# The effect of arbuscular mycorrhiza fungi on the growth of Lolium perenne under water stress conditions

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# Abstract

There is a growing need for livestock farmers to find alternate ways of producing forage grasses, in order to reduce their carbon footprint and to become more responsive to climate change. Arbuscular mycorrhizal fungi (AM) could help to make grass production more sustainable. AM have been shown to benefit plants by improving their uptake of nutrients and water and to mitigate abiotic stress. The level of interaction between plant host and the fungi is affected by the availability of nutrients. In a high nutrient environment where artificial fertilizers are used AM associations may be less abundant.

This study explored the research question, can arbuscular mycorrhiza fungi in symbiosis with *Lolium perenne* (perennial rye grass) mitigate water stress in a reduced nutrient environment? A pot study was carried out over 13 weeks, *L. perenne* was grown with and without AM and plants were subjected to 9 weeks of drought stress at 85% and 70% of field capacity.

The results showed there was a significant increase (F=13.28, P=6.04x10$^{-04}$, F crit= 4.02) in aboveground dry biomass for AM associated plants, but it didn't show a significant benefit (F=0.40, P=6.73x10$^{-01}$ F crit= 3.17) of AM association to the water stressed plants. This may be due to the level of drought stress being too low to highlight the benefits of AM association or the species of fungi used were sub-optimum for *L. perenne* in the drought stressed environment.

This study highlights the need for research in to specific AM species that may perform better with *L. perenne*, and at the same time the development of *L. perenne* strains that are more responsive to AM species.

(Word count 270)


# List of abbreviations

AM Arbuscular mycorrhizal fungi

+AM plants grown with arbuscular mycorrhizal fungi

-AM plants grown without arbuscular mycorrhizal fungi

WW well-watered or watered to field capacity

15W -15% water treatment or 85% of field capacity

30W -30% water treatment or 70% of field capacity

CAT catalase

MDA malondialdehyde

NPK nitrogen, phosphorus, potassium

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# 1 Introduction

The overuse of artificial fertilizers in livestock farming and forage grass production has caused the eutrophication of water ways (EPA, 2022) and contributed to greenhouse gas emissions (EPA, 2023). The pressure to reduce fertilizer inputs coupled with climate change predictions of hotter summers with more risk of drought (IPCC, 2023) has highlighted the need for alternate ways of growing crops and managing land. Arbuscular mycorrhizal fungi (AM) may form a small part of the solution by improving the uptake of nutrients and water by the grass plants (Gosling *et al*., 2006), and this could facilitate more efficient and environmentally friendly grass production.

AM are obligatory biotrophs that can form mutualistic relationships with 80% of terrestrial plants (Zhu *et al*., 2022). Their hyphae penetrate the cell wall of the root cortex, where they form arbuscules, a highly branched network that allows exchange of nutrients between the fungus and the plant (Open University 2022a). Fungal hyphae are much finer than plant root and the extraradical hyphal networks can go far beyond the root depletion zone (Gosling *et al*., 2006), effectively extending the root system of the plant (Camenzind and Rillig, 2013). AM provide the plant with nutrients particularly phosphorus, in exchange for photosynthates (Eason *et al*., 1999).

Research has shown that AM may also reduce abiotic stress caused by drought, salinity and heavy metals (Wang *et al*., 2018; Yang *et al*., 2019). Mirshad and Puthe's (2017) research on *Saccharum spontaneum* under drought stress conditions found that the AM associated plants accumulated more antioxidant enzymes, sugars and amino acids, than the non-AM plants. These molecules work to reduce the fall of water leaf potential with soil moisture levels and protect the cells from reactive oxygen species (Mirshad and Puthe 2017).

AM are not plant or species specific so they can form relationships with multiple plants and different species at the same time. The benefits of AM symbioses to the plant varies with plant species and mycorrhizal species. Kyriazopoulos *et al*., (2014) found that *Dactylis glomerata* (cock's-foot grass) performed better under drought stress conditions when inoculated with *Glomus intraradices* than with *Glomus mosseae* or a mixture of both. Wang *et al*., (2018) obtained similar results for *Chrysanthemum morifolium* under salt stress with *Diversispora versiformis* giving better results than *Funneliformis mosseae* (*Glomus mosseae*) or a combination of both. Cavagnaro (2014) looked at how different types of grasses responded to AM symbiosis in a nutrient deficit environment and found tropical grasses performed better than temperate grasses for the same mix of AM species

The reported rates of root colonisation by AM under drought stress vary considerably between studies for example, Yang *et al*., (2019) reported higher rates with drought stress while Huang *et al*., (2011) reported reduced rates with reduced water levels as did Lee *et al*., in their (2011) study. Ayling *et al*., (2021) in their field study found no variation in colonization rates of *Holcus lanatus* (Yorkshire Fog). These variation in colonisations may be due to the level of drought stress induced, species interactions or even the level of nutrients available.

