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# Imbalanced carbon-for-phosphorus exchange between European arbuscular mycorrhizal fungi and non-native *Panicum* grasses—A case of dysfunctional symbiosis

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ABSTRACT

Arbuscular mycorrhizal fungi (AMF) are globally widespread root symbionts of the majority of terrestrial plant species, they are present in almost all soils, and show particularly low levels of partner specificity. Usually, they benefit their plant hosts through increased nutrients (especially phosphorus, P) supply, improved growth, stress tolerance and fitness as compared to the non-mycorrhizal plants. In exchange for the symbiotic benefits, plant supplies the fungal partner with carbon (C), constituting symbiotic costs for the plant. Here we tested the effect of four soil treatments, combining removal of indigenous AMF communities and/or supplementation with mineral P to restore plant P nutrition, on plant growth and C fluxes from plant to soil as well as on mineral nutrition of a $C_3$ and a congeneric $C_4$ grass species. Contrary to all expectations, both plant species showed lower P and nitrogen contents, and grew smaller, though allocated more C belowground, when supplied with AMF-containing full soil inoculum as compared to AMF-free inoculum. Our results indicate possible incompatibility of symbiotic partners of different geographic origin (European AMF and tropical/subtropical grasses from Africa/Asia), leading to apparent parasitism of the plants by the AMF communities in terms of both growth and nutritional responses. Most likely, downregulation of the direct (root) P uptake pathway by the plants in response to mycorrhiza formation over-compensated the symbiotic (indirect) P acquisition via mycorrhizal hyphae. The observed effects could also have been caused (or contributed to) by the relatively young age of the experimental plants, and different composition of microbial communities in the two inoculant (containing or not the AMF).

## 1. Introduction

Arbuscular mycorrhizal fungi (AMF; subphylum Glomeromycotina; Spatafora et al., 2016) are among the most widespread and ecologically significant microbial groups in soils, colonizing roots of the vast majority of extant plant species (van der Heijden et al., 2015). These fungi play a crucial role in a number of terrestrial ecosystem processes, such as movement of mineral nutrients, carbon (C), and water between soil and plants (Allen, 2007; van der Heijden et al., 2003); stabilization of soil aggregates (Leifheit et al., 2014), plant coexistence (Bever et al., 2010; van der Heijden et al., 1998), interactions of plants with pathogens (Newsham et al., 1995a; Vigo et al., 2000), and plant tolerance to drought and osmotic stresses (Aroca et al., 2007; Augé et al., 2014, 2015). Efficient phosphorus (P) transfer from the soil to the plants mediated by the AMF hyphae is frequently considered to be the major benefit of arbuscular mycorrhizal (AM) symbiosis for the plants (Smith et al., 2011; Smith and Read, 2008). Under P-limiting soil conditions, AMF can be responsible for nearly all P uptake by a mycorrhizal host plant (Pearson and Jakobsen, 1993; Smith et al., 2004). The plant in return provides the AMF with the photosynthesis-derived carbohydrates (Lekberg et al., 2010; Řezáčová et al., 2017). The importance of the mycorrhizal symbiosis for a plant usually decreases along with increasing P availability in the soil. Under ample P supply (i.e., high P availability in the soils), plants often show lower AMF colonization rates of their roots than under low P availabilities (Menge et al., 1978; Smith and Read, 2008; Treseder and Allen, 2002). The P is

*Abbreviations:* AMF, arbuscular mycorrhizal fungi; NM, AMF-free treatment; M+, mycorrhizal treatment; ANOVA, analysis of variance; C, carbon; N, nitrogen; P, phosphorus; DW, dry weight

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an essential mineral nutrient for plants and its limited availability is often limiting plant growth (Smith et al., 2011; Smith and Smith, 2011). Improvement of plant P acquisition via symbiotic P uptake (Smith et al., 2011) is thus often translating to better plant growth under P limiting conditions, i.e., to net benefit for the plant due to mycorrhiza formation (Smith and Read, 2008 and multiple references therein). However, depending on the balance between gross symbiotic costs for the plant (i.e., the fraction of C resources allocated to the AMF) and the gross symbiotic benefits (e.g., improved transfer of nutrients from the soil to plants via the AMF hyphae), mycorrhizas can elicit a range of plant responses from positive to neutral to negative − constituting (an apparent) mutualism-parasitism continuum (Hoeksema et al., 2010; Johnson, 2010; Johnson and Graham, 2013; Johnson et al., 1997; Klironomos, 2003). Besides the soil conditions, a number of plant traits such as coarseness of root system or the type of photosynthesis determine plant responsiveness to mycorrhiza − with the $C_4$ plants being often more responsive to mycorrhiza formation than the $C_3$ plants (Grman, 2012; Hetrick et al., 1990; Hoeksema et al., 2010; Wilson and Hartnett, 1998).

A number of functionally non-redundant AMF taxa coexist in virtually every soil and ecosystem (Jansa et al., 2008; Koide, 2000) and the community thus cannot easily and without consequences be replaced by a single taxon (van der Heijden et al., 1998; but see Mathimaran et al., 2005). To understand the importance of these fungi in ecosystem functioning, the whole AMF community shall be considered rather than its individual components. Yet, due to the high complexity of the soil biota, it is still challenging to subtract the contribution of the AMF from that of the rest of soil biota without causing substantial artifacts (Brito et al., 2009; Kahiluoto et al., 2000).

