

Received: 30 April 2020 | Revised: 6 June 2020 | Accepted: 8 June 2020

DOI: 10.1002/ppp3.10128

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Contrasting effects of commercial and native arbuscular mycorrhizal fungal inoculants on plant biomass allocation, nutrients, and phenolics

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Societal Impact Statement

As the global population increases, the need to feed more people must be met while simultaneously conserving the long-term sustainability of our agroecosystems. There is mounting interest and discussion around the application of arbuscular mycorrhizal fungal (AMF) inoculants to enhance crop growth, nutrient uptake, and pest resistance. However, the effects of AMF inoculation are variable and context dependent. This study found that a multi-species AMF inoculant had a stronger effect on plant biomass allocation and chemistry than a single AMF species inoculant, however, neither of these had a stronger effect than re-inoculating plants with a field-sourced native AMF community.

KEYWORDS

arbuscular mycorrhiza, barley, phenolics, phosphorus, sorghum, sustainable agriculture

1 | INTRODUCTION

Global food security relies heavily on a selective number of plant species, with a particular reliance on certain plant groups such as cereals (FAO, 2019). Most of these associate with arbuscular mycorrhizal fungi (AMF) of the subphylum, Glomeromycotina (Smith & Smith, 2011). Arbuscular mycorrhizas can provide plants with an array of benefits including nutrient acquisition and protection from abiotic and biotic stressors. The fungi also play an important role in many ecosystem level processes, contribute to soil structure and health, and have strong effects on plant community ecology (Tedersoo, Bahram, & Zobel, 2020).

Given the capacity for the arbuscular mycorrhizal (AM) symbiosis to provide ecological and agricultural benefits, and the serious concern for global soil “health”, there is increasing recognition of the importance of managing AMF to the sustainable future of food production (Rillig et al., 2019; Thirkell, Charters, Elliott, Sait, & Field, 2017). One aspect of this is the application of AMF inocula

to encourage mycorrhization of crops. However, the outcome of engaging in the AM symbiosis can be highly context dependent, subject to AMF and plant species identities, and on local soil conditions. For example, nutrient exchange between fungus and plant can vary between crop cultivar (Elliott, Daniell, Cameron, & Field, 2020), and studies show a certain level of partner selectivity exists in these plant–fungal associations (Sepp et al., 2019). Additionally, evidence suggests that certain AMF taxa may be more associated with particular functions such as plant nutrient uptake, or plant resistance against pests and pathogens (Bennett & Bever, 2007; Wehner, Antunes, Powell, Mazukatow, & Rillig, 2010). Indeed, AMF have been shown to differentially affect plant secondary metabolites associated with resistance to insect herbivores including phenolics (Mithöfer & Boland, 2012) and benzoxazinoids (Frew, Powell, Glauser, Bennett, & Johnson, 2018).

Despite evidence of context-dependent functional diversity across AMF taxa, there are relatively few examinations at the fungal community level. Indeed, different combinations of AMF taxa

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differentially interact and can exhibit functional complementarity (Jansa, Smith, & Smith, 2008; Sikes, Powell, & Rillig, 2010). For example, studies have shown that inoculants containing more than one AM fungal species can have stronger or weaker effects on their plant hosts compared to single species inoculants (Grümborg, Urcelay, Shroeder, Vargas-Gil, & Luna, 2015; Veresoglou, Meneses, & Rillig, 2012). Yet, our understanding of how assemblages of AMF communities (including species richness) might correlate with different crop nutritional and stress resistance traits remains ambiguous at best. Consequently, it is a gamble whether the AMF taxa in a given inoculum will provide the desired outcomes, or are indeed “superior” to the native fungal community already present in the soil (Hart, Antunes, Chaudhary, & Abbott, 2018).

Therefore, this study examined the effects of inoculation with a single AM fungal species, a combination of four AM fungal species, and a native field soil inoculum. The effects on plant biomass allocation, nutrient uptake (phosphorus and nitrogen), and a group of resistance-associated metabolites (phenolics) were assessed in two globally significant crop species, one C_3 (barley; *Hordeum vulgare* L.) and one C_4 (sorghum; *Sorghum bicolor* L. Moench).