It has been shown that AM are more prevalent in low input or organically managed grasslands (Eason *et al*., 1999). Van Auken and Feridrich's (2006) pot study found there was a significant decrease (51% to 33%) in AM infection rates in *Helianthus annuus* and *H. paradoxus* as added nutrients increased. Thilagar *et al*., (2016) field study found that the lower fertilizer rates had a significant effect on root colonization and that reducing NPK inputs by 50% did not significantly impact yields of the chilly plants when compared with 75% and 100% of recommended application.

These studies highlight the benefits of AM association to host plants and also the complex nature of these interactions. *Lolium perenne* (perennial rye grass) grown in Ireland and around the world as a forage crop (Popay, 2013), has been the focus of many studies that show AM colonisation boost plant growth under different conditions (Clique *et al*., 1997; Yang *et al*., 2019; Lee *et al*., 2012) but few studies have looked at how AM performs under drought stress combined with a low nutrient environment.

This leads to the research question, can arbuscular mycorrhiza fungi in symbiosis with Lolium perenne mitigate water stress in a reduced nutrient environment?

This study addressed the following hypotheses:

$HA1_1$: There will be a difference in the hight of *L. perenne* between plants grown with and without AM.

$HA2_1$: There will be a difference in the height of *L. perenne* between plants grown with different water treatments; well-watered, water reduced by 15% and 30%.

$HA3_1$: There will be a difference in the height of *L. perenne* due to the interaction between the presence or absence of AM, and different water treatments of well-watered, water reduced by 15% and 30%.

$HB1_1$: There will be a difference in the dry biomass of *L. perenne* between plants grown with and without AM.

$HB2_1$: There will be a difference in the dry biomass of *L. perenne* between plants grown with different water treatments; well-watered, water reduced by 15% and 30%.

$HB3_1$: There will be a difference in the dry biomass of *L. perenne* due to the interaction between the presence or absence of AM, and different water treatments of well-watered, water reduced by 15% and 30%.

# 2 Method

To investigate the research question, a pot study using *L. perenne* was carried out over 13 weeks in county Limerick (52°33’03.29” N 8°39’54.00” S).

## 2.1 Germination and Potting

To remove any fungi or bacteria spores that may be beneficial or harmful to the growing *L. perenne* plants, the seeds were surface sterilized by washing with NaOCl (4.5% active chlorine) for 20 minutes and rinsing with boiled water (Cavagnaro *et al*., 2021). 300 seeds were spaced out on trays of blotting paper soaked with boiled water and then kept in the dark at 20°C for three days to allow germination (Cavagnaro *et al*., 2021).

Each 2 litre pot was filled with 1160 g of moist Plagron lightmix$_{@}$ potting compost (NPK 12-14-24 1.5 kg $m^{-3}$). 3 seedlings were planted in each pot, half of the pots had 5 ml of INOQ mycorrhizal product containing fungi *Rhizoglomus irregulare*, *Funneliformis mosseae*, *Funneliformis caledonium* (INOQ 2023), sprinkled on the soil under the seedlings. The pots were numbered 1 to 72 and marked +AM and -AM. After 10 days the seedlings were thinned to one per pot, by removing smaller less developed (fewer leaves) plants (Cavagnaro *et al*., 2021).

Pots were placed on a table on a patio in a randomized pattern and were rotated after each watering. The patio was open to the south and west, and had clear polycarbonate roof. The minimum nighttime temperature range over the 12 weeks was 0.1 to 18.0 °C, with a mean of 8.6 °C. The maximum daytime temperature ranged from 9.1 to 29.8 °C, with a mean of 20.6 °C.

## 2.2 Watering and Treatments

All pots were maintained as well-watered (WW) for the first 4 weeks to allow the plants to establish (Kyriazopoulos et al., 2014). The pots were watered from the bottom, by placing the pot in a saucer of water until the surface of the compost turned dark (Open University 2022b). The pots were watered every 3 to 4 days. A sample of 20 pots were weighed before watering to determine evapotranspiration rates. After watering the pots were allowed to drain for 30 minutes and then reweighed (Poorter *et al.,* 2012). During the first 4 weeks, all pots were weighed once a week to establish a WW base weight for each pot.

There were 3 treatments applied for 9 weeks (Kyriazopoulos *et al*., 2014), with 10 +AM and -AM pots in each treatment, well-watered (WW), -15% water treatment (15W) and -30% water treatment (30W). The water stressed pots were watered to bring their pot weight back to 85% (15W) and 70 % (30W) of their WW base weight. The water status of pots was monitored by weighing a sample of pots each day (Saneoka *et al*., 2004).

Plants were fed with OFP 5-2-5 at a rate of 1 ml/$m^2$ every 3 weeks rather than weekly as recommended (Plant Health Cure 2019). This reduces the nutrients available to the plants and encourages symbiosis with AM (Eason *et al*., 1999; Thilagar *et al*., 2016). The area of each pot was 0.0133 $m^2$, liquid feed was mixed 1 ml with 1500 ml of water and applied to plants at 20 ml per pot using a syringe.

## 2.3 Measurements and Statistics Analysis

At the end of the 13 weeks the aboveground plant was cut off from the root. The overall length of shoot was measured and the plants were dried in an oven at 72°C for 48 hours, until the weight of plants was stable (Iwuala *et al*., 2022). The aboveground dry biomass was determined by weighing on a digital scale (+/- 0.1 g) (Kyriazopoulos *et al*., 2014).

