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Arbuscular mycorrhizal fungi influence decomposition and the associated soil microbial community under different soil phosphorus availability

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ABSTRACT

Despite the general appreciation that arbuscular mycorrhizal fungi (AMF) can influence decomposition of organic materials, the underlying mechanisms remain unclear. Here we investigated whether AMF influence decomposition and change the associated soil bacterial and fungal communities through nutrient acquisition and whether this effect is altered by the level of soil phosphorus (P). We conducted a pot experiment using *Medicago truncatula* as host plant without or with AMF (a mixture of six species) and with two levels of soil P (4 and 24 mg kg^{-1}). Hyphal-ingrowth mesh bags (which excluded roots but not hyphae or other microbes) were used to measure the effect of AMF hyphae on decomposition. Maize leaves dual-labeled with ^{15}N:^{13}C stable isotopes were used as the organic substrate. Bacterial and fungal communities were accessed via sequencing of partial 16S rRNA and ITS genes. The results showed the ^{15}N and ^{13}C content of the organic matter remaining in the mesh bags under low soil P availability were significantly lower in the treatment with AMF than without AMF. Under high soil P availability, however, no significant difference of ^{15}N content remaining was found between AMF and non-AMF treatments, while ^{13}C content remaining was higher in AMF than in non-AMF treatment. Levels of mycorrhizal colonization and ^{15}N transport from organic matter to the host by AMF hyphae were higher at low P availability than at high P availability, suggesting that the host can acquire more nutrients through the AM from organic matter when soil P availability was low. The composition of bacterial and fungal communities were altered by AMF at both soil P levels. For bacterial community, the richness and diversity were higher with low P availability, especially for the phyla Saccharibacteria and Nitrospirae. For fungal community, AMF increased the richness but not the diversity under low P availability. Together, we show that AMF acquire nutrients from organic matter for host, influence decomposition and alter the bacterial and fungal communities, and that these effects were modulated by the soil P availability.

1. Introduction

Arbuscular mycorrhizal fungi (AMF) form symbiotic associations with approximately 80% of terrestrial plants (Smith and Read, 2008) and play a significant symbiotic role in mediating the flow of mineral nutrients, especially phosphorous (P) and nitrogen (N), from the soil to the host plant, and the flow of organic carbon (C) from the host plant to the soil (Allison et al., 2010; Orwin et al., 2011; Averill et al., 2014). The distribution of nutrients in natural soils, however, is patchy, and the nutrients may occur in complex organic forms rather than in readily available mineral forms (Kelly and Canham, 1992). AMF hyphae have been found to proliferate extensively in nutrient-rich organic materials (John et al., 1983; Ravnskov et al., 1999a; Camenzind and Rillig, 2013) and then acquire and transfer substantial amounts of P and N from the organic source to the host plant (Joner et al., 2000; Hodge, 2003). Because AMF are obligate biotrophic fungi (Read and Perez-Moreno, 2003) and mainly acquire inorganic nutrients (P and N) from the organic patches in the soil (Govindarajulu et al., 2005; Leigh et al., 2011; Hodge, 2014), the ability of AMF acquisition of nutrients for host plants may depend on the effects of AMF on soil organic material decomposition.

Several studies have suggested that decomposition is accelerated in the presence of AMF (Hodge, 2001, 2003). Cheng et al. (2012), for example, showed that AMF can stimulate decomposition of soil organic