2 | MATERIALS AND METHODS

2.1 | Experimental set-up

Hordeum vulgare L. cv. “Hindmarsh” (barley; 90 plants) and *Sorghum bicolor* L. Moench cv. “Enforcer” (sorghum; 90 plants) were grown in 3.7 L pots, one plant per pot, with a (50kGray) gamma-irradiated (80:20) soil: quartz sand mixture (Table S1; see Supporting Information for more detailed methodology). Plants were grown under one of the three AMF treatments (by directly pipetting ~400 spores onto roots) which comprised of either (a) a single AMF species from a commercial inoculum containing *Rhizophagus irregularis*; (b) four AMF species from a commercial inoculum containing *Claroideoglossum etunicatum*, *Funneliformis coronatum*, *F. mosseae*, and *Rhizophagus irregularis*; (c) native AMF community comprising AMF spores extracted from the field soil. All spores were extracted from the respective inoculum using the wet sieving and sucrose centrifugation method (Daniels & Skipper, 1982) and at the same time were examined ad libitum to confirm AMF identity and viability as per Souza (2015). There were 30 biological replicates per treatment. All pots received microbial filtrate (300 ml) made of equal parts of extraneous extraction solution (i.e., without AM fungal spores) from the three treatments (including the field soil prior to sterilization) to standardize the background non-AMF microbial community within each pot. Plants were grown in a growth chamber (Convion® PGW40) with day/night air temperatures of 27°C and 17°C ($\pm 4^\circ\text{C}$) respectively, daylight set at $900 \text{ mol}^{-2}\text{s}^{-1}$ on a 12 hours photoperiod. Every 2 weeks pots were rearranged within the chamber to reduce any spatial effects.

Ten weeks after sowing and prior to flowering, plants were removed from their pots, roots were washed and a 1–2 g subsample

of fine roots were taken from a random selection of 10 plants per treatment from each plant species for mycorrhizal colonization scoring. All leaf material was snap frozen in liquid nitrogen before being freeze dried then ground to a powder and homogenized prior to chemical analysis.

2.2 | Fungal colonization and plant chemistry

Root subsamples were cleared with 10% KOH and stained with 5% ink-vinegar (Vierheilig, Coughlan, Wyss, & Piché, 1998). Total, arbuscular, and vesicular colonization were assessed using the gridline-intersect method with at least 100 intersects per sample (McGonigle, Miller, Evans, Fairchild, & Swan, 1990). Freeze-dried ground plant material was analyzed for nitrogen concentrations using an elemental analyser (LECO TruMac CNS analyser; LECO) and for phosphorus concentrations using inductively coupled plasma-optical emission spectrometer (ICP-OES) (Varian 710-ES; Agilent Technologies Inc.) after digestion with nitric acid and hydrochloric acid (APHA, 1998). Total phenolics in leaves were determined using a Folin-Ciocalteu assay with gallic acid (Sigma-Aldrich) as the quantification standard (Salminen & Karonen, 2011).

2.3 | Statistics

R statistical interface (v3.6.1) was used for all statistical analysis.

The effects of the AMF treatments on measured parameters of the two plant species were assessed by fitting either generalized linear models or standard linear models followed by Tukey post hoc tests. To satisfy model assumptions response data were transformed where necessary (see Supporting Information for detailed statistical methods).

3 | RESULTS

Barley plants inoculated with four AMF species and the native AMF had 25% and 22% lower root:shoot, respectively, compared with those inoculated with a single AMF species (Table S2, Figure 1a). This was largely driven by reductions in belowground biomass (Figure S1c).

