Soil microbial community structure and function responses to successive planting of *Eucalyptus*

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Abstract

Many studies have shown soil degradation after the conversion of native forests to exotic *Eucalyptus* plantations. However, few studies have investigated the long-term impacts of short-rotation forestry practices on soil microorganisms. The impacts of *Eucalyptus* successive rotations on soil microbial communities were evaluated by comparing phospholipid fatty acid (PLFA) abundances, compositions, and enzyme activities of native *Pinus massoniana* plantations and adjacent 1st, 2nd, 3rd, 4th generation *Eucalyptus* plantations. The conversion from *P. massoniana* to *Eucalyptus* plantations significantly decreased soil microbial community size and enzyme activities, and increased microbial physiological stress. However, the PLFA abundances formed “U” shaped quadratic functions with *Eucalyptus* plantation age. Alternatively, physiological stress biomarkers, the ratios of monounsaturated to saturated fatty acid and Gram+ to Gram- bacteria, formed “∩” shaped quadratic functions, and the ratio of *cy17:0* to *16:1ω7c* decreased with plantation age. The activities of phenol oxidase, peroxidase, and acid phosphatase increased with *Eucalyptus* plantation age, while the cellobiohydrolase activity formed “U” shaped quadratic functions. Soil N:P, alkaline hydrolytic nitrogen, soil organic carbon, and understory cover largely explained the variation in PLFA profiles while soil N:P, alkaline hydrolytic nitrogen, and understory cover explained most of the variability in enzyme activity. In conclusion, soil microbial structure and function under *Eucalyptus* plantations were strongly impacted by plantation age. Most of the changes could be explained by altered soil resource availability and understory cover associated with successive planting of *Eucalyptus*. Our results highlight the importance of plantation age for assessing the impacts of plantation conversion as well as the importance of reducing disturbance for plantation management.

Key words: phospholipid fatty acid; microbial community structure; soil enzyme activity; *Eucalyptus*; successive rotation

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Introduction

A common global land-use change is the conversion of native forest to fast-growing exotic plantations (Burton et al., 2010; Iovieno et al., 2010) due to increased demand for timber and timber products. However, this form of land-use conversion often exhausts soil nutrients and decreases soil quality (Wei et al., 2009) because of the large demand for nutrients by fast-growing species (Laclau et al., 2010) and forestry-related activities (e.g., weed control, soil preparation, and plantation tending), which consequently influences the sustainability of plantations.

Soil microbial communities may provide early indication of soil changes (Harris, 2009; Banning et al., 2011), and are increasingly used to assess ecosystem responses

to environmental perturbations (Harris, 2003; van Dijk et al., 2009; Lewis et al., 2010; Banning et al., 2011) because of its rapid response to anthropogenic disturbances relative to soil biogeochemical properties. These responses have been assessed using soil biological indicators such as the phospholipid fatty acid (PLFA) profiles to reflect the structure of the soil microbial community (Iovieno et al., 2010), and soil enzyme activities to reflect their functional capacity (Rivas et al., 2009). “Signature” PLFA can provide information on specific functional groups of microorganisms present in a community (Frostegård et al., 1993). In an environmental sample, increases in the molar percentages of saturated and cyclopropyl fatty acids and Gram+ bacteria are indications of physiological stress or substrate depletion. Microorganisms prefer to use cis-monoenoic fatty acids, likely due to their faster

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turnover rate and ease of metabolism, and this could be a survival mechanism to maintain membrane integrity during starvation (Guckert et al., 1986; Balkwill et al., 1988). In addition, Gram+ bacteria thrive under more nutrient-limited environments than Gram- bacteria (Fierer et al., 2003). The composition of PLFA in soil samples has been used to detect sensitive shifts in soil microbial communities due to management and vegetation effects (Bossio and Scow, 1998; Drijber et al., 2000; Hackl et al., 2005; Moore-Kucera and Dick, 2008). The functional capacity of the soil microbial community, as reflected in the activities of the enzymes involved in mineralization processes, also varies among soils dominated by vegetation changes (Waldrop et al., 2000).

