effects of the symbiosis on plant mineral nutrition. AMF are usually considered beneficial, despite the demands for carbon placed on the plant, because they can help the plant to access minerals such as phosphorus (P) (Sanders and Tinker 1973) and micronutrients that are often immobile or present at low concentrations in the soil (see Smith and Read 2008 for examples). The effects of AMF are not limited to mineral nutrition; there are numerous examples of plants in which the presence of AMF appears to help the plant to tolerate drought (Auge 2001; Khan et al. 2003; Ouledali et al. 2018). Although the mechanisms involved in AMF mediated drought tolerance remain unknown, they are likely to include effects on plant physiology, such as improved water use efficiency (Koide 1985; Khan et al. 2003), and on soil properties (Marulanda et al. 2003). In grasses, under increasing water stress, the plants receive increasing benefits from AMF symbionts along a continuum (Worchel et al. 2013), with the effect being greatest in cool season grasses, such as those found in UK pastures.

Recent concerns about the potential effects of global climate change have stimulated interest in how soil microbiology may be affected but much of this work has focused on the direct effects of changing temperature or CO_2 levels (for example, Heinemeyer and Fitter 2004, Hawkes et al. 2008). Studies of the effect of drought on soil microbes have produced mixed results (Hueso et al. 2012; Siebert et al. 2019).

Research into AMF has tended to concentrate on how the presence of AM fungi helps plants withstand adverse conditions but there is little information on the effects, if any, of adverse conditions on the fungi themselves (Johnson et al. 2003; Turk et al. 2006; Walter et al. 2016), particularly under field conditions. The interactions between the fungal symbiont and the host plant are complex. Smith and Read (2008) emphasised the importance of considering negative impacts of each partner on the other; under certain conditions, the symbiosis may be of no advantage to the plant and may even put the host at a competitive disadvantage (Johnson et al. 2015).

Agricultural grasslands cover almost 40% of the UK. In 2015 there were 5 363 thousand hectares of pastures (22%), and 4 157 thousand hectares (17%) of semi-natural grasslands, making grasslands, by area, the most important agricultural crop in the UK (Office of National Statistics 2015). The perennial grass *Holcus lanatus* L. (Yorkshire Fog) is found in pastures and gardens worldwide (Hubbard 1984; Rose 1989; Weber 2003). *H. lanatus* is the second most abundant plant species in the UK (Natural Environment Research Council 2008) and is an important constituent of swards more than six years old and thus important for fodder production. It is common in damp pastures (Watt 1978), but is also considered moderately tolerant of dry conditions (Watt 1978). *H. lanatus* is easy to identify at all stages of growth and has been shown to form mycorrhiza with AMF (McGonigle and Fitter 1990; West 1996).

We wanted to know if AMF colonisation of the widespread pasture grass *H. lanatus* was affected, under field conditions, by reduced precipitation. In this study, we compared levels of AMF colonisation in roots of *H. lanatus* (Yorkshire Fog), growing within a semi-natural agricultural grassland, in the South West of England, under incident and reduced rainfall for three consecutive growing seasons (October 1, 2015 to September 30, 2018).

Methods and Materials

Field site

The field site was in South Gloucestershire, UK between the University of West of England Frenchay campus and the M32 motorway. National Grid reference: ST629 397. The site sloped gently towards the southeast.

The site was an agricultural field that had been used for arable crops until the late 1980s. The field has been permanent grassland with minimal inputs for about thirty years; for most of this time the field was grazed by sheep or cattle but in the four years preceding the start of this work, it had been subjected to a late September hay cut. To maintain the existing management regime, in late September the experimental areas were cut with hand shears, to 1 - 2 cm, and the grass cuttings removed.

The soil is a Worcester series dark brown silty clay loam (gleyed brown earth) developed over Mercia mudstone (Findlay 1976); chromic vertic luvisol (http://www.landis.org.uk/services/soilsguide/). The site is within an area of grade 1 and 2 soils, often referred to as the Bristol Blue Finger (Sponsler 2013). Soil properties were pH 5 - 5.5 ($CaCl_2$ method, Rayment and Higginson 1992), nitrate 4 - 6 mg/kg (ion-selective electrode, Hadjidemetriou 1982), phosphate 0.3 - 0.4 mg/kg (Palintest Ltd., Gateshead, UK), potassium 4 – 6 mg/kg (Palintest).