Data were collated and examined in excel. As 2 variables were manipulated within the study, 2-way ANOVA was used to test the null hypotheses and the Tukey test was used to assess differences in pairs of means.

Null Hypotheses:

$HA1_0$: There will be no differences in the plant height of *L. perenne* between plants grown with and without AM.

$HA2_0$: There will be no differences in the plant height of *L. perenne* between plants grown with different water treatments; well-watered, water reduced by 15% and 30%.

$HA3_0$: There will be no difference in the plant height of *L. perenne* due to the interaction between the presence or absence of AM, and different water treatments of well-watered, water reduced by 15% and 30%.

$HB1_0$: There will be no differences in the dry biomass of *L. perenne* between plants grown with and without AM.

$HB2_0$: There will be no differences in the dry biomass of *L. perenne* between plants grown with different water treatments; well-watered, water reduced by 15% and 30%.

$HB3_0$: There will be no difference in the biomass of *L. perenne* due to the interaction between the presence or absence of AM, and different water treatments of well-watered, water reduced by 15% and 30%.

## 2.4 Literature searching strategy

The Web of Science database was used for literature searching. Key search words used included, mycorrhizal fungi, forage grass, *Lolium perenne*, water/drought stress, and low nutrients. Some of the functions used within Web of Science included, marked lists to store the names of papers of interest, filters to exclude or include review articles and areas of interest (particularly where search terms returned over 100 papers), the sort function to sort by date or number of citations. A word file was created with notes of all papers read and included title, citation, points of interest and main conclusion. Google search was used to find more general information on plants, climate change and nutrients. PROMT was used to evaluate papers and web pages, particularly looked at provenance and objectivity for web pages and relevance and method for scientific papers.

No ethical approval was required for this study.

# 3 Results

The following are the results of plant height and aboveground dry biomass of *L. perenne* after 13 weeks of growth with and without AM and drought stress.

## 3.1 Plant Height

The mean plant height, measured from leaf tip to tiller base, for all +AM plants is 8% higher than for all -AM plants. Across the water treatments see figure 1 below, the height difference for +AM/-AM is greatest for the most drought stressed plants, at more than 12%. The variation between water treatments is less pronounced, (WW/+AM and 15W/+AM 1%, WW/+AM and 30W/+AM 2% figure 1), with -AM plants having the greater differences, (WW/-AM and 30W/-AM 10% figure 1). The standard error bars figure 1 indicate the level of overlap, and show that the variation within samples is similar to the variation between water treatments.

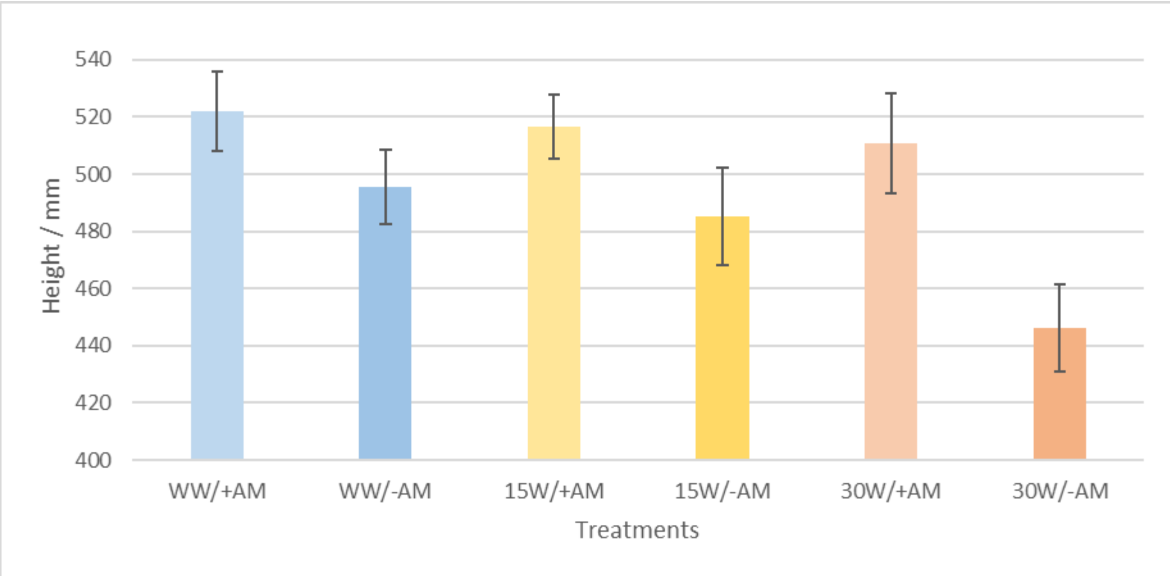


*Figure 1 Mean heights of Lolium perenne plants, with standard error bars, WW well-watered, 15W/30W -15% and -30% below field capacity, +AM/-AM with and without AM fungi, n=10.*

The 2-way ANOVA results show a statistically significant difference only for +AM/-AM treatments, the p-value of $1.45 \times 10^{-03}$, is below significance level of 0.05, (F=11.26, P=$1.45 \times 10^{-03}$, F crit= 3.17, Table 1). $HA1_0$ can be rejected: There will be no differences in plant height of *L. perenne* between plants grown with and without AM.

