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Long-term fertilizer application effects on the soil, root arbuscular mycorrhizal fungi and community composition in rotation agriculture

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ABSTRACT

Arbuscular mycorrhizal fungi (AMF) play key roles in plant growth, ecosystem sustainability and stability. However, it is still unclear how the soil, root AMF growth and community composition are affected by fertilizer application in subtropical wheat–rice rotation agro-ecosystems. We investigated the impact of different organic and/or inorganic fertilizers on AMF growth and community composition in a long-term experiment field in Zhejiang Province, east China. AMF biomass was determined using 16:1ω5 phospholipid fatty acids (PLFAs) and neutral lipid fatty acids (NLFAs) content. Soil and root AMF community compositions were determined by DGGE analysis, cloning, sequencing and phylogenetic analyses. The root colonization rate was not significantly affected by different fertilizer regimes. Manure amendment significantly enhanced both AMF hyphal (i.e., 16:1ω5 PLFA) and spore (i.e., 16:1ω5 NLFA) biomass content, while inorganic fertilizer only increased the AMF spore biomass. A total of 10 and 11 *Glomeromycota* phylotypes were detected in soil and root samples, respectively. *Glomus* was the dominant genus in both soil and root samples, while *Acaulospora* genus occurred in roots only. Although the different fertilizers altered soil AMF communities, the root AMF community structure was not significantly altered. Soil pH ($F = 5.695$, $P < 0.01$) and available K ($F = 4.888$, $P < 0.01$) contributed to both soil and root AMF community composition, while the C/P ratio ($F = 3.893$, $P < 0.01$) only contributed to soil AMF community. The data suggests that high soil organic C content benefits AMF growth, while soil pH and available K concentration strongly influence AMF community. The root AMF community was much more resilient to the changes caused by long-term fertilizer application than the soil community.

1. Introduction

Applying fertilizers to agricultural soils may potentially influence soil properties, nutrient availability and crop yields. Traditional organic farming using organic fertilizers, such as crop residues, animal manure and green manure crops, allows soil to retain beneficial qualities (Watson et al., 2002). To the contrary, inorganic fertilizers allow higher crop productivity, but this comes at an environmental cost to soil and water quality (Chirinda et al., 2010). Therefore, applying inorganic fertilizer and manure is an essential component of soil management in arable crop production systems. Applying fertilizers affects soil nutrient availability and crop production, as well as soil microorganisms and microbial communities (Insam and Merschak, 1997; Ros et al., 2006a). Different fertilizers directly affect soil microbial community structure and function (Marschner et al., 2003; Ros et al., 2006b; Hu et al., 2010) and the direct influence on soil arbuscular mycorrhizal fungi has been intensively studied in the past decade (Bhadalung et al., 2005; Beauregard et al., 2010; Lin et al., 2012; Chen et al., 2014).

Arbuscular mycorrhizal fungi (AMF) form obligate symbiotic associations with over 80% of all terrestrial plant families by associating with their roots (Schüβler et al., 2001), where they play

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a vital role in plant growth by providing mineral nutrients such as phosphorus (P), nitrogen (N), and trace elements to the plants (Smith and Read, 2008). Because of their importance in plant productivity and soil structure (Gosling et al., 2010), many studies have focused on the response of AMF abundance and community composition to agricultural management, especially to fertilizer application in agro-ecosystems. However, results published thus far are contradictory. For example, Chen et al. (2014) found that P fertilizers significantly decreased AMF abundance, Hammer et al. (2011) observed P to selectively stimulate AMF growth, while Beauregard et al. (2010) reported that the AMF community was unaffected by long-term P application. Similarly, the response of AMF abundance to N addition is also highly variable (Oehl et al., 2004; Wilson et al., 2009; Chen et al., 2014). According to Egerton-Warburton et al. (2007), the N:P ratio could be an important factor determining AMF abundance and diversity. Soil pH is one of the most powerful determinants of the microbial community composition (Rousk et al., 2011). Different AMF respond differently to soil pH: *Glomus mosseae* is not tolerant of soil pH below about 5, while other species are distinctly acid-tolerant (Mosse et al., 1973; Wang et al., 1993). Thus, AMF growth and community diversity in field soils often appeared to be independent of pH. However, Hazard et al. (2013) observed that AMF community composition was influenced by soil pH and not by land use, where the pH ranged from 3.8 to 7.9 over 40 geographically dispersed sites in Ireland. It is not known that whether a number of factors contribute to stimulate AMF growth and community composition, or if a single factor is dominant.

In this study we investigated the effect of long-term application of different fertilizers (organic and/or inorganic) on AMF growth and community composition in a rice–wheat rotation agro-ecosystem in eastern China. Both soil and root samples were collected to examine the variations of AMF biomass and community diversity during the wheat season. Furthermore, the contributions of soil characteristics to these variations were studied to test the following hypotheses: (1) long-term application of different fertilizers may alter AMF growth and the fungal community; (2) the ratio of organic to inorganic fertilizers significantly affects soil and root AMF communities.