Eucalyptus, a fast-growing tree species, has been introduced to many tropical and subtropical regions as an afforestation species (Turnbull, 1999). The short-rotation forestry practices performed in *Eucalyptus* plantations can be considered representative of most *Eucalyptus* industrial plantations worldwide. Therefore, afforestation with fast-growing *Eucalyptus* has received considerable attention with regard to its impacts on soil properties, which is highly relevant to the sustainable productivity of *Eucalyptus* plantations. Studies have reported that soil organic carbon, total nitrogen, microbial biomass, and metabolic quotient were significantly lower in *Eucalyptus* plantations compared with natural and regenerated forests (Behera and Sahani, 2003), native plantations (Chen et al., 2013) or pastures (Sicardi et al., 2004). However, Pulrolnik et al. (2009) found no change in total organic carbon, microbial biomass carbon or nitrogen after the conversion of native savanna to *Eucalyptus* plantations. A series of studies conducted in Hawaii reported no change in soil carbon after the conversion of sugarcane fields to *Eucalyptus* plantations, because increased soil carbon caused by *Eucalyptus* afforestation was offset by the loss of sugarcane-derived carbon (Bashkin and Binkley, 1998; Binkley and Resh, 1999; Binkley et al., 2004). Although the impacts of plantation conversion on soil properties of native ecosystems to *Eucalyptus* plantation have been well documented, the following two questions remain to be answered: (1) What are the effects of successive planting of *Eucalyptus* on soil microbial community structure and function after the conversion from native *Pinus massoniana* to exotic *Eucalyptus* plantation? (2) Which factors will influence the direction and degree of variation in soil microbial community in *Eucalyptus* plantations during their short-rotation? In this study, we investigated the variations in soil microbial communities for different-aged *Eucalyptus* plantations quantified by PLFA abundance, composition, and soil enzyme activities, in Guangxi Province, southern China.

1 Materials and methods

1.1 Study area

The study sites were located in the Dongmen Forest Farm, Dongmen Town, Fusui County of Guangxi Province (107°47'–107°56'E, 22°14'–22°21'N). The mean annual temperature is 21.2–22.3°C, and the mean annual precipitation is 1100–1300 mm. Soils of the region are mainly lateritic red earths, derived from arenaceous shale, with a typical soil depth of 80 cm or greater.

In this area, *Eucalyptus* plantations underwent considerable site preparation and removal of shrubs and weeds prior to reforestation. Special eucalypt fertilizer was applied to promote growth, with 500 g/plant as base fertilizer (N:P:K = 10:15:5) at plantation establishment and 250 g/plant as supplemental fertilizer (N:P:K = 15:10:8) at 6, 12 and 24 months after plantation establishment.

1.2 Experimental design and sampling

We selected four *Eucalyptus* plantation sites (*Eucalyptus urophylla* × *grandis*) forming a chronosequence of 1st, 2nd, 3rd, and 4th generation plantations (abbreviated as G1, G2, G3 and G4, respectively) representing plantation ages 3, 8, 14, and 19 years in the Dongmen Forest Farm. Plantation generation refers to the management regime in which *Eucalyptus* trees were cut every five years and replanted. For example, the 19-year old plantation underwent 4 rotations and was considered 4th generation. Prior to reforestation, all the *Eucalyptus* plantation sites were native *Pinus massoniana* plantations, thus one adjacent *P. massoniana* plantation (MP, ca. 20 years) was selected as the standard for comparing native plantation soil properties to non-native. Each site covered an area of approximately 10 ha and all were situated close to each other on a gently sloping area with an elevation approximately 150 m above sea level.

In October 2010, the top 10 cm of mineral soil was sampled using a 3.6-cm diameter soil corer. Three 20 × 20 m² plots were established in each plantation. Ten soil cores from random locations in each plot were collected and mixed together as a composite sample. Three composite soil samples were collected at each site, and soil samples were stored on ice for transport to the laboratory. During soil collection, the plant communities were also surveyed. At each site, we identified all tree species within three 20 × 20 m² plots, all shrub species within five 5 × 5 m² plots, and all herb species within nine 1 × 1 m² plots.