Samples of *H. lanatus* were collected from vegetation plots set up as part of the Drought Risk and You (DRY) project (McEwen 2019 and Thompson et al. 2019 https://aboutdrought.info/report-back-from-drought-water-scarcity-conference/). Reduced precipitation (RP) plots were surrounded by a steel frame (3 m x 3 m) that supported a roof of V-shaped transparent gullies arranged so that they intercepted approximately 50% of incident rainfall. Intercepted water was carried away from the plots by plastic pipes (S1a).

Control plots, also 3 m x 3 m, had no frame and received full incident rainfall (S1b). There were six Control plots and six RP plots. Automatic weather stations (CR-1000, Campbell Scientific, Loughborough, UK) located at the field site recorded rainfall (tipping bucket, ARG 100, Campbell Scientific), soil moisture tension (kPa) at 10 cm depth (MPS6, Decagon Devices, Pullman, USA) and soil moisture content (%) at 10 cm depth (SMP1, Adcon Ltd, Vienna, Austria).

Sample collection

Holcus lanatus plants with roots and soil attached were collected on five occasions between November 2015 and August 2018. The first samples were collected in November 2015, shortly after the roofs were fitted. These samples established baseline levels of mycorrhizal colonisation, under Control conditions, and allowed us to refine the processing methods. Thereafter samples were collected in February 2016 and late July/early August of 2016, 2017 and 2018.

Sampling points were chosen by using random number tables (Murdoch and Barnes 1998) to generate coordinates. The *H. lanatus* plant closest to the selected sampling point was located and a plug of soil and roots 10 cm deep and 5 cm diameter centred on the base of the plant was removed. We wanted to be certain that the roots we used were *H. lanatus*; this could only be achieved by sampling roots attached to a growing shoot. In pastures in the UK, and elsewhere, most roots (65-90%) occur in the top 10 cm of soil (Macklon et al. 1994; Dawson et al. 2000 and Brown et al. 2010). After removal from the ground, the sample of soil and root with shoot attached was placed in a sealed plastic bag. Twenty samples were collected on each occasion, ten per treatment (RP and Control). Samples were stored in a refrigerator at 4 °C, for a maximum of 48 h, until processed.

Sample preparation

Each plant, with the attached soil, was placed in a water filled plastic tray and the soil carefully washed from the roots. Dead plant material, moss and any pieces of root not attached to the shoot were carefully removed. Once the roots were clean of all debris they were placed in a plastic sieve and gently washed using a jet of clean water to remove any remaining soil. The cleaned roots were cut into 1 cm segments and placed into 10% KOH at 4 °C for 48 h. The shoot was then discarded. The roots were stained using the ink and vinegar method (Vierheilig et al. 2005). The cleared roots were acidified for 10 min in 10% vinegar and water, then transferred to solution of 5% Parker blue-black Quink ink (Parker, Nantes, France) and vinegar (5% acetic acid) and heated at 100 °C for 5 min. The roots were removed and de-stained for 25 min in 10% vinegar before storing in 50% ethanol and glycerol mixture.

Assessment of fungal colonisation

Fungal colonisation was assessed, in a sub-sample of 30 - 40 root segments, using the grid line intercept method (Newman 1966), as described by Giovannetti and Mosse (1980), recording presence, or absence, of fungi at each intersection of root with the grid. Any further debris and any hyphae that were not attached to a root were removed before colonisation was assessed. Colonisation was calculated as the number of fungal intercepts as a percentage of the number of root intercepts. In late July/early August 2016, 2017 and 2018 each sample was independently counted by at least two different observers to minimise observer bias, and the counts pooled. In 2015/16 and in 2018 (the first and final year of the experiment), the number of arbuscules and vesicles was recorded. The number of arbuscule (or vesicle) intercepts was divided by the total number of fungal intercepts to give arbuscules (or vesicles) as a proportion of total fungus.

Root samples were observed (x 50 magnification) using a BMS Stereo microscope (Brunel Microscopes Ltd, Chippenham, Wiltshire).

Data on fungal colonisation were analysed with the statistical package SPSS 26 (IBM, Armonk, USA). A generalised linear model using a binomial distribution was fitted. Fungal count was the events variable and root count was the trials variable, treatment and time were predictors. Sample coding is shown in S2. For arbuscules (or vesicles), arbuscule (or vesicle) count was the events variable and fungal count the trials variable.

