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Rapid soil fungal community response to intensive management in a bamboo forest developed from rice paddies

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ABSTRACT

Although heavy winter mulch and high rate fertilizer application are commonly practiced in intensively managed bamboo (*Phyllostachys praecox*) plantations, little is known about the effects of these practices on soil microbial activities. Therefore a field study was conducted to investigate the long term intensive management on the development and composition of soil fungal communities. Fungal biomass (fungal phospholipid fatty acid marker), fungal DNA (18S rDNA real-time qPCR) and fungal community composition (culture-independent methods: DGGE, cloning and sequencing) were determined across a bamboo plantation that included seven stand age-classes (1, 4, 6, 8, 10, 12 and 20 years old). Although soil microbial PLFA biomass and fungal DNA abundance were unaffected during the first two years of intensive management, all increased significantly after three years of intensive management. The total microbial PLFA and bacterial PLFA increase linearly ($P < 0.001$) with increasing stand age, while soil fungal PLFA and 18S gene abundance increase was best described using a quadratic equation ($P < 0.01$). The fungal/bacterial ratio generally remained constant, but did increase for the 8 and 12 year stand soils. Sequencing of commonly-occurring bands revealed that the majority of the soil fungi were species of either *Sordariomycetes* or *Chytridiomycetes*. Cluster analysis by Ward's method revealed rapid short-term change in fungal communities that returned to its original composition within one to two years when the soils were not disturbed. This indicated a robust original fungal community that was resilient to transient perturbations resulting from intensive land management when allowed breaks from nutrient loading and soil disturbance. Redundancy analysis indicated that soil chemical characteristics, such as pH, N_{tot} and C_{org}, could account for 12.7%, 12.1% and 10.3% of the variance in soil fungal community composition, respectively. Stand age contributed to 12.6% of the variance of soil fungal community.

1. Introduction

Bamboo is a fast growing and widespread forest crop that has great potential to improve economic development. An example is Lei bamboo (*Phyllostachys praecox*), which is a cash crop commonly grown in eastern China for high-value edible bamboo shoots. *P. praecox* has an intensive management regime that includes applying winter mulch and large amounts of mineral fertilizer to stimulate earlier bamboo shoot emergence (Jiang et al., 2006). Our previous study demonstrated that long-term intensive management has led to significant soil acidification as well as the accumulation of soil nutrients such as nitrogen, phosphorus and potassium (Qin et al., 2011). The application of winter mulch and organic manure greatly increased the soil organic matter and water-soluble organic carbon (WSOC) and the intensive management is now considered as an important option for soil carbon sequestration and a regional scale counter to climate change (Zhou et al., 2011). However, the effect of long-term intensive management on the soil microbial community remains largely uncertain.

Soil fungi play an important role in soil ecosystems where they break down plant residues, promote nutrient cycling and stimulate plant growth (He et al., 2005). Forest conversion to cropping land typically results in declining soil organic matter (SOM) (Trasar-Cepeda et al., 2008), which has been linked to shifts in the fungi to bacteria ratio caused by reductions in fungal biomass (Montecchia et al., 2011), and increased bacterial diversity (Upchurch et al., 2008). However, little information is available on the changes of soil fungal community following the conversion of crop land to an intensive managed forest. Soil microbial biomass in conventionally-managed arable and grassland soils is usually

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dominated by bacteria (Bloem et al., 1994). Fungal biomass generally increases in more natural, untilled soil systems not receiving fertilizer (Bardgett et al., 1998; Helgason et al., 2009), although supplementing soils with organic matter that has a high C/N ratio stimulates fungal growth (Henriksen and Breland, 1999; Vinten et al., 2002). Fungi are adversely affected by a high mineral N content (Bardgett and McAlister, 1999) and fertilizer application, especially N application, lowers soil fungal biomass (Bittman et al., 2005; de Vries et al., 2006; de Vries et al., 2007).

Changes in microbial community composition are often observed after organic or inorganic amendment. Soil fungi differ in their response to NPK fertilizers (Donnison et al., 2000), and N fertilizers alter not only the fungal biomass, but also the abundance of fungal species (Sarathchandra et al., 2001). Different organic amendments may differ in composition (eg. C/N ratio) and thereby affect the decomposition rate as well as the subsequent fungal community composition (Marschner et al., 2003). The soil microbial communities are also strongly influenced by pH, as it affects abiotic factors such as carbon and nutrient availability. In addition, soil pH may control biotic factors, such as the community composition of fungi and bacteria (Fierer and Jackson, 2006). A lower soil pH favours fungal growth and enzyme activity (Bååth and Anderson, 2003). Other factors such as soil moisture (Hawkes et al., 2011), prescribed burning (Bastias et al., 2006), soil tillage (Helgason et al., 2009) and grazing (Jirout et al., 2011) may negatively or positively affect fungal biomass and community structure.

We expected long-term intensive management to alter the composition of fungal communities in the soils under bamboo stands. In this study, 21 bamboo stands with seven different stand ages were sampled in Zhejiang Province, China. The objective was to explore PLFA and 18S rDNA-based molecular approaches to assess the impact of high rate mineral fertilizer application and winter mulch on soil fungal abundance and community composition in long-term intensive managed bamboo stands. Possible links between the environmental factors and fungal parameters were also evaluated, such as using soil characteristics as predictors for the fungal biomass, community composition and changes to the *F*/*B* ratio.