Soil samples were homogenized and sieved (2 mm) to remove roots and gravel. A portion of soil was immediately frozen at –20°C for subsequent PLFA analysis. Subsamples were air-dried for soil biogeochemical analyses.

1.3 Soil physicochemical analysis

Soil pH was determined in a 1:2.5 soil-water slurry using a Delta 320 pH meter (Mettler-Toledo Instruments Co., Ltd., China). Soil total C and total N were determined by combustion in a Vario EL III Elemental Analyzer (Elementar Analysensysteme GmbH, Germany). Soil organic carbon was measured by wet oxidation using dichromate in acid medium followed by the FeSO_4 titration method (Bao, 2000). Alkaline hydrolytic nitrogen was measured using the boric acid absorption – hydrochloric acid titration method (Bao, 2000). Soil was digested using sulfuric acid and perchloric acid and total phosphorus was measured using a Prodigy High Dispersion ICP-OES (Teledyne Technologies Incorporated, USA). Sulfuric acid and hydrochloric acid were used to extract and measure available phosphorus in the soil (Bao, 2000). Available phosphorus was measured using a Prodigy High Dispersion ICP-OES. Fresh soils were analyzed for ammonium (NH_4^+ -N) and nitrate (NO_3^- -N) using KCl extraction and a Continuous Flow Analyzer (San++, SKALAR, Netherlands). **Table 1** shows the basic characteristics and measured soil properties of the sites.

1.4 Phospholipid fatty acid analysis

The biomass and composition of the soil microbial community was measured using PLFA analysis with a modified Buyer method (Buyer et al., 2010). Four grams of lyophilized soil was placed in a 30-mL glass centrifuge tube with a Teflon-lined screw cap. To each soil sample 3.2 mL of 100 mmol/L phosphate buffer (pH 7.4), 8 mL of methanol, and 4 mL of chloroform were added. Tubes were sonicated for 10 min in a sonicating water bath and then rotated end-over-end for 1 hr at room temperature. After centrifugation for 10 min at 2500 r/min, the liquid phase was transferred to a 30-mL separatory funnel. Another 3.2 mL of 100 mmol/L phosphate buffer and 4 mL of chloroform were added, shaken vigorously, and allowed to separate overnight. The bottom (organic phase) was evaporated under N_2 .

Lipid classes were separated by solid phase extraction (SPE) chromatography using a 500 mg silica gel column (Part No. 5982-2265, Agilent Technologies, Wilmington, USA), which was washed with 5 mL of chloroform in advance. After loading the extract in chloroform, neutral lipids were eluted with 10 mL of chloroform, polar lipids were eluted with 10 mL of acetone, and phospholipids

were eluted with 5 mL of methanol. The phospholipid fraction was evaporated under N_2 .

Fatty acids were trans-esterified with 1 mL of 1:1 methanol:toluene and 1 mL of 0.2 mol/L methanolic KOH at 35°C for 15 min. After adding 2 mL of a 1:4 chloroform:hexane solution, 1 mL of 1 mol/L acetic acid, and 2 mL of ddH_2O , the mixture was vortexed for 5 min at 2000 r/min and the phases allowed to separate. The top phase was removed and the bottom phase was extracted with an additional 2 mL of 1:4 chloroform:hexane. The combined organic phase was evaporated under N_2 and stored at –20°C.

The extracts were cleaned by SPE using an NH_2 SPE column (Part No. 8B-S009-EAK, Phenomenex, Torrance, CA) washed with 1 mL of chloroform in advance. Samples were eluted with 1 mL of chloroform and loaded onto the column. After evaporation under N_2 , samples were dissolved in 1 mL of hexane including 10 $\mu\text{g/mL}$ methyl nonadecanoate (19:0) before analysis.