2. Materials and methods

2.1. Experimental design

The long-term field experiment was conducted in West Lake District, Hangzhou, Zhejiang Province, China (30.098°N, 120.062°E). The silty clay loam soil (about 21% clay and 54% silt) was derived from alluvial sediment from the Qiantang River and classified as waterloggogenic paddy soil. The soil contained 29.0 g kg^{-1} organic matter, 1.70 g kg^{-1} total N, 0.53 g kg^{-1} total P, 22.6 g kg^{-1} total K and had a pH of 6.69 at the beginning of the experiment in 1996. The crop succession was winter wheat (*Triticum aestivum* L.) and summer rice (*Oryza sativa* L.). Six treatments with three replicates in completely randomized blocks were established, with a total of 18 plots (7.5 × 4 m^2 of each). The treatments consisted of: (1) a control with no fertilizer application; (2) straw (dried rice straw applied at 1500 kg hm^{-2}, equaling 10.95 kg hm^{-2} total N, 11.34 kg hm^{-2} P_2O_5 and 35.61 kg hm^{-2} K_2O); and (3) manure (composted pig manure, applied at 7500 kg hm^{-2}, equaling 135 kg hm^{-2} total N, 187.23 kg hm^{-2} P_2O_5 and 261 kg hm^{-2} K_2O); (4) NPK, applied as urea (135 kg hm^{-2} total N), superphosphate (45 kg hm^{-2} P_2O_5), and potassium chloride (75 kg hm^{-2} K_2O); (5) S + NPK (applied with both straw and NPK fertilizer); and (6) M + NPK (applied with both manure and NPK fertilizer). Each plot received the same treatment from 1996 onwards. Half of the fertilizers were applied in winter wheat and the other half in summer rice, i.e., two applications yearly. Pig manure from livestock farm was collected and composted for 2 weeks. Nutrient concentrations (N, P, and K) in the compost and rice straw were determined prior to application and P and K were generally supplemented to equal the prescribed doses of superphosphate and KCl. Winter wheat was sown in November and harvested in early June of the next year. Summer rice was sown in late June and harvested in November.

2.2. Soil and root sampling and DNA extraction

Five individual soil samples were collected from each plot at a depth of 0–15 cm in May 2013 (at wheat harvesting stage). After the wheat shoots and roots were separated, soil samples from the same plot were combined to form a composite sample and homogenized through a sieve (<2 mm). The composite samples were separated into two subsamples: one for determining soil pH and nutrient concentrations; the other subsample was freeze–dried and stored at −80 °C for determining DNA and lipid content. Fresh roots samples were carefully hand-washed with tap water (removing soil and particulate matter), rinsed twice with deionized water, and also separated into two subsamples. One subsample was used for determining the root colonization rate and the other was used for DNA extraction. The DNA was extracted from 50 mg of mixed, homogenized plant roots from each of the 18 plots using MoBio PowerPlant™ DNA isolation kits as per the manufacturer's instructions (MoBio Laboratories, Inc., Carlsbad, CA, USA). The genomic DNA from each soil sample was extracted from 0.5 g of freeze–dried soil using a MoBio PowerSoil™ soil DNA isolation kit (MoBio Laboratories, Inc., Carlsbad, CA, USA) as per the manufacturer's instructions. The extracted root and soil DNA were dissolved in 100 μl TE buffer and quantified spectrophotometrically, then stored at −20 °C until further amplification and sequencing.

2.3. AMF community analysis

Species composition of the soil and root AMF communities was assessed by a genotypic fingerprinting approach using PCR–DGGE. A nested PCR amplification for the AMF community was performed according to Liang et al. (2008). Nested PCR make use of two sets of primers, in two successive PCR runs. As the second PCR amplifies a secondary target within the first run's product, false positives are eliminated. Briefly, PCR reactions were performed in 25 μl of reaction mixtures, which was prepared with 10 ng of template DNA, 12.5 μl of Premix Taq (TaKaRa, Japan), 0.2 μl of each primer (10 μM), 80 ng μl^{-1} of bovine serum albumin (BSA), and adjusted to a final volume of 25 μl with RNase-free water. Negative controls consisted of 1 μl molecular grade water in all reaction sets to check for contamination. In nested PCR there was a second control, consisting of a re-amplified negative control from the first PCR set. Soil and root DNA were first amplified with the AMF specific primer pair AM1 (5′-GTT TCC CGT AAG GCG CCG AA-3′) and NS31 (5′-TTG GAG GGC AAG TCT GGT GCC-3′) to obtain an approximately 580 bp fragment of the 18S rRNA gene. PCR was performed in a S1000 Thermal Cycler (Bio-Rad, USA) using the following conditions: 1 cycle (1 min at 94 °C; 1 min at 66 °C; 1 min 30 s at 72 °C); 30 cycles (30 s at 94 °C; 1 min at 66 °C; 1 min 30 s at 72 °C); final extension (10 min at 72 °C, with cooling to 4 °C). PCR products were verified by agarose gel electrophoresis (1.0% w/v agarose; 100 V, 30 min) using ethidium bromide staining.

Amplification product from the first PCR reaction was diluted 1/10 and 1 μl of this dilution was used as the template in a second round of PCR using the NS31-GC (5′-CGC CCG GGG CGC GCC CCG GGC GGG GCG GGG GCA CGG GGG TTG GAG GGC AAG TCT GGT GCC-3′) and the Gol1 (5′-GCC TGC TTT AAA CAC TCT A-3′) primer