In barley, foliar phosphorus concentrations were 24% and 35% greater in plants inoculated with four AMF and the native AMF, respectively, compared to those inoculated with a single AMF species (Figure 1b). Sorghum displayed a similar response with 22% and 23% greater foliar phosphorus concentrations in plants inoculated with four AMF and native AMF, respectively, compared to the single AMF species inoculant (Figure 1b). These effects on foliar phosphorus concentrations in sorghum were reflected by foliar N:P response which was significantly lower in plants treated with four AMF and native AMF inocula compared with those plants under the single AMF treatment (Figure 1c). In contrast, the AMF inocula did not

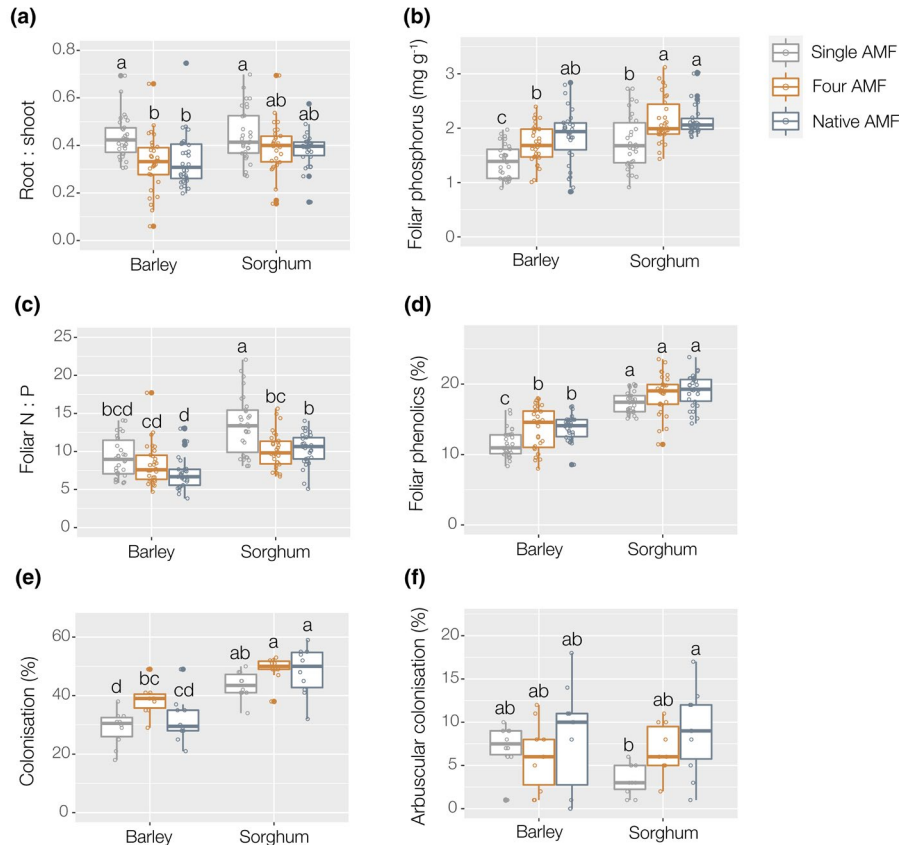


FIGURE 1 Effects of inoculation with one arbuscular mycorrhizal fungal (AMF) species, four AMF species or with a native AMF inoculant extracted from field soil on the (a) root:shoot, (b) phosphorus concentration (mg/g), (c) N:P, (d) total phenolics (%DM), (e) total AMF root colonization (%), and (f) arbuscular root colonization (%) in barley (*Hordem vulgare* L. cv. "Hindmarsh") and sorghum (*Sorghum bicolor* L. Moench cv. 'Enforcer'). Different letters indicate boxes that are significantly different from each other ($p < .05$, Tukey HSD)

differentially affect foliar N:P in barley. Foliar nitrogen differed overall between the two plant species but was unaffected by the AMF treatments (Table S2, Figure S1d).

Sorghum had 41% more foliar phenolics than barley (Table S2, Figure 1d). Phenolic concentrations did not differ between AMF treatments in sorghum, while barley plants inoculated with four AMF species and the native AMF had higher phenolic concentrations than plants inoculated with a single AMF species (Table S2, Figure 1d).

Overall, total AM fungal root colonization was 42% higher in sorghum compared with barley (Table S2, Figure 1e). Total colonization only differed between the different AMF inocula in barley roots (Figure 1e), while formation of arbuscules differed between AMF inocula in sorghum roots (Figure 1f).