The fatty acid methyl esters were separated on a gas chromatograph with a flame ionization detector (GC-FID) (HP 6890, Agilent Technologies, Santa Clara, USA) using an HP-5 fused silica capillary column (30 m $\times$ 0.25 mm $\times$ 0.25 μm). Helium was used as the carrier gas at 1.0 mL/min. Splitless injection was employed. Temperature specifications were set to injector temperature 230°C, detector 270°C, temperature program: 50°C for 1 min, then 15°C/min to 150°C, maintained for 2 min, and then 3°C/min to 250°C with a final hold of 15 min. The individual PLFA peaks were identified by GC-MS (gas chromatograph combined with mass selective detector HP 5973, Agilent Technologies, Santa Clara, USA).

Mass spectrometer peak identification was based on comparison with the spectra of commercially available bacterial methyl ester (BAME) mix standards (Sigma, St Louis, USA) and the software library NIST05.L. The following PLFAs identified bacterial groups: i15:0, a15:0, 15:0, i16:0, 16:1 ω 9t, 16:1 ω 7c, i17:0, a17:0, cy17:0, 17:0, i18:0, 18:1 ω 9t, 18:1 ω 7c, cy19:0; Gram+ bacteria: i15:0, i16:0, i17:0, a15:0, a17:0, Gram– bacteria: 16:1 ω 9t, 16:1 ω 7c, 18:1 ω 9t, 18:1 ω 7c, cy17:0, cy19:0; fungal groups: 16:1 ω 5c, 18:2 ω 6,9c, 18:1 ω 9c; actinomycetic groups: 10Me17:0 and 10Me19:0; and 14:0, 16:0, 18:0 were normal PLFAs (Frostegård and Bååth, 1996; Zelles et al., 1997, 1999; Olsson, 1999).

Table 1 Soil characteristics of native *P. massoniana* plantation, 1st, 2nd, 3rd, and 4th generation *Eucalyptus* plantations

Stand	Stand age (yr)	pH	TN (g/kg)	TP (mg/kg)	SOC (g/kg)	AN (mg/kg)	AP (mg/kg)	NO_3^- -N (mg/kg)	NH_4^+ -N (mg/kg)	C:N	N:P
MP	≈ 20	4.21 ± 0.10	1.64 ± 0.04 a	406.89 ± 1.05 d	26.87 ± 0.39 a	57.34 ± 1.54 a	2.98 ± 0.11 b	2.09 ± 0.17 a	12.99 ± 0.31 a	19.00 ± 0.34 a	4.02 ± 0.10 a
G1	3	4.28 ± 0.05	1.01 ± 0.03 d	1067.45 ± 33.27 ab	13.35 ± 0.85 bc	43.43 ± 1.60 b	5.03 ± 0.41 a	0.82 ± 0.03 b	11.21 ± 0.20 b	15.05 ± 0.09 b	0.95 ± 0.03 c
G2	8	4.32 ± 0.10	0.95 ± 0.02 d	896.18 ± 1.08 c	10.83 ± 0.67 c	41.35 ± 1.67 b	5.14 ± 0.60 a	0.54 ± 0.02 b	12.30 ± 0.43 ab	13.70 ± 0.19 c	1.06 ± 0.01 c
G3	14	4.24 ± 0.04	1.31 ± 0.03 c	969.37 ± 5.14 bc	15.54 ± 0.20 b	60.27 ± 0.24 a	2.56 ± 0.11 b	1.80 ± 0.13 a	10.63 ± 0.53 b	14.45 ± 0.09 b	1.35 ± 0.03 b
G4	19	4.02 ± 0.03	1.43 ± 0.05 b	1074.81 ± 33.34 a	17.17 ± 0.79 b	59.57 ± 0.61 a	2.93 ± 0.10 b	1.83 ± 0.13 a	11.62 ± 0.24 ab	13.38 ± 0.17 c	1.33 ± 0.07 b
p-Value	–	NS	< 0.001	< 0.001	< 0.001	< 0.001	< 0.01	< 0.001	< 0.01	< 0.001	< 0.001

TN: total nitrogen; TP: total phosphorus; SOC, soil organic carbon; AN, alkaline hydrolytic nitrogen; AP, available phosphorus; NO_3^- -N and NH_4^+ -N, nitrate nitrogen and ammonia nitrogen; NS: no significance.