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matter within AMF-active zones when the level of atmospheric CO_2 is elevated with N amendments. Other studies, by contrast, found that AMF did not accelerate decomposition (Nottingham et al., 2013; Shahzad et al., 2015) or inhibited decomposition (Leifheit et al., 2015; Carrillo et al., 2016). So far, the mechanisms underlying the effects of AMF on decomposition remain unclear (Hodge, 2014). One hypothesis is that AMF affect decomposition processes in soil by influencing associated microbes (Herman et al., 2012; Nuccio et al., 2013; Zhang et al., 2016). AMF hyphal exudates contain H^+, OH^- or $HCO3^-$, low-molecular-weight sugars (i.e., glycoprotein glomalin) and organic acids (Toljander et al., 2007; Nuccio et al., 2013). These ions and compounds likely change the local pH, phosphatase activity, and soil C economy in the hyphosphere (Wright and Upadhyaya, 1998; Rillig and Mummey, 2006) and consequently influence microbial community composition and activity in the hyphosphere (Toljander et al., 2007; Veresoglou et al., 2012). Approximately 10% of the bacterial community in a study by Nuccio et al. (2013) responded to a single species of AMF, *Glomus hoi* by using high-throughput molecular techniques (e.g., the 16S rRNA gene microarray). By using phospholipid fatty acid (PLFA) analysis, some studies found that AMF increased the biomass of soil saprotrophic fungi and Gram-negative bacteria (Ravnskov et al., 1999b; Artursson et al., 2005; Albertsen et al., 2006) or suppressed most microbial groups (i.e. Gram-positive and Gram-negative bacteria, other fungi) (Olsson and Wilhelmsson, 2000; Welc et al., 2010). Bacteria and fungi play key roles in decomposition (Schneider et al., 2010). These changes of microbes affected by AMF may alter the production of bioactive metabolites and decomposition processes (Schmidt et al., 2011).

The other hypothesis is that AMF affect decomposition and associated decomposers through changing soil nutrients availability (Geisseler et al., 2010; Cheng et al., 2012; Hodge, 2014). For example, AMF may immobilize N through their external hyphae (Hodge and Fitter, 2010) and alleviate a commonly observed N-induced suppression of microbial biomass and activity (Treseder, 2008; Geisseler et al., 2010; Cheng et al., 2012), and thereby increase decomposition. Under N deficient soil, however, AMF may exacerbate nutrient limitation on decomposers, thus reducing their activity and decomposition (Schimel and Bennett, 2004; Jannoura et al., 2012). Soil P often limits plant growth in terrestrial ecosystems (Bonser et al., 1996). Studies showed that the availability of soil P affected decomposition (Jing et al., 2017) and shaped the population of bacteria and general fungi (Beauregard et al., 2010). AMF play a key role in P nutrition of the plant hosts and they are expected to be more important for plant growth when soil P availability in soil is low, but less beneficial for plants when soil P availability is high (Smith and Smith, 2011). It is unclear, however, whether AMF forage more P through promoting decomposition under low soil P availability and whether AMF affect the associated decomposers (e.g. bacterial and fungal communities) during the decomposition.

We hypothesize that nutrients (e.g. N and P) acquisition by AMF would concomitantly promote the decomposition of organic materials and the associated microbial communities when soil P availability is low. In this study, we determined the effect of AMF on decomposition under conditions of low and high soil P availability, and tested whether this effect associates with microbial communities (bacterial and fungal communities) in the AMF hyphosphere. We used *Medicago truncatula* as a host plant. Decomposition influenced by AMF hyphae was quantified by analyzing the organic remains of maize leaves (dual labeled with the stable isotopes ^{15}N:^{13}C) in hyphal-ingrowth mesh bags, i.e., in bags made with mesh that enabled passage of hyphae and bacteria but not roots (Cheng et al., 2012). The acquisition of nutrients from the leaves in the mesh bags by the hyphae and then transport to the plant was assessed by determining the $\delta^{15}N$ in the shoots of *M. truncatula* (Hodge and Fitter, 2010; Cheng et al., 2012). The soil microbial community in the hyphosphere was analyzed using Illumina MiSeq sequencing.