4 | DISCUSSION

This study demonstrated that inoculation with four AMF species had stronger effects on plant allometric partitioning, foliar nutrient, and phenolic concentrations than inoculation with a single AMF species, depending on the host plant. This finding is generally consistent with previous studies where inocula with more AMF taxa tended to have stronger effects on different host plant traits of interest (Frew, 2019;

Jansa et al., 2008; Veresoglou et al., 2012). However, the results here have also shown that the effects of inoculating with four AMF species were no different from the effects of applying a native AMF inoculant, extracted from field soil. Thus, applying commercial AMF inocula to soil does not necessarily deliver additional benefit over and above the effects obtained from the resident AMF community. However, these results also point out that AMF inocula may provide significant benefits to plants grown in substrates with impoverished AMF diversity.

Although inoculating with four AMF species or the native AMF had similar outcomes, the effects differed between the two crop species. For example, in barley the four and native AMF treatments reduced root:shoot, and increased phosphorus and phenolics compared to the inoculant with a single AMF species. Contrastingly, in sorghum the four and native AMF treatments did not affect root:shoot ratio between AMF treatments, but did increase phosphorus and reduce foliar N:P compared to the one AMF species treatment. Indeed, the plants also differed in their mycorrhization responses such that only barley exhibited differences in total fungal colonization under the different inocula, while arbuscular colonization differed between treatments only in sorghum.

The allocation of biomass away from the roots observed here is a commonly reported effect of engaging in an AM symbiosis

(Veresoglou et al., 2012), which can be attributed to improved nutrition. Although the root to shoot ratio is a relatively crude measure, it is proposed that biomass investment toward roots decreases as nutrient requirements are met. Although root:shoot did not differ between AMF treatments in sorghum, both plant species exhibited greater phosphorus concentration under the four and native AMF treatments compared to inoculation with a single AMF species. Thus, it is notable that N:P was reduced by the four and native AMF treatments in sorghum and not barley, as the reduced biomass allocation toward the roots observed in barley under these same treatments might have otherwise suggested nutrient limitation under the single AMF treatment.

The increased foliar phenolics in barley under the four and native AMF compared to the single AMF treatment is also noteworthy. Previous studies report increases in phenolics from the AM symbiosis (Jung, Martinez-Medina, Lopez-Raez, & Pozo, 2012), yet this is the first evidence, to my knowledge, that inoculation with different AMF communities differentially affects phenolics between plant species. Although a relatively simplistic measure, total phenolics have previously been associated with resistance to insect herbivory (Mithöfer & Boland, 2012). Thus, the findings here call for a more detailed examination of how differences in AMF community assembly might affect phenolic-based resistance to herbivory.

Despite controversies around AMF inoculants and the variability of their efficacy, the agricultural management of mycorrhizal fungi is likely to have an increasingly important role in future sustainable food production. It is worthwhile pointing out that this study did not assess the combined effects of the native and commercial inocula, which may have uncovered potential interactive effects that might be observed in the field. Although this study was under controlled conditions, the results presented here highlight that the application of multispecies AMF inoculants can have beneficial outcomes for the host plants, but also that inoculant AMF communities may provide little to no additional benefit compared with the resident AMF community. Our knowledge around effectively managing the AM symbiosis in plant production systems is still developing and therefore practitioners should take a cautious approach when it comes to applying AMF inoculants in the field.

ACKNOWLEDGEMENTS

This study was supported by a Charles Sturt University Faculty of Science Postdoctoral Research Fellowship. The author would like to thank the technical teams at Charles Sturt University for their support including the technical team in the National Life Sciences Hub, in particular Vincent West and Yameng Tian. The author is grateful to the four reviewers for their constructive comments on this paper.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Frew A. Contrasting effects of commercial and native arbuscular mycorrhizal fungal inoculants on plant biomass allocation, nutrients, and phenolics. *Plants, People, Planet*. 2020;00:1–5. <https://doi.org/10.1002/ppp3.10128>