Water treatments (F=1.86, P=0.17 F crit= 4.02, Table 1) and the interactions between water treatments and AM treatment (F=0.73, P=0.49 F crit= 3.17, Table 1) did not return a statistically significant result

$HA2_0$ must be accepted: There will be no differences in plant height of *L. perenne* between plants grown with different water treatments; well-watered, water reduced by 15% and 30%.

$HA3_0$ must be accepted: There will be no difference in plant height of *L. perenne* due to the interaction between the presence or absence of AM, and different water treatments of well-watered, water reduced by 15% and 30%.

Table 1 *ANOVA results for analysis of influence of water treatments (well-watered, -15% and -30% below field capacity) and inoculation with and without arbuscular mycorrhiza fungi; on plant height. * Indicates a statistically significant result at p < 0.05. n=10.*

| **ANOVA Results Plant Height** | | | |
|---|---|---|---|
| *Source of Variation* | F | P-value | F crit |
| Water treatments | 1.86 | $1.65x10^{-1}$ | 3.17 |
| +AM / -AM | * 11.26 | $1.45x10^{-3}$ | 4.02 |
| Interaction | 0.73 | $4.86x10^{-1}$ | 3.17 |

## 3.2 Aboveground Dry Biomass

Aboveground dry biomass was greater for +AM plants than for -AM plants at 11%. Across water treatments, the differences increased with drought stress, see figure 2 below. There is twice the difference (14%) between 30W/+AM and 30W/-AM as there is between WW/+AM and WW/-AM (7%). The difference between water treatments is greatest for WW/-AM and 15W/-AM (7%), 7 time greater than WW/+AM and 15W/+AM (1%).

The standard error bars figure 2 show there is considerable overlap between sample values for WW treatment.

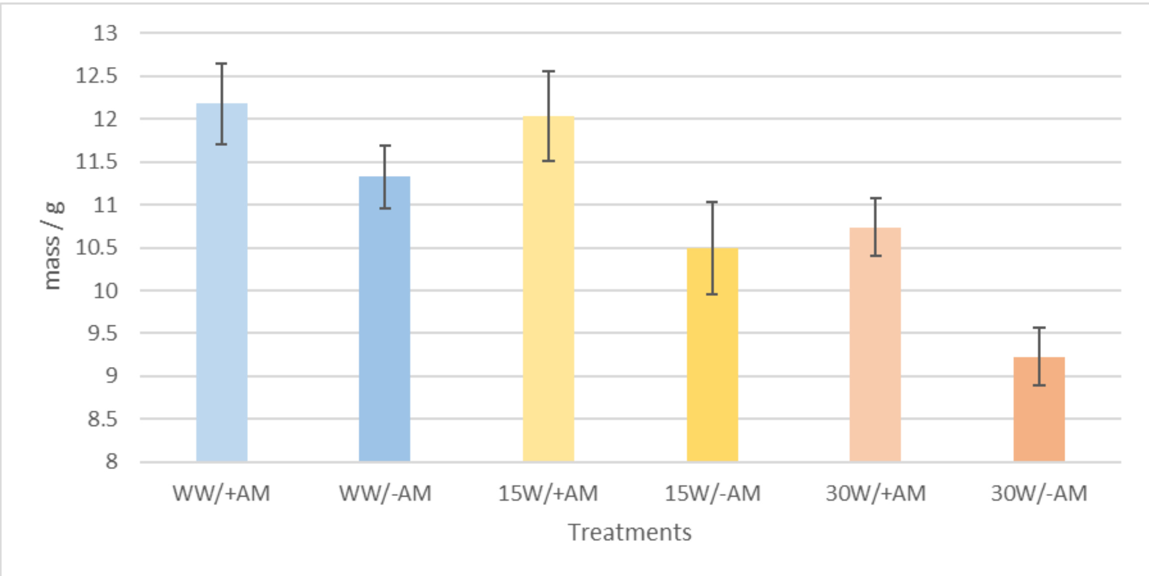


*Figure 2 Means of Above ground dry biomass of Lolium perenne plants with standard error bars, WW well-watered, 15W/30W -15% and -30% below field capacity, +AM/-AM with and without AM fungi, n=10.*

The 2-way ANOVA shows a statistically significant difference in aboveground dry biomass for the water treatments and for AM treatments.

The AM treatments had a p-value well below significance level of 0.05, (F=13.28, P=6.04x$10^{-04}$, F crit= 4.02, Table 2). $HB1_0$ can be rejected: There will be no differences in dry biomass of *L. perenne* between plants grown with and without AM.

The water treatments had a p-value, well below significance level of 0.05, (F=8.74, P=5.16x$10^{-04}$, F crit= 3.17, Table 2). $HB2_0$ can be rejected: There will be no differences in dry biomass of *L. perenne* between plants grown with different water treatments; well-watered, water reduced by 15% and 30%.