1.5 Enzyme analysis

A range of extracellular enzyme activities involved in C, N, and P cycling processes was investigated using microplate assays.

Soil β -glucosidase, phenol oxidase and peroxidase activities were measured according to Sinsabaugh et al. (2003). The substrate for β -glucosidase assays was 200 μ mol/L *p*-nitrophenol (pNP) β -D-glucopyranoside. The phenol oxidase substrate was 10 mmol/L 1-dihydroxyphenylalanine (DOPA). The peroxidase substrate was 10 mmol/L DOPA solution and 0.3% H_2O_2 . All substrates were made using 50 mmol/L pH 5.0 acetate buffer. 5 g fresh soil was added to 50 mL of 50 mmol/L acetate buffer solution (pH 5.0), briefly shaken by hand and stirred on a stir plate for 1 min. Approximately 50 μ L of the mixture was added to each lane of a 96-well microplate that contained 150 μ L of 50 mmol/L pNP-substrate solution or acetate buffer (sample control). There were four analytical replicates and four sample controls for each extract. Plates were incubated at 25°C for 2 hr, then 50 μ L of the solution in each well was transferred to another 96-well plate containing 50 μ L of 1.0 mol/L NaOH solution to terminate the reaction. For phenol oxidase and peroxidase, however, aliquots were transferred to empty wells. Absorbance was measured at 410 nm for pNP-substrates and at 469 nm for DOPA assays using a Spectramax plus spectrophotometer. Peroxidase activity was calculated as the difference between samples reacted with and without H_2O_2 .

Soil cellobiohydrolase activity was measured using the fluorimetric method according to Saiya-Cork et al. (2002). A 50- μ L of 200 mmol/L 4-methylumbelliferone- β -D-cellobioside solution was added to each sample well. Blank wells received 50 μ L of acetate buffer plus 200 μ L of sample suspension. Negative control wells received 50 μ L substrate solution plus 200 μ L of acetate buffer. Quench standard wells received 50 μ L of standard (10 mmol/L 4-methylumbelliferone) and 200 μ L of sample suspension. Reference standard wells received 50 μ L of standard and 200 μ L of acetate buffer. There were eight replicate wells for each blank, negative control and quench standard. The microplates were incubated in darkness at 25°C for 4 hr. To stop the reaction, a 10 μ L aliquot of 1.0 mol/L NaOH solution was added to each well. Fluorescence was measured using a microplate fluorometer with 365 nm excitation and 450 nm emission filters.

Soil protease activity was estimated by determining the amino acids released after incubation of soil with sodium caseinate for 2 hr at 50°C using Folin–Ciocalteu reagent according to Ladd and Butler (1972). Soil urease activity was assessed by determining ammonia released after incubation of the soil sample with urea solution for 2 hr at 37°C, using a UV-1700 spectrophotometer (Shimadzu, Japan) at $\lambda = 690$ nm after a 30-min color development period (Kandeler and Gerber, 1988). Soil acid phosphatase

activity was measured as for β -glucosidase, but with substrate pNP-phosphate (Sinsabaugh et al., 2003).

1.6 Data processing and analysis

The macro in SigmaPlot 11.0 was used to fit the curve of changes in soil microbial size, structure, and enzyme activity to the different aged *Eucalyptus* plantations. The composition of the soil microbial community was summarized using principle components analysis on the relative molar percentages of the PLFAs in each sample. The SPSS 16.0 (SPSS Inc., Chicago, IL, USA) software package was employed for the analysis of variance (ANOVA). We analyzed the relationships between environmental variables and the soil microbial community through redundancy analysis. It was performed with CANOCO 4.5. “Species” data was represented by the relative molar percentages of PLFAs or the enzyme activities, which were $\log_{10}(x+1)$ transformed. “Species” data were centered and “environment” data were standardized.