Here, we inoculated our experimental plants with unsterile field soil harboring a native AMF community that has previously been thoroughly characterized (Řezáčová et al., 2016), constituting the mycorrhizal (M+) treatment, and established a non-mycorrhizal (NM) treatment by selective removal of AMF from the soil. To this end, we repeatedly heated (pasteurized) the soil to suppress eukaryotic microorganisms and then back-inoculated the soil with soil filtrate to establish a treatment free of AMF but containing the other microbes. We also manipulated P availability in the soil to test if the growth/nutritional benefits of mycorrhiza formation could be explained by improved P nutrition of the plant. To disentangle the contribution of indigenous AMF to plant growth and nutrition and to test if the effect was dependent on plant photosynthesis type, we assessed biomass production, P and nitrogen (N) content and C allocation of two closely related grasses (genus *Panicum*), one with $C_3$ and the other one with $C_4$ photosynthesis. We expected to get (1) M+ plants with higher total biomass, higher P content and higher belowground C allocation as compared to the NM plants; (2) greater mycorrhizal benefits in $C_4$ than $C_3$ plants because previously, perennial $C_4$ prairie grasses were found to be more dependent on mycorrhiza than annual $C_3$ grasses (Hetrick et al., 1990; et al., 1998); and (3) smaller difference between M+ and NM treatments at high than at low P availabilities, because the main benefit of mycorrhizal symbiosis is increasing P uptake at low P availabilities, and also because AMF colonization is often reduced at high P supply (da Motta et al., 2016; Johnson et al., 2015).

## 2. Material and methods

### 2.1. Experimental design

The experiment was setup in a fully factorial design with four factors (plant photosynthesis type – $C_3$ or $C_4$; microbial inoculum – full soil inoculum including indigenous AMF, M+, or AMF-free inoculum, NM; P availability – low, unamended with P, or high, amended with P fertilizer; and harvest time – 1 h and 72 h post-$^{13}C$ labeling) with 5 replicates per treatment combination, resulting in a total of 80 pots. The positions of the pots in the glasshouse were completely randomized and the positions were further re-randomized every week throughout the entire experiment.

### 2.2. The plants

Two *Panicum* species were used in this study, *P. bisulcatum* Thunb. and *P. maximum* Jacq. These two plant species are well characterized in terms of their photosynthesis types, with *P. bisulcatum* being a typical $C_3$ plant and *P. maximum* having a $C_4$ (PCK subtype) type of photosynthesis (Pinto et al., 2014). Seeds of both plant species were kindly provided by Dr. Oula Ghannoum, Hawkesbury Institute for the Environment, University of Western Sydney, Australia.

### 2.3. Pots and the substrate

Plants were grown in 2-l pots (11 × 11 × 20 cm, w × d × h) lined with a plastic mesh (opening of 1 mm) at the bottom, all sterilized with 96% ethanol and filled with potting substrate. The substrate consisted of thoroughly mixed (volume-based) 10% γ-irradiated ( > 25 kGy) field soil from Litoměřice, Czech Republic (N50°31′54.53", E14°06′7.10") described in Řezáčová et al. (2016), 45% autoclaved zeolite MPZ 1–25 from Zeopol (www.zeolity.cz, grain size 1–2.5 mm) and 45% autoclaved quartz sand (grain size < 3 mm). Further details and physico-chemical properties of both the soil and the potting substrate are provided in the electronic supplement (Table A.1 in the Supplementary data).

### 2.4. Microbial inoculation and P fertilization

Each pot was augmented with either full soil inoculum containing the indigenous AMF (M+; being the non-sterile field soil collected at the same site as the soil component of the potting substrate) or AMF-free microbial inoculum (NM). The latter was prepared by pasteurization of the field soil (incubation of the moist field soil at 80 °C in a closed vessel for 12 h, twice, 24 h apart) and adding it with unsterile field soil wash (1:10 w:v), twice filtered through a fast filter paper (Munktell Filtrak 388, Verkon, Praha, Czech Republic), holding back particles above 10 μm in diameter (own observation) and incubated for 3 weeks in the dark. Fifty grams of the inoculum (either M+ or NM) were added to each pot as a layer 5–8 cm below the surface.

The potting substrate of the high P pots were supplemented with 30 mg P $kg^{-1}$ in form of aqueous solution of $NaH_2PO_4$ mixed in the entire volume of the substrate (except the inoculum), resulting in approximately 7-fold increase of P availability (i.e., the water-extractable P levels), as compared to P-unamended substrate measured at different time points between 4 and 18 days after the P addition (Fig. A.1 in the Supplementary data). This treatment was supposed to reduce efficiency of mycorrhizal P uptake and thus the plant mycorrhizal responses.

### 2.5. Planting and growth conditions

Seeds of both plant species were pre-germinated in 15 cm Petri dishes on wet filter paper at 37 °C for 12 h and then incubated at 30 °C for 4 days under ambient light before transplanting them into the pots. Three to four seedlings of the respective plant species were planted per pot and thinned to two individuals per pot two weeks after planting. Planting of the pots with *P. bisulcatum* preceded planting of the pots with *P. maximum* by one week due to differences in rapidity of seed germination and also to facilitate isotopic labeling, harvest and sample processing. The pots were incubated in a glasshouse of the Institute of Microbiology, Prague, Czech Republic during early summer 2014 (May–July), with temperatures ranging between 14 °C and 42 °C (mean of temperature measurements logged every 15 min with a Testo 435-2 datalogger being 25.9 °C) and the day length extended to 14 h with supplemental lighting (metal halide lamps, 400 W each) providing a