*Table 2 ANOVA results for analysis of influence of water treatments (well-watered, -15% and -30% below field capacity) and inoculation with and without arbuscular mycorrhiza fungi; on the growth of above ground dry biomass. * Indicates a statistically significant result at p < 0.05. n=10*

| **ANOVA Results Aboveground Dry Biomass** | | | |
|---|---|---|---|
| *Source of Variation* | F | P-value | F crit |
| Water treatments | * 8.74 | 5.16x$10^{-4}$ | 3.17 |
| +AM / -AM | * 13.28 | 6.04x$10^{-4}$ | 4.02 |
| Interaction | 0.40 | 6.73x$10^{-1}$ | 3.17 |

A Tukey test on the water treatments means showed the significant differences lie between WW and 30W, and between 15W and 30W

*Table 3 Tukey test results for pairs of means for water treatments. T = 1.05, MS = 1.91, q=3.40, n=20 * Indicates a statistically significant result.*

| **Tukey test** | |
|---|---|
| Treatment Pairs | Result |
| WW / 15W | 0.50 |
| WW / 30W | * 1.77 |
| 15W / 30W | * 1.28 |

The interactions between water treatments and AM treatment did not return a statistically significant result (F=0.40, P=6.73x$10^{-01}$ F crit= 3.17, Table 2). $HB3_0$ must be accepted: There will be no difference in dry biomass of *L. perenne* due to the interaction between the presence or absence of AM, and different water treatments of well-watered, water reduced by 15% and 30%.

# 4 Discussion

## 4.1 Effects of Arbuscular Mycorrhizal fungi on plant growth

AM increased plant growth as measured by aboveground dry biomass and plant height (11% and 8%), over all the water treatments, (figure1 and 2). This is consistent with other research on AM fungi and plant interactions (Huang *et al*., 2011, Lee *et al*., 2012, Cavagnaro et al. 2014, Endresz *et al*., 2015). It shows the benefits of symbioses to the host plant in a low nutrient environment and indicates that colonisation of the plants by AM was probably successful.

However, the level of increase shown in this study is half the increase in aboveground dry biomass shown by Lee *et al*. (2012) in their study of *L. perenne* (27%). Their study was over 11 weeks and plants were grown with full nutrients which probably benefitted the overall growth of the plants.

Other studies show the level of benefit from AM association varies between the plant species. Both Endreze and Cavgnaro compared different grass species. Endreze *et al*., (2015) study showed a 27% to 33% increase in aboveground biomass for non-invasive *Danthonia alpina* and *Chrysopogon gryllus* associated with AM while the invasive species *Calamagrostis epigejos* and *Cynodon dactylon* association with AM had a negative effect on growth. Cavagnaro *et al*., (2014) also reported a negative effect of AM association for temperate grass *Dactylis glomerata* (-27%) while in the same study tropical grass *Panicum coloratum* showed a positive effect (162%). These studies highlight the carbon cost to the plant of association with AM (Eason *et al*., 1999).

## 4.2 Effects of water treatment on plant growth

As expected, water treatments caused a significant reduction in aboveground dry biomass, but this did not translate into a significant difference in plant height (table1). Comparing this to other studies, Yang *et al*. (2019) observed a significant decrease in plant height for *L. perenne* with drought stress, but exactly what was measured is not specified in their study. They had 10 plants per pot which may have encouraged more upright growth and less tillering due to shade on the plant base (Hunt 1998). In this current study, height was measured from leaf tip to the base of the tiller. Leaf length may be a phenotypical trait, not influenced by water stress. *L. perenne* can only support three leaves at a time, as a fourth leaf emerges the leaf at the base begins senescence (teagasc 2019). Leaf base to tiller base or stem length might have been a better indicator of plant height.

## 4.3 Effects of combined treatments

The combined effects of water and AM treatments did not have a significant effect on plant growth as measured by this study (table1 and 2). This result is contrary to some studies that found a significant effect (Lee *et al*., 2012, Huang *et al*., 2011), but these other studies induced a more severe drought stress for a shorter period of time. For example, Lee *et al*., (2012), had 12 days of drought stress (20 ml water $d^{-1}$), and Huang *et al*., (2011) study on *Cucumis melo* had 10 days of drought stress at 63% of field capacity.

While other studies that did not see a significant effect in plant aboveground biomass, they did see significant effects on other variables. Interestingly Yang *et al*., (2019) observed a significant increase in plant height but not in plant biomass with 20 days of drought stress at 40% and 20% of field capacity. Li *et al*., (2019) in a field study comparing $C_3$ and $C_4$ grasses, found a significant increase in leaf carbon assimilation rates and catalase (CAT) activity with reduced levels of malondialdehyde (MDA) for $C_3$ grass *Leymus chinensis* under water restrictions of 30% and 50%. CAT aids the breakdown of hydrogen peroxide (Yang and Poovaiah 2002) protecting the plant from oxidative stress, MDA is a biomarker of oxidative stress (Khoubnasabjafari and Jouyban 2020). Their levels change as the plant comes under drought stress and can indicate how well the plant is coping with the drought event (Mirshad and Puthur 2017)

It is probable that even though AM symbioses does not always show benefits in increased biomass, it is still protecting the plant from the drought stress and thereby aiding its recovery after the event (Legay *et al*., 2018). Cavagnaro *et al*., (2021) also showed that AM association aided recovery, both *Agropyron elongatum* and *Brachiaria brizantha* recovered better after defoliation particularly at low P supply, when inoculated with AM.