2 Results

2.1 Soil microbial community size

The soil microbial community size decreased significantly after the plantation conversion from native MP to exotic *Eucalyptus* plantation ($P < 0.05$), and the stand ages of the *Eucalyptus* plantation had a substantial effect on PLFA abundance (Fig. 1). All of the measures of PLFA abundance (e.g., single PLFAs, the sum of the abundances of bacterial, fungal, and actinomycetic characteristic PLFAs, and total PLFA) formed “U” shaped quadratic function relationships with stand age of *Eucalyptus* plantations. The minimum value appeared in the G2 stand (Fig. 1).

2.2 Soil microbial community structure

The *Eucalyptus* plantation age had a significant effect on the PLFA composition of soil microbial communities. Principle component (PC) analysis showed there were significant differences between the microbial PLFA compositions among the different *Eucalyptus* plantations and MP on PC1 ($P < 0.05$, Fig. 2). The axis scores of *Eucalyptus* plantations on PC1 were quadratic functions of plantation age ($y = 0.01x^2 - 0.25x + 0.72$, $R^2 = 0.8349$, $P < 0.001$), while the axis scores on PC2 decreased with plantation age ($y = 5.75e^{-0.05x} - 3.32$, $R^2 = 0.9206$, $P < 0.0001$). The axis scores on PC1 were significantly negative with molar percentages of i15:0, i16:0, i17:0 and 17:0, but were significantly positive with molar percentages of 16:1 ω 7c, 18:1 ω 9t, 16:1 ω 5c, 18:1 ω 9c and 10Me17:0. The axis scores on PC2 were significantly negative with molar percentages of cy19:0 and 18:0, but were significantly positive with molar percentages of a15:0, a17:0, 18:2 ω 6,9c and 16:0 (Fig. 4).

The changes of the above PLFAs led to changes in the ratios of saturated to monounsaturated fatty acid, Gram+ to

Fig. 1 PLFA abundances in the soils of native *P. massoniana* plantation (MP), 1st, 2nd, 3rd, and 4th generation *Eucalyptus* plantations (G1–G4).

Fig. 2 Principle components (PC) analysis of soil microbial PLFA composition from native *P. massoniana* plantation (MP), 1st, 2nd, 3rd, and 4th generation *Eucalyptus* plantations (G1–G4).

Gram- bacteria, and cyclopropyl fatty acid to precursors. The ratios of normal saturated to monounsaturated fatty acids (SAT/MONO), Gram+ to Gram- bacteria (GP/GN),

and cy17:0 to its precursor, 16:1 ω 7c, significantly increased after plantation conversion from native MP to *Eucalyptus* plantations ($P < 0.05$), and they showed significant temporal variations with the *Eucalyptus* stand age (**Fig. 3**). SAT/MONO and GP/GN reached maximum at G2 and subsequently slowly decreased with stand age, and were unimodally related to *Eucalyptus* stand age. The ratio of cy17:0 to 16:1 ω 7c was an exponential function of *Eucalyptus* stand age.

2.3 Soil enzyme activity

Plantation conversion from native MP to exotic *Eucalyptus* plantation significantly decreased soil enzyme activities ($P < 0.05$), and the activities of phenol oxidase, peroxidase, cellobiohydrolase and acid soil acid phosphatase showed significant temporal variations with the *Eucalyptus* stand age (**Fig. 4**). The activities of phenoloxidase ($P < 0.0001$), peroxidase ($P < 0.0001$) and soil acid phosphatase ($P < 0.0001$) increased gradually with the stand age of the *Eucalyptus* plantations, while the activities of cellobiohydrolase were “U” shaped quadratic functions ($P < 0.01$), and the minimum value appeared in the G2 stand. There were no significant correlations between the activities of β -glucosidase, soil protease, soil urease and the *Eucalyptus* stand age.

Fig. 3 SAT/MONO ratio of normal saturated to monounsaturated fatty acids and GP/GN ratio of Gram+ to Gram- bacteria.