Environmental data were summarised using Excel 2016 (Microsoft Corp., Redmond, USA).

Soil moisture

We calculated the numbers of days per month, during the main growing season (1 March to 31 July), for each year (2015/16, 2016/17 and 2017/18) when soil moisture tension was between 0 and -59 kPa, between -60 and -490 kPa or ≥ -491 kPa. In the soil used in this study -59 kPa occurs at a soil water content of about 44% and -491 kPa at a soil water content of 36-37% (S3).

The amount of water in a soil can be divided into that which is freely available to plants, available, slightly available and unavailable; these amounts are different for every soil and depend on the water holding properties (texture and structure) of the soil. Freely available water can be defined as that when the soil moisture tension is between 0 and -59 kPa, and available as that when soil moisture tension is between -60 and -490 kPa (Berglund 2020). When soil moisture tension is greater (more negative) than -491 kPa little water is available for plants and they will experience water stress. On the soil moisture content vs. soil moisture tension curve (S3) for the soil used in this study -59 kPa is where the slope of the curve increases and -491 kPa is where the relationship between water content and tension is affected by wetting and drying processes within the soil.

Results

Levels of colonisation

All of the root samples observed were colonised by fungi; aseptate intra-root hyphae, aseptate external hyphae, vesicles, arbuscules and spores were present. The presence of arbuscules indicates that the roots were colonised by arbuscular mycorrhizal fungi (AMF) (Smith and Read 2008). While we were not able to identify fungal species, the presence of vesicles suggests that the AMF present were not members of the family Gigasporaceae (Smith and Read 2008).

At the start of the study, in November 2015, the level of colonisation in roots of *H. lanatus* was 36.1% (SE ± 1.8). The results from fitting a binomial generalised linear model suggested that there was a strong interaction between year and treatment ($p<0.001$), therefore we also analysed the data on a year-by-year and on a seasonal basis. In February 2016 and August 2016 roots in the Control treatment had slightly higher levels of colonisation (38.2% (SE ± 3.9) and 44.3 % (SE ± 1.1)) than those in the RP treatment (31.2% (SE ± 2.9) and 39.6% (SE ± 1.7)) ($p=0.001$, Figure 1). In August 2017 and August 2018 there was no statistically significant difference in the level of colonisation in roots from the RP treatment and those from Control plots. In August 2017 levels of fungal colonisation, in roots of *H. lanatus* from both Control and RP plots, were higher (121%, SE ± 2.7) than in either August 2016 (41.9% SE ± 1.1) or August 2018 (42.0% SE ± 1.5) (Figure 1).

Numbers of arbuscules and vesicles

The number of arbuscules and vesicles, as a proportion of total fungus, was greater in August 2016 (0.09, SE ± 0.01 arbuscules and 0.14, SE ± 0.02 vesicles) and August 2018 (0.08, SE ± 0.01 arbuscules and 0.11, SE ± 0.01 vesicles) than in November 2015 (0.06, SE ± 0.01 arbuscules and 0.06, SE ± 0.01 vesicles) or February 2016 (0.05, SE ± 0.01 arbuscules and 0.06, SE ± 0.01 vesicles) (p<0.001) (Figure 2). Roots collected in August 2016 had a greater proportion of fungus as arbuscules or vesicles than in those collected in August 2018 (p<0.001) (Figure 2). In roots collected in August 2016 and August 2018 there was a higher proportion of fungus as vesicles (0.13, SE ± 0.01) than as arbuscules (0.09, SE ± 0.01) (p=0.005, t-test, Fig 2). There was little difference in the proportion of fungus as arbuscules or as vesicles between the roots of plants growing in the RP treatment and those of plants growing in the Control plots.

Amount of precipitation

The experiment ran for three growing seasons and three complete hydrological years (1 October 2015 to 30 September 2018). In 2015/16 total rainfall for the hydrological year was 968.4 mm, in 2016/17 748.1 mm and in 2017/18 773.4 mm. The 1961 to 2019 average for the Frome catchment, for the hydrological year, is 817 mm (National River Flow Archive) (Figure 3a). Over the three years of this study rainfall recorded in the RP plots was 53% of that in the Control plots (Table 1).

