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Changes in soil microbial community structure and activity in a cedar plantation invaded by moso bamboo

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ABSTRACT

Moso bamboo is fast-growing and can invade a neighboring forest with its rhizome system. We investigated the effect of bamboo invasion on an adjacent Japanese cedar plantation in terms of soil microbial biomass, activities and community structure by analysis of phospholipid-derived fatty acid (PLFA) and denaturing gradient gel electrophoresis (DGGE) profiles. In the cedar-plantation soil invaded by bamboo, soil microbial biomass C (C_{mic}) decreased and biomass N (N_{mic}) increased, which reduced C_{mic}/N_{mic}. Similarly, soil cellulase and xylanase activities decreased with invasion, indicating that bamboo invasion into the cedar plantation facilitated changes in microbial biomass and activities by changing soil biochemical properties. The proportion of total PLFAs that was attributed to all bacteria, Gram-positive (G+) bacteria and Gram-negative (G−) bacteria, was reduced with invasion. The ratio of G+/G− bacteria, 16:ω7t to 16:1ω7c, and cyclopropyl fatty acids to their precursors (i.e., cy17:0/16:1ω7 and cy19:0/18:1ω7c) was highest in cedar-plantation soil, suggesting that environmental stress for soil bacterial communities is alleviated in bamboo invaded soils. Low ratio of G+/G− in the bamboo-plantation and transition-zone soil was associated with increased level of easily decomposable organic matter (C_{mic}/C_{org} and N_{mic}/N_{tot}) in bamboo-invaded soil. Principle component analysis of PLFA content separated the cedar-plantation soil from bamboo and transition-zone soil. DGGE analysis revealed that change in both bacterial and fungal community structure was associated with bamboo invasion. Bamboo invasion caused significant changes in soil microbial activities and community structure in the Japanese cedar plantation.

1. Introduction

Soil microorganisms are essential for maintaining soil fertility and plant growth because they play an important role in nutrient cycling and availability (Fritze et al., 1994). Forest species substitution has resulted in forest ecosystem changes (Van Calster et al., 2007). Such changes directly affect microbial communities and may alter physico-chemical properties, such as soil C, N and other biological properties (Chen et al., 2004; Ushio et al., 2010; Lucas-Borja et al., 2012). This influence may be attributed to species differences in litter quality, root exudates, and nutrient uptake (Grayston and Prescott, 2005; Lucas-Borja et al., 2012).

Bamboo is one of the most important economic plants in East Asia. Bamboo's culms are used similar to wood as a construction material, and its shoots are widely consumed as a healthy and delicious food source. Bamboo is one of the fastest-growing plants in the world (Cho et al., 2011) because of a unique rhizome-dependent system that spreads laterally and generates sprouts at the soil surface. Consequently, bamboo spreads and easily invades natural and secondary forests.

The invasion of bamboo into adjacent forests has become a critical problem in forest management. With an aggressive rhizome system, bamboo expands easily into adjacent forests. The fast-growing shoots persistently occupy the gaps of trees under the canopy, making the forest floor too dark to regenerate the seedlings of young trees. Finally, bamboo gradually takes over the territory of adjacent forest. Thus, the expansion of bamboo leads to reduced biodiversity (Okutomi et al., 1996). Meanwhile, bamboo leaves can release allelochemicals that reduce the growth of understory plants (Chou and Yang, 1982) and cause changes in plant community composition and species diversity (Larpkern et al., 2011). Plant species composition plays a major role in governing soil microbial community structure (Grayston et al., 2004). However, there are few reports on the impact of bamboo invasion on the structure and diversity of the microbial community of forest soils (Wang et al., 2009).

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Our previous bar-coded pyrosequencing study of 16S rRNA gene clone libraries (Lin et al., 2014) indicated that soil bacterial diversity increased and the bacterial community changed when bamboo invaded a cedar plantation. However, little is known about the effect of bamboo invasion on soil microbial activities, or on the microbial biomass of bacteria and fungi.

Denaturing gradient gel electrophoresis (DGGE) (Lorenzo et al., 2010) and phospholipid-derived fatty acid (PLFA) analysis (Sun et al., 2013) have been used to determine changes in the soil microbial community structure associated with plant invasion in forest ecosystems. PLFA analysis can provide information on the viable biomass of the microbial community, such as Gram-positive bacteria, Gram-negative bacteria, actinomycetes and fungi (White et al., 1996), because PLFAs are quickly degraded after cell death (Tabuchi et al., 2008; Frostegård et al., 2011). PLFA analysis has been used to determine the changes in soil microbial community structure resulting from different forest management practices, including different coniferous species composition (Demoling et al., 2008), moisture stress (Wilkinson et al., 2002) and prescribed burning regimes (Sun et al., 2011). In addition, PLFAs have been used to assess the physiological status of microorganisms by measuring specific fatty acids indicative of stresses, such as nutrient limitation and severe pH conditions (Bååth and Anderson, 2003). Although versatile, the use of PLFA analysis to examine the microbial community at the species level is limited. DGGE is a simple, rapid and reproducible technique to interrogate diverse taxa, such as bacteria, fungi and target groups, using different primers to amplify from the same soil DNA extract (Nakatsu, 2007).

Here, we examined the microbial biomass and community structure by measuring PLFAs and used DGGE profiling to clarify how the microbial activity, biomass and community structure changed in Japanese cedar-plantation soil with invasion by bamboo.