Table 2 Proportion of microbial community variation explained by significant soil and plant variables based on redundancy analysis in *Eucalyptus* plantations

	PLFA	Enzyme activity
Soil		
Soil organic carbon	10.1%**	
Alkaline hydrolytic nitrogen	25.6%**	10.2%**
C:N	3.9%*	
N:P	48.0%**	73.7%**
Plant		
Understory cover	38.6%**	35.7%**

*, ** indicate significant differences at $P < 0.05$ and $P < 0.001$, respectively. Blank spaces indicate lack of significant difference.

2.4 Relationships between microbial community composition, function and environmental factors

Microbial community composition and its functional capacity could be influenced by aboveground vegetation and soil properties. The soil variables that explained the greatest amount of variation in the PLFA composition were N:P (48.0%), alkaline hydrolytic nitrogen (25.6%), and organic carbon (10.1%); for variation in the enzyme activities, the variables were N:P (73.7%) and alkaline hydrolytic nitrogen (10.2%) (Table 2). For the plant variables, understory cover explained 38.6% of the variation in PLFA composition and 35.7% of the variance in the enzyme activities.

3 Discussion

The simultaneous availability of *Eucalyptus* plantation sites of different stand ages and *Pinus massoniana* plantation enabled us to apply the ‘space-for-time substitution’ approach as a surrogate for a long-term study on microbial succession (Pickett 1989; Chauvat et al., 2003). Despite a few methodological shortcomings, this approach is often considered to be the only way of determining long-term changes in forest ecosystems when long-term monitoring datasets of succession are unavailable (Trofymow and Porter, 1998; Chauvat et al., 2003). By using the ‘space-for-time substitution’ approach, we found that successive rotations had significant effects on the size, structure, and enzyme activities of soil microbial communities in *Eucalyptus* plantations after plantation conversion from native *Pinus massoniana*.

Understanding soil microbial community changes could help to broadly predict how biogeochemical cycles respond to plantation conversion. Previous studies (Sicardi et al., 2004; Berthrong et al., 2009; Chen et al., 2013) have shown that *Eucalyptus* plantations have significantly different microbial community compositions, smaller sizes and lower functional activities than the soil microbial communities of native species plantations. However, microbial communities recovered with increased stand age of the *Eucalyptus* plantations (Figs. 1–4). Soil microbial biomass has traditionally been used as an indicator of soil fertility, with decreases indicating a decline in soil quality (Powlson et al., 1987; Wardle, 1992; Zheng et al., 2005). In this study, the PLFA abundances were “U” shaped quadratic functions of the *Eucalyptus* plantation age (Fig. 1), which indicates that soil fertility recovered gradually after a decrease in the early generation *Eucalyptus* plantations.

Successive short-rotations of *Eucalyptus* plantations had a significant effect on the PLFA composition of soil microbial communities (Fig. 2). These changes of PLFA compositions altered the ratios of saturated to monounsaturated fatty acid, Gram+ to Gram- bacteria, and cyclopropyl fatty acid to precursors, which correlate positively with soil nutrient stress or negatively with resource availability (Bossio and Scow, 1998; Fierer et al., 2003; McKinley et al., 2005; Moore-Kucera and Dick, 2008). The ratios of saturated to monounsaturated fatty acid and Gram+ to Gram- bacteria were “∩” shaped quadratic functions of the *Eucalyptus* plantation age, and the ratio of cy17:0 to 16:1ω7c decreased gradually with *Eucalyptus* plantation age (Fig. 3). This suggests that soil resource availability increased gradually after a decrease in the early generation *Eucalyptus* plantations.

Soil enzymes are directly linked to the turnover rates of organic matter and may provide key insights into the preservation or loss of specific compounds. Soil acid phosphatase may activate phosphorus, which is crucial for improving soil phosphorus availability in this area. Phenol oxidase and peroxidase not only reflect soil quality, but also correspond to the allelochemicals produced by *Pinus* (Cao et al., 2006) and *Eucalyptus* (Zhang and Fu, 2009, 2010). Cellobiohydrolase breaks cellulose chains into smaller units, and the enhanced activity of cellobiohydrolase could indicate high litter or root turnover in the soils (Sanaullah et al., 2011). In the study, the activity

Fig. 4 Soil enzyme activities in native *P. massoniana* plantation (MP), 1st, 2nd, 3rd, and 4th generation *Eucalyptus* plantations (G1–G4).

of soil acid phosphatase, phenol oxidase, and peroxidase increased gradually with the stand age of the *Eucalyptus* plantations. The activities of cellobiohydrolase decreased at first generation and reached their minimum at the 2nd rotation, and subsequently recovered during the 3rd and 4th rotations of *Eucalyptus* plantations.