The autumn, winter and spring of 2015/16 were much wetter than the equivalent periods in 2016/17 or 2017/18, but the summer 2015/16 was the driest with only 158 mm rainfall recorded (Table 1). Autumn, winter and spring 2016/17 were the driest seasons, but the summer 2016/17 was the wettest during this study, with 243 mm rainfall. The amount of rainfall received in autumn, winter and spring 2017/18 was intermediate between that recorded in 2015/16 and 2016/17 but the summer 2017/18, when only 159 mm of rainfall fell, was equally as dry as summer 2015/16 (158 mm) (Table 1).

Soil moisture

In 2015/16, there were no days when the soil moisture tension in Control plots was greater (more negative) than -491 kPa; even in June 2016, for 25 of 30 days, soil moisture was freely available (Figure 3b). In the RP plots, in 2015/16, soil moisture was freely available during March, April and most of May 2016; only in July was soil moisture tension > -491 kPa (Table 2). For technical reasons we do not have a complete soil moisture tension record for the Control plots in March 2016; however, extrapolating from the RP plots (Table 2) suggests that soil moisture tension in the Control plots, for March 2016, was between 0 and -59 kPa. In contrast, during 2016/17, only in March 2017 was soil moisture freely available in Control plots for each day of the month. In May 2017, there were only ten days on which soil moisture was freely available to plants, in Control plots (Figure 3b), and by June 2017 plants in Control plots experienced soil moisture stress on some days. In the RP plots, in 2016/17, soil moisture was freely available each day only in March 2017. In May 2017, soil moisture tension, in RP plots, was between 0 and -59 kPa on only 2 days and by June the plants in RP plots were experiencing water stress (Table 2). In 2017/18, in Control plots, soil water was freely available in March, April and most of May 2018 (Figure 3b); plants experienced soil moisture stress only in July 2018. In 2017/18, in RP plots, soil water was freely available to plants in March and April and most of May; plants began to experience water stress in June 2018, and for most of July 2018 soil moisture tension was ≥ -491 kPa (Table 2).

Discussion

Levels of colonisation

Average levels of AMF colonisation in *Holcus lanatus* roots in Control plots, across the three growing seasons were around 53% (or 40% if values for 2017 are excluded). These values are well within the range reported for *H. lanatus* in other studies of mycorrhiza (McGonigle and Fitter 1990; West 1996). Slightly lower levels of colonisation were observed in root samples collected in winter (November 2015 and February 2016) than in those collected in summer (August 2016, 2017 and 2018) (Figure 1). Seasonal variation in AMF colonisation in grassland has also been reported by other researchers (Heinemeyer et al. 2003; Lutgen et al. 2003;). These findings give us confidence in our methods.

In August 2017, levels of colonisation above 100% were recorded in samples collected from all plots (Control and RP). The gridline intercept method records presence or absence of fungal infection at each intersection of a root with the grid (Giovannetti and Mosse 1980) and can include external hyphae closely associated with the roots as well as intra-root hyphae and fungal structures, thus in heavily colonised roots it is possible to have values of colonisation above 100%. In August 2017, we observed large numbers of external hyphae in all samples.

Levels of AMF colonisation in the roots of *H. lanatus* plants growing in the reduced precipitation (RP) plots were lower in February and August 2016, than in roots of plants in the Control plots (Figure 1); however this difference, though statistically significant, is small compared with the year-to-year variation. In August 2017, and August 2018, differences in colonisation, between roots of plants from Control plot and roots of plants from RP plots, were small and not statistically significant. In roots of *H. lanatus* growing in a mixed sward, in Central Europe, Walter et al. (2016) found that 42 days summer drought (starting in mid-May) did not significantly alter levels of AMF colonisation observed in July. The observations of Walter et al. (2016) were made over one growing season, but the timing of the drought treatment they used (starting in mid-May), is comparable to the 2017/2018 period of low rainfall in our study in (Figure 3a), and the effect on mycorrhizal colonisation was similar (Figure 1). After 6 years of repeated summer (July and August) drought Staddon et al. (2003) found decreases in plant species relative abundance and small increases in the levels of AMF colonisation in mixed root samples collected in July of the seventh drought year from a mixed permanent pasture in Yorkshire (UK). Drought-induced changes in plant species relative abundance of grassland can alter mycorrhizal colonisation (Johnson et al. 2003; Staddon et al. 2003); by using a single species, we avoided any compounding effect of drought on the species composition of the grassland. In other studies on different types of plant, reducing water availability has produced variable results on measured levels of AMF colonisation. Steven and Peterson (1996) found no effect of water availability on mycorrhizal colonisation of *Lythrum salicaria* L. in growth room experiments but natural populations in the field were more highly colonised under dry conditions. In *Hordeum vulgare* L., in growth room conditions, drought reduced AMF colonisation levels (Sendek et al. 2019).