## 4.4 Limitations of method

The lower drought stress in this study of 85% and 75% of field capacity may not have been enough to demonstrate the benefits of AM association. The Tukey test shows there was no significant difference in aboveground dry biomass between WW and 15W water treatments (table 3) but there was a significant difference between WW and 30W.  Suggesting lower water treatment of 40% or 50% such as used by Li *et al*., (2019) were needed to show significant effects.

This lack of variation between the results, particularly WW/+AM and 15W/+AM, figure 1 and 2, may also be due to the pot size, as plants became pot bound in the latter part of the study and this may have limited growth. Using larger pots would have prevented this issue and would have facilitated root measurements to be taken, which was not feasible once the plants were pot-bound.

The means of the aboveground biomass in figure 2 show a trend of increasing benefit of AM association as water stress increases (diff +AM/-AM, WW 7%, 15W 13%, 30W 14%) but these treatments combined were not statistically significant because of the variability within the samples. Some of this variability may be due to the limitations of the pot study or the effectiveness of the AM species to the host. Remke *et al*., (2021) research on *Bouteloua gracilis* found that plants and their associated AM were adapted to local conditions, while Cavagnaro *et al*., (2014) showed the growth response of 6 grass species varied for the same AM inoculation. Kyriazopoulos *et al*., (2014) found that *Dactylis glomerata* (cock's-foot grass) performed better under drought stress conditions when inoculated with *Glomus intraradices* than when inoculated with *Glomus mosseae* or a mixture of both. In this study commercially available AM was used, which is produced for the horticulture industry (INOQ 2023) and may not be the optimal for forage grasses.

## 4.5 Opportunities for future study

To keep livestock farming sustainable into the future, forage grasses that can respond to climate change and perform with low inputs of fertilizer, need to be developed. The research undertaken in this study and other research undertaken in the past, (Endresz *et al*., 2015, Remke *et al*., 2021, Cavagnaro *et al*., 2021), demonstrate the value that could be obtained from the development of these forage grasses in conjunction with AM.

To find the optimal combinations of species to promote grass growth and recovery it may be beneficial to investigate AM fungi from local grasslands. Further research could investigate the performance of local AM fungi with different grass species in a similar pot study.

# 5 Conclusion

The benefits of AM to the host plants are numerous but the interactions between plant and AM are complex and mitigated by many environmental variables. This study showed that AM association significantly increased the growth of *L perenne* in a low nutrient environment (F=13.28, P=$6.04 \times 10^{-04}$, F crit= 4.02, Table 2). While there was a trend of increasing benefit with drought stress (figure2), the increase was not significant (F=0.40, P=$6.73 \times 10^{-01}$ F crit= 3.17, Table 2). This may be partly due to the fact that the levels of water treatments used did not create enough of a difference in conditions and also other limitations within the study.

AM are a valuable resource that could aid farmers to use fertilizers more efficiently and make forage grass production more sustainable into the future. Further study is needed to find the optimal species combinations that suit different environments and production systems.

# 6 References

Ayling, S.M., George, B.H. and Rogers, J.B., (2021) 'Mycorrhizal colonisation in roots of *Holcus lanatus* (Yorkshire Fog) in a permanent pasture under conditions of reduced precipitation', *Botany*, 99(4), pp.199-208. Available at https://web-s-ebscohost-com.libezproxy.open.ac.uk/ehost/pdfviewer/pdfviewer?vid=0&sid=6c9a022d-b1e0-4f3d-bd71-f95b50a34501%40redis (accessed 21 Apr 2023).

Camenzind, T. and Rillig, M.C., (2013) 'Extraradical arbuscular mycorrhizal fungal hyphae in an organic tropical montane forest soil', *Soil Biology and Biochemistry*, 64, pp.96-102. Available at https://doi.org/10.1016/j.soilbio.2013.04.011 .

Cavagnaro, R.A., Oyarzabal, M., Oesterheld, M. and Grimoldi, A.A., (2014) 'Screening of biomass production of cultivated forage grasses in response to mycorrhizal symbiosis under nutritional deficit conditions', *Grassland Science*, *60*(3), pp.178-184. Available at https://doi-org.libezproxy.open.ac.uk/10.1111/grs.12057 .

Cavagnaro, R.A., Oyarzabal, M., Oesterheld, M. and Grimoldi, A.A., (2021) 'Species-specific trade-offs between regrowth and mycorrhizas in the face of defoliation and phosphorus addition', *Fungal Ecology*, 51, p.101058. Available at https://doi-org.libezproxy.open.ac.uk/10.1016/j.funeco.2021.101058 .