2. Materials and methods

2.1. Soil, plants, AMF, and organic material

Soil was collected from a paddy field at the Changxing Agricultural Sci-Tech Park of Zhejiang University. The paddy field had been continuously planted with rice without application of P fertilizer for at least 10 years. The soil had the following properties: pH 5.9 ± 0.13, total P 246 ± 15.8 mg kg^{-1}, Olsen-soluble P 2.33 ± 0.103 mg kg^{-1}, total N 1.27 ± 0.082 g kg^{-1}, and soil organic carbon 15.3 ± 0.32 g kg^{-1}.

Medicago truncatula seeds (cv. Jemalong A17) were obtained from the laboratory of Professor Rujin Chen at the Oklahoma Center for the Advancement of Science and Technology, USA.

We used an AMF inoculum containing six species including *Acaulospora scrobiculata* (As, BGC HK02A), *Gigaspora margarita* (Gma, BGC ZJ03), *Funneliformis geosporum* (Fg, BGC GZ01), *Rhizophagus intraradices* (Ri, BGC BJ09), *Funneliformis mosseae* (Fmo, BGC XJ01), and *Glomus tortuosum* (Gt, BGC NM03A). Each of the six original AMF isolates was deposited in the Glomales Germplasm Bank in China (the Beijing Academy of Agriculture and Forestry Sciences) and was propagated with *Zea mays* L. and *M. truncatula* in sterilized sand in a growth chamber for 5 months until sporulation in the greenhouse of Zhejiang University. The sand substrates with chopped roots, extraradical mycelium, and spores were stored in plastic bags at room temperature until used. The density of spores in the inocula of each AMF species was estimated by microscopic examination (Nikon SMZ800) after wet-sieving and centrifugation (Gerdemann and Nicolson, 1963). Spore numbers in the 50-g inoculum of species were 584 in As, 1137 in Gma, 1750 in Ri, 342 in Fg, 1046 in Fmo and 498 in Gt, respectively.

We used dual labeled with ^{15}N:^{13}C stable isotopes leaves of maize (*Zea mays* L.) to access decomposition rates and nutrients transform between organic material and host plants. Because maize is a C_4 plant that has a higher $^{13}C/^{12}C$ ratio ($\delta^{13}C$ is −13.6‰, Smith and Epstein, 1971), the maize leaves can be treated as the materials naturally labeled with ^{13}C stable isotope. For ^{15}N labeling, we prepared the maize leaves material by adding ammonium sulfate-^{15}N with 98.09 atom% of enrichment to the soil in which maize plants were grown.

To ensure the decomposition and microbial community changes were mediated by the AMF, we used hyphae-ingrowth bags made with 25-μm nylon mesh (VS-Monoprint polyester, Germany). These bags allowed penetration by AMF hyphae and passage by bacteria but did not permit penetration by roots. Each bag contained 50.0 g of sterilized soil and 0.5 g of oven-dried and chopped maize leaves; when filled with soil, the bags were round and had a volume of 35 cm^3. The maize leaves were chopped to ensure that the organic material formed a thin, homogeneous, and concentrated layer in the middle of each bag. The soil that was used to fill the bags was the same as the plant cultivation substrate. Each bag contained 1.007 ± 0.0163 mg of ^{15}N and 8.445 ± 0.013 mg of ^{13}C with a C:N ratio of 10.36 ± 0.055.

2.2. Experiment design

We conducted a microcosm experiment with a two-factor randomized design that included two concentrations of soil P (4 mg kg^{-1}, and 25 mg kg^{-1}) and two AMF treatments (soil amended with a living mixture of AMF inoculum or amended with an autoclaved mixture). Each treatment was replicated four times resulting in a total of 16 microcosms. Hereafter, the abbreviations AMF and NAMF refer to the treatments with and without AMF, respectively, and Low P and High P refer to the treatments with low and high P availability, respectively. In some cases, the abbreviations are combined. NAMF-Low P, for example, indicates a treatment with autoclaved inoculum and a low P availability.

The soil was γ-sterilized a month before the experiment. Each microcosm (volume 2 L, height 21 cm) was filled with 2 kg of soil. Each