Numbers of arbuscules and vesicles

Arbuscules are accepted to be one of the main sites at which exchange of phosphate (P) from the fungus to the plant occurs (Smith and Read 2008). The arbuscules are normally short-lived structures, four to 20 days, whose presence demonstrates that the symbiosis is active. Vesicles are important for storage of P, and may be involved in stress tolerance or fungal survival (Smith and Read 2008). The proportion of fungus as arbuscules, or vesicles, was lower in root samples collected in November and February than in samples collected in August. Other researchers (Lutgen et al. 2003; Merrild 2013) have also reported seasonal variation in the numbers of arbuscules in the roots of plants in temperate pastures. This seasonality may reflect the fact that both plant and fungus grow more slowly during the winter when water, P and some other mineral nutrients are more readily available to plants. During the summer, when plants are growing rapidly, demand for water and P is higher, but water and P are less available to the plant, and exchange of water and mineral nutrients in return for carbon, via the arbuscules, becomes more important. There is little published information about vesicles. Lingfei et al. (2005) reported that maximum and minimum numbers of vesicles occurred at different times of year in different grassland plant species.

In August 2018 the proportion of fungus as arbuscules and vesicles was less than in August 2016 (Fig 2); this may be a response to drier conditions of 2016/17 and 2017/18 when annual rainfall in the control plots was 50 -75 mm less than the long-term average.

In roots of *H. lanatus* collected in August, more of the fungus was in the form of vesicles than arbuscules (Fig 2), perhaps reflecting seasonal changes in the dynamics of the relationship between plant and fungus. In a northern temperate grassland, most grasses will have reached maximum height and either be flowering or setting seed by August. In a grassland growing in a monsoon climate, Lingfei et al. (2005) found low numbers of vesicles, compared to arbuscules, at all times of year. However, without identifying the fungal species present we cannot rule out seasonal variation in fungal species relative abundance.

There was no statistically significant difference in the proportion of fungus as arbuscules or as vesicles between the roots of *H. lanatus* growing in Control plots and those growing in RP plots (Fig 2). Walter et al. (2016) also found no significant change in the level of arbuscular colonisation of *H. lanatus* in July associated with drought. In contrast, in growth room experiments, drought reduced both arbuscule and vesicle colonisation of *Ephedra foliata* (Al-Arjani et al. 2020) and *Hordeum vulgare* (Sendek et al. 2019).

Why were levels of colonisation higher in August 2017?

In 2016/17 the median maximum height of *H. lanatus* (across Control and RP plots) was around 965 mm, much lower than in either 2015/16 (1410 mm) or 2017/18 (1200 mm) (unpublished data from DRY project records 2019); this pattern was also seen in the amount of above ground dry matter produced (Thompson et al. 2019 https://aboutdrought.info/report-back-from-drought-water-scarcity-conference/). In 2016/17 the autumn, winter and spring were drier than in the other two years (Table 1 and Fig 3a) and this was reflected in the amount of soil water available to plants. In April 2016/17, even in the Control plots, soil water was not freely available for plants on all days (Figure 3b and Table 2). This information suggests that water availability in the spring, when plants are entering the phase of maximum growth, has a strong influence on the symbiosis. The balance between plants and their mycorrhizal fungi is continuously changing and can be considered as a continuum from parasitic to beneficial depending on the demands of both organisms and the environmental conditions (Smith and Smith 2013). Mycorrhizal colonisation can help plants to withstand drought conditions; this is partly because the hyphae of mycorrhizal fungi can help plant roots access a larger volume of soil and thus larger amounts of soil water (Smith and Read 2008; Ouledali et al. 2018). It seems likely that the increased levels of colonisation recorded in August 2017 reflect the demands of the plant for water. The reduced plant height seen in 2016/17 is likely due to a combination of reduced water supply and increased demand for carbohydrate by the fungus. In contrast, in both 2015/16 and 2017/18, although the summer quarter was dry (Table 1 and Fig 3a), rainfall in the winter and spring was sufficient to replenish the soil moisture reserves. Even in the RP plots, soil water was freely available to plants for 31 days in May and June 2015/16 and for 22 days in May and June 2017/18 (Figure 3b and Table 2). In contrast, in May and June 2016/17 soil water was only freely available for two days. Therefore, in 2016/17 the plants would likely have been more dependent on AMF to access water. In an Australian grassland with 50% rainfall reduction, the fungal community associated with a mixed root sample was influenced more by the specific rainfall conditions than by the difference between the control and rainfall reduction treatment (Deveautour et al. 2019). In our study, the timing of water stress in relation to the plant demands for water seems to have a greater effect on the level of mycorrhizal colonisation than the absolute reduction in rainfall. We did not characterise the fungal community and thus cannot say if there were specific changes in the fungal community or in the number of AMF propagules within the soil.