2. Materials and methods

2.1. Experimental site and soil sampling

The bamboo stands investigated in this study were located in Taihuyuan township, Lin'an County, Zhejiang Province, China (30°16.072′–16.263′ N, 119°34.117′–34.977′ E). The soils were classified as Ferrisols in the FAO soil classification system. This area was formerly used as paddy field for rice production, where it has been used for at least 300 years according to the county records. Paddy fields in this area were converted to bamboo stands progressively from 1980, because of the higher profit margins associated with edible bamboo shoot production. The paddy field is usually drained and tilled in October after rice harvest prior to conversion to a bamboo forest. After that, the primary bamboo with rhizome is transplanted at a stem density of approximately 1000 plants ha^{-1}. Bamboo stands received no fertilizer during the first four years after conversion, but were intensively managed from the fifth year. The annual intensive management regime of bamboo stands is illustrated in Fig. 1. Bamboo stand soils underwent a cycle where they were intensively managed for three successive years and then left with little disturbance for two years. The total mineral fertilizers used per year were about 1.1 t ha^{-1} of urea and 2.2 t ha^{-1} of compound fertilizer ($N:P_2O_5:K_2O$ = 15:15:15), which amount to 0.8 t N ha^{-1}. These were applied in May (35%), September (30%) and December (35%). Besides the mineral

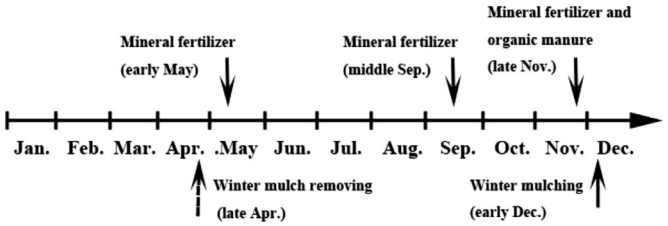

Fig. 1. Winter mulch and fertilizer application on the study sites.

fertilizer, 100 t ha^{-1} of organic manure from poultry and piggery was applied every year. Surface applications of winter mulch (15 cm rice straw and 20 cm rice grain hulls) was placed on the soil in December and removed the following April before fertilizer application.

A space-for-time substitution procedure was used to reconstruct chronological sequence for a bamboo stand. Twenty-one bamboo stands were selected to represent 7 different stand age-classes: 1 year (1 YR), 4 years (4 YR), 6 years (6 YR), 8 years (8 YR), 10 years (10 YR), 12 years (12 YR) and 20 years (20 YR), each with triplicate. For example, the 1 YR treatment represented a bamboo stand which was converted from paddy field for the first year, while the 8 YR treatment represented a bamboo stand was converted from paddy field for 8 years and had received three years of intensive management. The distribution of sampling plots was considered as a completely randomized design. Samples of topsoil (0–15 cm) were collected on the same day in August 2011. Five random sampling points were chosen for each bamboo stand with a minimum distance of 2 m between individual sampling points. These five samples were thoroughly mixed to make a single composite sample. Field samples were stored at 4 °C prior to analysis. Small sub-samples of each composite sample were extracted for DNA within 1 day of collection. Soil sub-samples were then air-dried for measurement of basic chemical parameters. The organic carbon (C_{org}) was determined by the combustion method (McGeehan and Naylor, 1988), total nitrogen (TN) by the Kjeldahl procedure (Kirk, 1950), available phosphorus (AP) by the Bray procedure (Bray and Kurtz, 1945), available nitrogen (AN) by the hot alkaline permanganate method (Subbiah and Asija, 1956), and the available potassium (AK) by the flame photometric method (Lu, 2000). The pH was determined in a 1:2.5 w/v mixture of soil and distilled water, while the soil moisture was determined by mass difference after drying at 105 °C until constant weight (Lu, 2000).

2.2. Microbial biomass by PLFA measurement

Phospholipid fatty acids (PLFAs) were analysed using modified methods (Wu et al., 2009) similar to that of Frostegård et al. (1991). Lipids were extracted from 3.00 g of soil using a chloroform: methanol: citrate buffer (pH 4) mixture (V/V = 5:10:8 and citrate concentration of 0.15 M). In the extract, phospholipids were separated from neutral and glycolipids using silica acid columns (Supelco, Inc., Bellefonte, PA, USA). Following methylation of the phospholipids, the PLFA methyl esters were separated and identified by gas chromatography (N6890, Agilent, USA) fitted with a MIDI Sherlocks microbial identification system (Version 4.5, MIDI, USA). 19:0 phosphatidylcholine (Avanti Polar Lipids, Alabaster, AL) was used as an internal standard and was added prior to methylation. Bacterial groups were represented by a15:0, i15:0, 15:1ω9c, i16:0, a17:0, i17:0, 17:1ω7c, 17:1ω8c, 18:0 2OH, 18:1ω5c, and fungal groups were represented by 16:1ω5c (AMF), 18:1ω9c, 18:2ω6,9c, and 18:3ω6,9c (Frostegard et al., 1993; Zelles, 1999; Balser and Firestone, 2005).