Cliquet, J.B., Murray, P.J. and Boucaud, J., (1997) 'Effect of the arbuscular mycorrhizal fungus *Glomus fasciculatum* on the uptake of amino nitrogen by *Lolium perenne*'. *The New Phytologist*, *137*(2), pp.345-349. Available at https://nph-onlinelibrary-wiley-com.libezproxy.open.ac.uk/doi/pdfdirect/10.1046/j.1469-8137.1997.00810.x (Accessed 20 April 2023).

Eason, W.R., Scullion, J. and Scott, E.P., (1999) 'Soil parameters and plant responses associated with arbuscular mycorrhizas from contrasting grassland management regimes', *Agriculture, ecosystems & environment*, *73*(3), pp.245-255. https://doi-org.libezproxy.open.ac.uk/10.1016/S0167-8809(99)00054-7 .

Endresz, G., Mojzes, A. and Kalapos, T., (2015) 'Deficit watering reduces plant growth to a smaller extent with arbuscular mycorrhizal association than without it for non-invasive grass species but not for invasive grass species', *Applied Ecology and Environmental Research*, *13*(2), pp.551-567. Available at https://www.aloki.hu/pdf/1302_551567.pdf (Accessed June 20 2023).

EPA (2022) 'Water Quality in Ireland 2016 – 2021' *The Environmental Protection Agency*. Available at https://www.epa.ie/publications/monitoring--assessment/freshwater--marine/water-quality-in-ireland-2016--2021-.php (Accessed 01 March 2023).

EPA (2023) 'Greenhouse gas emissions and projections – Agriculture' *The Environmental Protection Agency.* Available at https://www.epa.ie/our-services/monitoring--assessment/climate-change/ghg/agriculture/ (Accessed 01 March 2023).

Gosling, P., Hodge, A., Goodlass, G. and Bending, G.D., (2006) 'Arbuscular mycorrhizal fungi and organic farming', *Agriculture, ecosystems & environment*, *113*(1-4), pp.17-35. Available at https://doi.org/10.1016/j.agee.2005.09.009 .

Huang, Z., Zou, Z., He, C., He, Z., Zhang, Z. and Li, J., (2011) 'Physiological and photosynthetic responses of melon (*Cucumis melo L.*) seedlings to three *Glomus* species under water deficit', *Plant Soil* **339**, 391–399. Available at https://doi-org.libezproxy.open.ac.uk/10.1007/s11104-010-0591-z .

Hunt, W.F. and Easton, H.S., (1989) 'Fifty years of ryegrass research in New Zealand'. In *Proceedings of the New Zealand Grassland Association* pp. 11-23. Available at https://doi.org/10.33584/jnzg.1989.50.1876 .

INOQ (2023) 'Mycorrhiza for Agriculture, INOQ Advantage', [web site] https://inoq.de/en/application/mycorrhiza-for-agriculture/ (Accessed 20 February 2023).

IPCC (2023) 'SYNTHESIS REPORT OF THE IPCC SIXTH ASSESSMENT REPORT (AR6) Summary for Policymakers'. Available at https://report.ipcc.ch/ar6syr/pdf/IPCC_AR6_SYR_SPM.pdf (Accessed 21 April 2023).

Iwuala, E., Adu, M.O., Odjegba, V., Unung, O.O., Ajiboye, A., Opoku, V.A., Umebese, C. and Alam, A., (2002) 'Mechanisms Underlying Root System Architecture and Gene Expression Pattern in Pearl Millet (*Pennisetum glaucum*)', *Gesunde Pflanzen* **74**, 983–996. Available at https://doi-org.libezproxy.open.ac.uk/10.1007/s10343-022-00674-7 .

Khoubnasabjafari, M. and Jouyban, A., (2020) 'Challenges on determination of malondialdehyde in plant samples', *Arch Crop Sci*, *4*(1), pp.64-66. Available at https://doi.org/10.36959/718/604 .

Kyriazopoulos, A.P., Orfanoudakis, M., Abraham, E.M., PARISSI, Z.M. and Serafidou, N., (2014) 'Effects of arbuscular mycorrhiza fungi on growth characteristics of *Dactylis glomerata L*. under drought stress conditions'. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, *42*(1), pp.132-137 Available at https://doi.org/10.15835/nbha4219411 .

Lee, B.R., Muneer, S., Avice, J.C., Jung, W.J. and Kim, T.H., (2012) 'Mycorrhizal colonisation and P-supplement effects on N uptake and N assimilation in perennial ryegrass under well-watered and drought-stressed conditions', *Mycorrhiza*, *22*, pp.525-534. Available at https://doi-org.libezproxy.open.ac.uk/10.1111/j.1399-3054.2012.01586.x .

Legay, N., Piton, G., Arnoldi, C., Bernard, L., Binet, M.N., Mouhamadou, B., Pommier, T., Lavorel, S., Foulquier, A. and Clément, J.C., (2018) 'Soil legacy effects of climatic stress, management and plant functional composition on microbial communities influence the response of *Lolium perenne* to a new drought event', *Plant and Soil*, *424*, pp.233-254. Available at https://doi.org/10.1007/s11104-017-3403-x .