Conclusions

In this ex-arable permanent pasture, roots of *Holcus lanatus* were strongly colonised by arbuscular mycorrhizal fungi over a wide range of soil moisture conditions. The level of colonisation did not appear to be directly affected by the amount of incident rainfall; colonisation levels, assessed in late July /early August, across all plots (Control and RP) in 2015/16 and 2017/18 were similar, even though incident rainfall in Control plots was almost 200 mm higher in 2015/16 than in 2017/18. Year-to-year variation in percentage AMF colonisation was much greater than that associated with reduced precipitation, and possibly associated with yearly weather patterns. The results suggest that, within the range of precipitation that we studied, the timing of periods of water stress may be more important for the development of the symbiosis, and for the growth of the host plant, than the absolute level of the stress.

The results of our study are further evidence of the complexity of the relationship between plants, mycorrhizal fungi and abiotic stress. Our final samples were collected after three years and it would be interesting to know if we would have seen a different result if our study had lasted longer. Further work is needed to elucidate the effects of drought and the timing of drought periods on AMF colonisation and communities under field conditions. Our results also illustrate the difficulty in extrapolating results from growth room studies that can produce quite different results to field studies.

In a permanent pasture our work indicates that levels of mycorrhizal colonisation in roots of the grass *H. lanatus* (Yorkshire Fog) can remain stable even when as in 2016/17 incident precipitation is reduced to 36% of the long-term average.

Competing interests

The authors declare there are no competing interests.

Contributors' statement

All authors planned the work, S. A. and B. G. collected the data, S.A. prepared the first draft of the manuscript, all authors contributed to the writing.

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Copies of the data sets are available on application to Dry@uwe.ac.uk.

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Table 1. Quarterly rainfall totals (mm) for Control and Reduced Precipitation plots at UWE based on the hydrological year.

Date	Season	Control	Reduced Precipitation
Oct to Dec 2015	Autumn 2015/16	282.1	
Jan to March 2016	Winter 2015/16	309.2	190.3
April to June 2016	Spring 2015/16	218.5	106.7
July to Sept 2016	Summer 2015/16	158.6	71.3
Oct to Dec 2016	Autumn 2016/17	199.2	108.2
Jan to March 2017	Winter 2016/17	179.8	98.1
April to June 2017	Spring 2016/17	126	63.7
July to Sept 2017	Summer 2016/17	243.1	123.2
Oct to Dec 2017	Autumn 2017/18	230.2	124.6
Jan to March 2018	Winter 2017/18	244	142.6
April to June 2018	Spring 2017/18	139.6	71.1
July to Sept 2018	Summer 2017/18	159.6	79.2

Table 2. Number of days during the main growing season when soil moisture tension was between 0 and -59 kPa (soil moisture freely available to plants) or > -491 kPa (little soil moisture available to plants) under conditions of reduced precipitation (RP). Data for Control plots are shown in Figure 3b.