The succession of soil microbial community may result from the following factors.

(1) The effects of disturbance on understory vegetation decreased with increases in plantation age. During conversion of *Pinus* to *Eucalyptus* plantations, clear-cutting, slash-burning, and plowing destroyed vegetation cover. However, after establishment of *Eucalyptus* plantations, only smaller disturbances occurred from plowing and tending. Disturbance effects on understory vegetation decreased with time, which would be beneficial to understory vegetation recovery of *Eucalyptus* plantations (Fig. 5), and thus an improvement in the structure and functional activity of the soil microbial communities (Wu et al., 2011). This may explain why understory plant cover explained the greatest amount of variation in the PLFA composition and enzyme activity (Table 2). Understory species in northern boreal forests have also been shown to drive changes in the soil microbial community (Nilsson and Wardle, 2005).

(2) Improper land management and significant carbon import from *Eucalyptus*. After the conversion of native *P. massoniana* plantation to *Eucalyptus* plantations, artificial tending caused deterioration of the soil structure by breaking up soil macro-aggregates and exposing pre-

Fig. 5 Understory cover percentage in native *P. massoniana* plantation (MP), 1st, 2nd, 3rd, and 4th generation *Eucalyptus* plantations (G1–G4).

viously protected organic matter in the macro-aggregates (Zheng et al., 2005). This may be the main reason for the observed decreases in soil organic matter in 1st and 2nd generation. However, the higher growth rate and water use efficiency of *Eucalyptus* (Hou, 2006) suggest that *Eucalyptus* stands fix more plant carbon. This fixed carbon will be transported to the soil through litter fall and root exudates, which will increase soil organic matter with increased time after planting (Lima et al., 2006; Ma et al., 2010). In our study, soil organic carbon formed quadratic functions with *Eucalyptus* plantation age (Table 1), and partially explained the variation in the PLFA composition and enzyme activity of the soil microbial communities

(Table 2). The increased organic matter could provide more carbon sources for the soil microbial community and lead to eventual recovery of the structure and activities of the soil microbial community.

(3) Fertilization compensates for the losses of N and P caused by *Eucalyptus* uptake. The high nutrient demands of *Eucalyptus* lead to higher losses of nutrients in *Eucalyptus* plantations compared to native *Pinus* (Lacrau et al., 2003), thereby causing decreases in soil resource availability, as indicated by the lower soil N:P, and alkali-hydrolysable N (Table 1). However, fertilization was used in the first two years after *Eucalyptus* planting, which could in part lead to increases in soil N:P, and alkali-hydrolysable N with successive rotations of *Eucalyptus* plantations. This may explain why soil N:P and alkali-hydrolysable N explained the greatest amount of variation in the PLFA composition and enzyme activity.

4 Conclusions

The *Eucalyptus* plantations compared to the native *P. massoniana* plantation had significantly different microbial community compositions, and had smaller sizes and reduced functional activities of soil microbial communities. Successive short-rotations of *Eucalyptus* plantations after plantation conversion from native *Pinus massoniana* had significant impacts on the soil microbial community. The size, structure, and function of the soil microbial community recovered with the stand age of *Eucalyptus* plantations. The responses of soil microbial communities are likely regulated by the availability of soil resources and the effects of successive rotations on understory communities. In the long run, understory conservation and reduction of soil disturbance during plantation management could contribute to the recovery of soil microbial structure and function. Our results also highlight the importance of plantation age for assessing the impacts of plantation conversion, as well as the importance of reducing disturbance for plantation management.

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