Li, J., Meng, B., Chai, H., Yang, X., Song, W., Li, S., Lu, A., Zhang, T. and Sun, W., (2019) 'Arbuscular mycorrhizal fungi alleviate drought stress in C3 (*Leymus chinensis*) and C4 (*Hemarthria altissima*) grasses via altering antioxidant enzyme activities and photosynthesis', *Frontiers in Plant Science*, *10*, p.499. Available at https://doi.org/10.3389/fpls.2019.00499 .

Mirshad, P.P. and Puthur, J.T., (2017) 'Drought tolerance of bioenergy grass *Saccharum spontaneum L*. enhanced by arbuscular mycorrhizae', *Rhizosphere*, *3*, pp.1-8. Available at https://doi-org.libezproxy.open.ac.uk/10.1016/j.rhisph.2016.09.004 .

Plant Health Cure (2019) 'OFP 5-2-5 Technical data sheet' *Plant Health Cure B.V.* Available at https://www.fruithillfarm.com/wordpress/wp-content/uploads/OPF-5-2-5-ENG.pdf (Accessed 2 March 2023).

Poorter, H., Fiorani, F., Stitt, M., Schurr, U., Finck, A., Gibon, Y., Usadel, B., Munns, R., Atkin, O.K., Tardieu, F. and Pons, T.L., (2012) 'The art of growing plants for experimental purposes: a practical guide for the plant biologist', *Functional Plant Biology*, *39*(11), pp.821-838. Available at http://dx.doi.org/ 10.1071/ FP12028 .

Popay, I. (2013) '*Lolium perenne* (perennial ryegrass)', *CABI Compendium*. Available at https://doi.org/10.1079/cabicompendium.31166 .

Remke, M.J., Johnson, N.C., Wright, J., Williamson, M. and Bowker, M.A., (2021) 'Sympatric pairings of dryland grass populations, mycorrhizal fungi and associated soil biota enhance mutualism and ameliorate drought stress', *.Journal of Ecology*, *109*(3), pp.1210-1223. Available at https://doi-org.libezproxy.open.ac.uk/10.1111/1365-2745.13546 .

Saneoka, H., Moghaieb, R.E., Premachandra, G.S. and Fujita, K., (2004) 'Nitrogen nutrition and water stress effects on cell membrane stability and leaf water relations in *Agrostis palustris Huds*', *Environmental and Experimental Botany*, *52*(2), pp.131-138. Available at https://doi.org/10.1016/j.envexpbot.2004.01.011 .

Teagasc (2019) 'Understanding how the perennial ryegrass plant grows', Available at https://www.teagasc.ie/publications/2019/understanding-how-the-perennial-ryegrass-plant-grows.php (Accessed 26 July 2023).

The Open University (2022a) 'Different types of mycorrhizas', *S397: Block 2 – Balances and cycles*. Available at https://learn2.open.ac.uk/mod/oucontent/view.php?id=1940958&section=1.2 (Accessed 22 April 2023).

The Open University (2022b) 'Protocol for growing plants and measuring water uptake', *S390-23B resources for research projects*. Available at https://learn2.open.ac.uk/mod/oucontent/view.php?id=2083420 (Accessed 10 February 2023).

Thilagar, G., Bagyaraj, D.J. and Rao, M.S., (2016) 'Selected microbial consortia developed for chilly reduces application of chemical fertilizers by 50% under field conditions', *Scientia Horticulturae*, *198*, pp.27-35. Available at https://doi.org/10.1016/j.scienta.2015.11.021 .

Van Auken, O.W. and Freidrich, R., (2006) 'Growth and mycorrhizal infection of two annual sunflowers with added nutrients, fungicide or salts', *The Texas Journal of Science*, *58*(3), pp.195-219. Available at https://link-gale-com.libezproxy.open.ac.uk/apps/doc/A152237675/AONE?u=tou&sid=bookmark-AONE&xid=5a7c9662 (accessed 21 Apr 2023).

Wang, Y., Wang, M., Li, Y., Wu, A., and Huang, J., (2018) 'Effects of arbuscular mycorrhizal fungi on growth and nitrogen uptake of *Chrysanthemum morifolium* under salt stress', PLoS ONE 13(4): e0196408. Available at https://doi.org/10.1371/journal.pone.0196408 .

Yang, T.P.B.W. and Poovaiah, B.W., (2002) 'Hydrogen peroxide homeostasis: activation of plant catalase by calcium/calmodulin', *Proceedings of the National Academy of Sciences*, 99(6), pp.4097-4102. Available at https://doi.org/10.1073/pnas.052564899 .

Yang, Q., Zhao, Z., Bai, Z., Hou, H., Yuan, Y., Guo, A. and Li, Y., (2019) 'Effects of mycorrhizae and water conditions on perennial ryegrass growth in rare earth tailings', *RSC advances*, *9*(19), pp.10881-10888. Available at https://doi.org/10.1039%2Fc8ra10442e .

Zhu, B., Gao, T., Zhang, D., Ding, K., Li, C. and Ma, F., (2022) 'Functions of arbuscular mycorrhizal fungi in horticultural crops', *Scientia Horticulturae*, 303, p.111219. Available at https://doi-org.libezproxy.open.ac.uk/10.1111/grs.12057 .

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