Year	Month	Reduced precipitation (RP) Plots	
		Number of days 0 - -59 kPa	Number of days > -491 kPa
2015-16	March	31	0
	April	30	0
	May	25	0
	June	6	0
	July	4	1
2016-17	March	31	0
	April	20	0
	May	2	0
	June	0	4
	July	1	8
2017-18	March	31	0
	April	30	0
	May	18	0
	June	4	5
	July	0	29

Figure Legends

Figure 1. Mycorrhizal colonisation of roots of *Holcus lanatus* grown with full incident precipitation (Control) or reduced precipitation (RP). Values are mean of 10 samples per treatment (November 2015 20 samples) with standard error.

Control □ RP ■ .

Figure 2. Number of arbuscules and vesicles in roots of *Holcus lanatus* grown with full incident precipitation (Control) or reduced precipitation (RP) as a proportion of total fungal intercepts. Values are mean of 10 samples per treatment (November 2015 20 samples) with standard error.

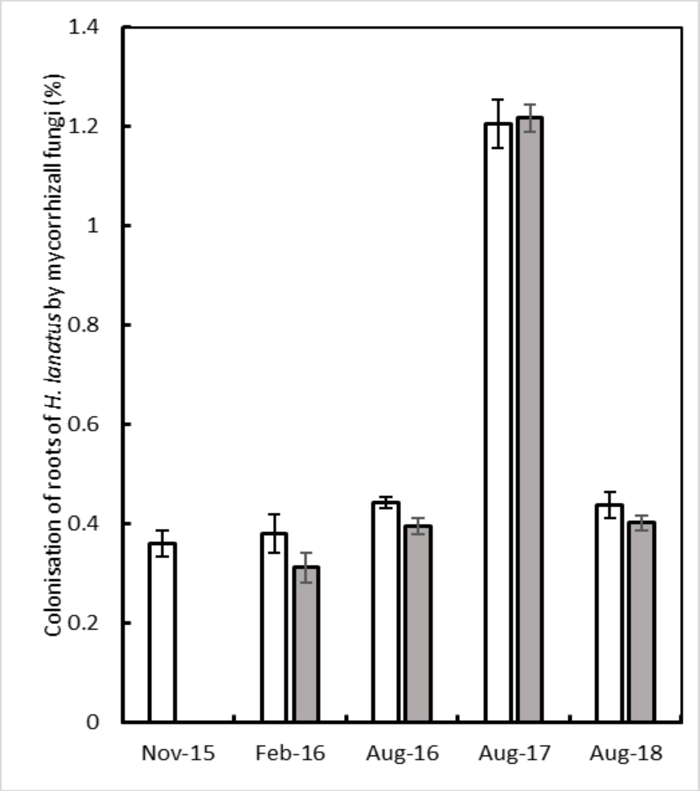
Control arbuscules □ Control vesicles ▒ RP arbuscules ■ RP vesicles ▨ .

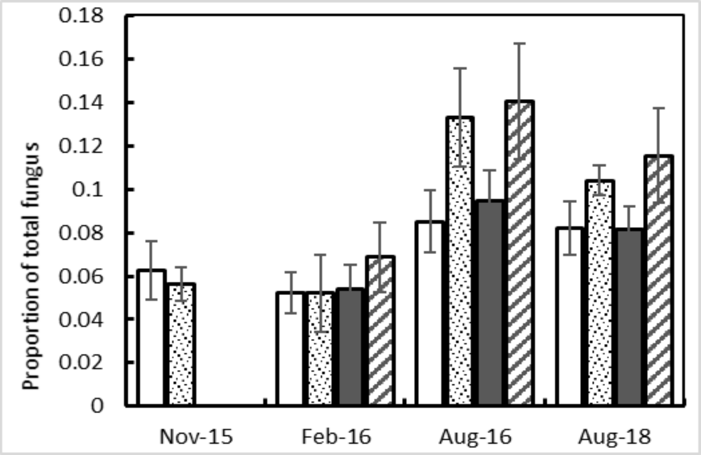
Figure 3. Monthly rainfall and Soil moisture availability in Control plots at UWE October 2015 to September 2018.