2. Materials and methods

2.1. Site description and soil sampling

This study was conducted at Shanlinshi (23°40′N, 120°46′E), a subtropical montane area in Nantou County in central Taiwan. The elevation is approximately 1350 m a.s.l., the mean annual precipitation approximately 2600 mm and the mean annual temperature 17 °C. Parts of this area were reforested with Japanese cedar (*Cryptomeria japonica*) about 40 years ago after large-scale cutting of the natural camphor forest. Almost at the same time, moso bamboo (*Phyllostachys edulis*), a temperate species of giant timber bamboo, was introduced and established adjacent to the cedar plantation. Currently, a transition zone, with both cedar and bamboo plants of 30–50 m wide, stretches in the boundary between the bamboo and cedar plantations. Farmers occasionally cut bamboo stems to induce the regeneration of bamboo, leaving many stumps in the bamboo plantation. In addition, frequent harvesting of bamboo shoots (e.g., monthly during harvesting season) also caused severe or moderate disturbance for the soils in bamboo plantation and transition zone. By contrast, the soil in cedar plantation is largely undisturbed and has lush understory plants.

Four parallel transect lines separated by more than 50 m within each vegetation type (moso bamboo plantation, the transition zone and cedar plantation) were surveyed in February 2011. Four replicate plots (25 × 25 m) were selected for each vegetation zone, for 12 sampling plots in total. The soils were derived from sandstone, and classified as Dystrudept in Soil Taxonomy (Soil Survey Staff, 2010). The studied soils were clayey loam and usually moderately well drained. Two to 3 cm depth of litter covered on the surface of soils. Soil samples were collected with use of a soil core 8 cm in diameter by 10 cm deep. Three cores collected from each plot composed a sample in each plot. Visible detrital material, such as roots and litter, were manually removed when soil was passed through a 2-mm sieve. The 10 cm depth soil samples from each sampling plot were all in A horizon. Samples were stored at 4 °C in the dark. Biochemical analyses, including analysis of microbial biomass and enzymatic activities, were completed within 1 month after field collection. Portions of soil samples were freeze-dried at −20 °C immediately after sampling for analyses of PLFAs and DNA extraction.

2.2. Analytical methods

Organic C and total N contents in soil samples were calculated by use of an NSC elemental analyzer (NA1500 Series 2, Fisons, Italy). Soil subsamples were weighed and oven-dried for 72 h at 105 °C to determine moisture content. Soil pH values in air-dried samples were measured by use of a combination of glass electrodes (soil: water ratio 1:2.5) (McLean, 1982). Soil microbial biomass was analyzed by the chloroform fumigation–extraction method (Vance et al., 1987). Total organic C in the extracted solution was measured by use of a total organic C analyzer (Model 1010 O.I. Analytical, Texas) and converted to microbial biomass C (C_{mic}) assuming a conversion factor of 2.22 (Wu et al., 1990). Microbial N (N_{mic}) was calculated from ninhydrin-reactive N released from the biomass and determined colorimetrically at 560 nm (Amato and Ladd, 1988).

Cellulase activity (EC 3.2.1.4) was determined as described by Schinner and von Mersi (1990). Fresh soil samples (5 g) were incubated with water-soluble carboxymethylcellulose (1.4%) for 24 h at 50 °C (pH 5.5). The low-molecular-weight products and sugars resulting from the enzymatic degradation of carboxymethylcellulose were measured at 690 nm absorbance. Xylanase activity (EC 3.2.1.8) was determined as described by Schinner and von Mersi (1990). Fresh soil samples (1 g) were incubated with 5 ml xylan (3.4%) and 5 ml acetate buffer (pH 5.5) at 50 °C for 24 h. Absorbance was measured at 690 nm. Substrate concentrations were tested to maximize enzyme activities in soil samples. Phosphatase activity (EC 3.1.3.2) was analyzed colorimetrically by measuring the absorbance by *p*-nitrophenol at 400 nm after incubation of 1 g fresh soil with 4 ml modified universal buffer (pH 6.5) and 1 ml of 100 mM *p*-nitrophenyl phosphate at 37 °C for 1 h, following Tabatabai and Bremner (1969). Urease activity (EC 3.5.1.5) was determined as described by Kandeler and Gerber (1988). A fresh soil sample (2.5 g) was mixed with 2.5 ml urea (80 mM) and 20 ml of 0.1 M borate buffer (pH 10.0). The mixture was allowed to react for 2 h at 37 °C. After incubation, 30 ml of KCl (2 M) was added before shaking for 30 min. Ammonium content was determined through the indophenol reaction. The color intensity of the final solution was measured at 690 nm.

Extraction and analysis of PLFAs were as described by Frostegård et al. (1993). Lipids were extracted in a single-phase mixture of chloroform–methanol–citrate (1:2:0.8). Phospholipids were split into neutral, glyco- and phospholipids by use of a solid-phase extraction column and eluted with chloroform, acetone and methanol. Phospholipids then underwent methylation to form fatty acid methyl esters (FAMEs). FAME identification and quantification were analyzed by capillary gas chromatography (GC) with a flame ionization detector (Thermo Finnigan Trace chromatograph) as described by Chang et al. (2011). Fatty acid nomenclature was in accordance with Frostegård et al. (1993). The fatty acids i15:0, a15:0, 15:0, i16:0, 16:1ω7c, 17:0, i17:0, cy17:0, 18:1ω7c, and cy19:0 represent bacteria; 18:2ω6 fungi; i15:0, a15:0, i16:0, and i17:0 Gram-positive (G+) bacteria; 16:1ω7c, cy17:0, 18:1ω7c, and cy19:0 Gram-negative (G−) bacteria; and 10Me18:0 actinomycetes (Zogg et al., 1997; Zelles, 1999).