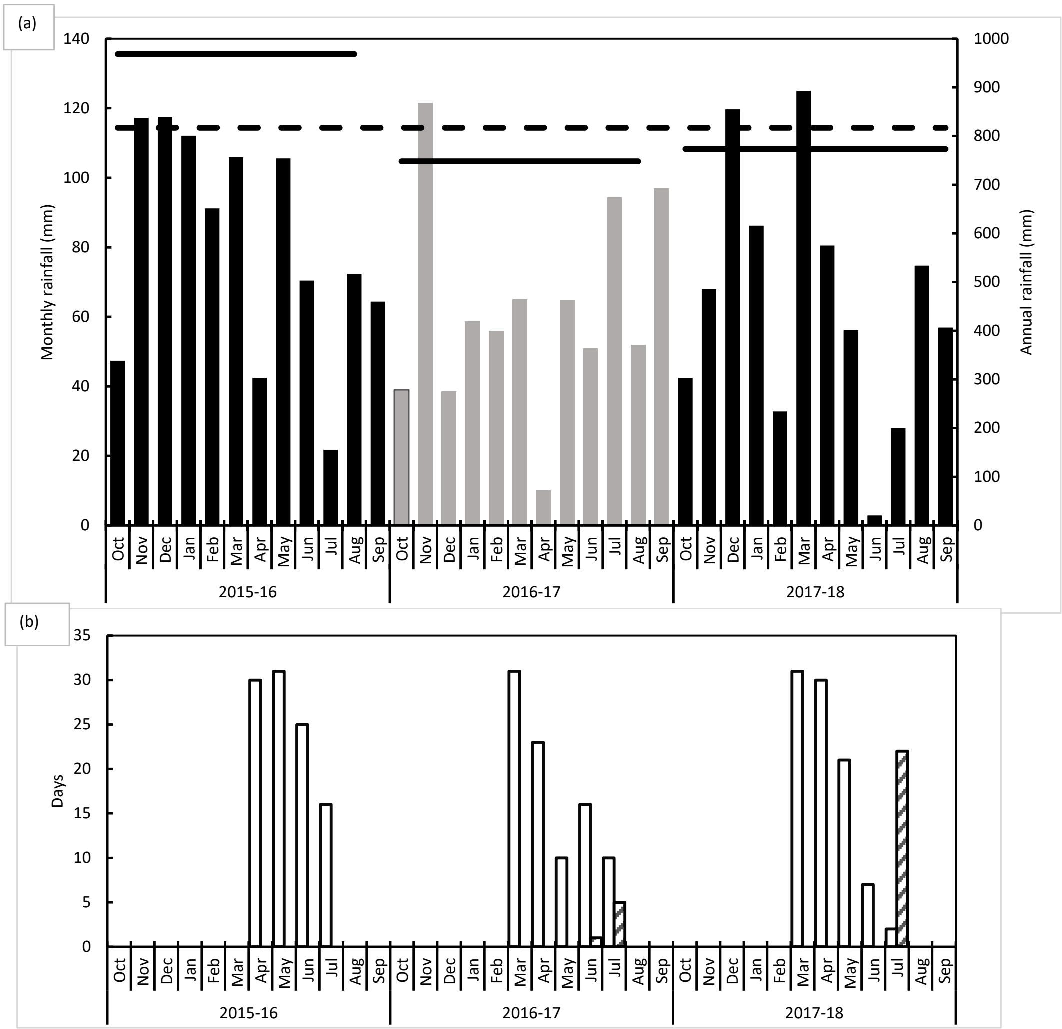
3a. Monthly rainfall for the Control plots and the annual water year total rainfall compared to the long-term average rainfall for the period 1961 to 2019. For clarity, 2016-17 monthly rainfall is shown in grey.

Long-term average rainfall - - - - Water year rainfall —— .

3b. Number of days per month between March and July when soil moisture tension was between 0 and -59 kPa □ or > -491 kPa ▨ .


Colonisation of roots of H. lanatus by mycorrhizall fungi (%)
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Nov-15
Feb-16
Aug-16
Aug-17
Aug-18


0.18
0.16
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
Proportion of total fungus
Nov-15
Feb-16
Aug-16
Aug-18


(a)
Monthly rainfall (mm)
140
120
100
80
60
40
20
0
Annual rainfall (mm)
1000
900
800
700
600
500
400
300
200
100
0
Oct
Nov
Dec
Jan
Feb
Mar
Apr
May
Jun
Jul
Aug
Sep
2015-16
2016-17
2017-18
(b)
Days
35
30
25
20
15
10
5
0
Oct
Nov
Dec
Jan
Feb
Mar
Apr
May
Jun
Jul
Aug
Sep
2015-16
2016-17
2017-18