Microbial community composition under adjacent coniferous and broadleaf plantation forests

S. H. Xing[A,C], C. R. Chen[B,C,E], H. Zhang[D], B. Q. Zhou[A] and Z. M. Nang[D]

[A]College of Resource and Environment, Fujian Agriculture and Forestry University, Fuzhou 350002, China.
[B]Institute of Crop Research, Fujian Academy of Agriculture Sciences, Fuzhou 350003, China.
[C]Environmental Futures Centre, Griffith School of Environment, Griffith University, Nathan 4111, Australia.
[D]Institute of Soil and Fertilizer, Fujian Academy of Agriculture Sciences, Fuzhou 350003, China.
[E]Corresponding author. Email c.chen@griffith.edu.au

Abstract
The PLFA profile technique was used to analyse soil microbial community composition under adjacent coniferous Chinese fir (*Cunninghamia lanceolata* (Lamb) Hook) (12-year old) (designated as 'Fir'), coniferous-broadleaf mixed species of broadleaf Nagai Podocarpus (*Nageia nagi* (thumb.) O. Ktze) and coniferous Pond cypress (*Taxodium ascendens* Brongn) (12-year old) (designated as 'Mixed Species') and broadleaf Phoebe Nanmu (*Phoebe Bournei* (Hemsl.) Yang) (12-year old) (designated as 'Broadleaf Phoebe'). The phospholipid fatty acid (PLFA) profiling analysis showed that amounts of total bacteria in the soils under the Mixed Species were higher than under the Fir and Broadleaf Phoebe, while amounts of fungi in the soils under the Broadleaf Phoebe and Mixed Species were lower than under the Fir. Results from the principal component analysis (PCA) of PLFA data showed that PC1 and PC2 explained 44.5% of the variations in the PLFA. Different forest types could be clearly separated along PCs 1 and 2, which were mainly attributed to 14 major PLFAs. The different organic inputs and availability of soil C substrates may be responsible for the discrimination of the soil microbial communities among different forest types.

Introduction

Microbial community plays a key role in soil carbon (C) and nutrient transformation (Chen and Xu 2008). A number of techniques, such as community level physiological profiling (CLPP), phospholipids fatty acid (PLFA) analysis and PCR-based molecular methods, have been used for detecting shifts in microbial community composition as affected by land-use change, forest species and management practices (e.g. Grayston and Prescott 2005; Ramsey *et al.* 2006). It has been suggested PLFA analysis can better differentiate the treatment effects on microbial community composition than CLPP or PCR-based methods (Ramsey *et al.* 2006). The PLFA profiles and microbial communities in forest soils have been reported to be affected by liming, organic matter removal, soil compaction and moisture stress and tree species (e.g. Frostegård *et al.* 1993; Grayston and Prescott 2005). The objective of this study was to investigate effects of coniferous, broadleaf and mixed forest species on soil microbial community composition as revealed by the PLFA profiling technique.

Methods

Site description and sample collection

The research site was located within the Forest Research Station, Fujian Agriculture and Forest University in Nanping, Fujian Province (26°38′S, 117°57′E) in the subtropical area of southeastern China. Three adjacent plantation forests planted in 1996 were selected for this study. These included: a) coniferous Chinese fir (*Cunninghamia lanceolata* (Lamb) Hook) (12-year old) (designated as 'Fir'); b) coniferous-broadleaf mixed species of broadleaf Nagai Podocarpus (*Nageia nagi* (thumb.) O. Ktze) and coniferous Pond cypress (*Taxodium ascendens* Brongn) (12-year old) (designated as 'Mixed Species'); and c) broadleaf Phoebe Nanmu (*Phoebe Bournei* (Hemsl.) Yang) (12-year old) (designated as 'Broadleaf Phoebe'). The experimental site measured 0.3 ha in area (ca. 0.1 ha for each of forest types) on the slope facing the sun. The soil was a Typic Alliti-Udic Ferrosols (Soil Survey Staff 1999), with the parent material being slope and residual deposits weathering from gneiss. Three replicate sampling plots (10 x 15 m^2) of each of the three plantation forests were established in the positions of upper, middle and lower slopes. Fifteen soil cores were randomly collected from each plot at the 0-20 cm and 20-40 cm, using a 7.5 cm diameter auger and bulked.

Analysis of phospholipid fatty acid in soil

Soil phospholipid fatty acid (PLFA) was extracted and measured using the modified methods described by Frostegard (1993) and Kourtev (2002). The amounts of individual PLFAs were expressed as mole % of total PLFA.

Identification of microbial community in soil

The composition of soil microbial community was identified by microbial analysis software (Sherlock MIS 4.5 System, MIDI, USA) based on the spectrogram of specific PLFA. The amount of bacteria in soils was estimated from the sum of percentages of the following PLFAs: i15:0, a15:0, 15:0 3OH, i16:0, a16:0, 16:1 2OH, 16:1ω5c, 10Me 16:0, 16:1ω7c, i17:0, a17:0, 10Me 17:0, 18:1ω7c, cy19:0ω8c (Frostegård *et al.* 1993; Frostegård and Bååth 1996). The PLFAs i15:0, a15:0, i16:0, i17:0, a17:0, 10Me 16:0, 10Me 17:0 represent Gram positive bacteria, while 16:1ω5c, 16:1ω7c, 18:1ω7c, cy19:0ω8c represent Gram negative bacteria (Frostegård *et al.* 1993; Frostegård and Bååth 1996). The sum of percentages of 18:2ω6,9, 18:1ω9c and 18:3ω6c (6, 9, 12) was considered to represent the percentage of fungi (Frostegård and Bååth 1996; Karliński *et al.* 2007). The percentages of actinomycetes was estimated from the percentage of 10Me 18:0 (Frostegård and Bååth 1996).

Statistical analysis

PLFA profiling data were also conducted in SAS Version 9.1.3 for Windows. Data (mole %) on the PLFA profiles were log-transformed [log (n+1)] and were subject to principal component analysis (PCA) using Statistica Version 6.1 (Statsoft, Inc.).

Results

Across the three plantation forests, the PLFAs 16:0, 18:1ω9c, cy19:0ω8c, 18:2ω6,9c, 18:0, i16:0 and i15:0 were predominant in both 0-20 cm and 20-40 cm layers, accounting for 61.8% to 71.9% of total PLFA. In particular, the PLFAs 16:0 and 18:1ω9c were the most abundant in all soils under the three plantation forests (Table 1). The PLFA profiles in the 0-20 cm and 20-40 cm layers varied greatly across the three plantation forests. Over 25 types of PLFA were detectable in the 0-20 cm soil under the Mixed Species, while only 20 types of PLFA were found in the corresponding depth under both Fir and Broadleaf Phoebe (Table 1). In the 20-40 layers, the number of soil PLFA in three plantation forests followed the order: Mixed Species (20)＞ Fir (18)＞Broadleaf Phoebe (16) (Table 1).

Table 1. PLFA profiles from soils under adjacent coniferous Chinese fir (Fir), coniferous-broadleaf mixed species of broadleaf Nagai Podocarpus and coniferous Pond cypress (Mixed Species), and broadleaf Phoebe Nanmu (Broadleaf Phoebe) plantation forests in subtropical China.

PLFA	0-20 cm (Mole %)			20-40 cm (Mole %)		
	Fir	Mixed species	Broadleaf Phoebe	Fir	Mixed species	Broadleaf Phoebe
12:0	0.3a	0.3a	0.0a	0.0a	0.0a	0.0a
14:0	1.7a	1.8a	1.2a	1.8a	1.3a	1.2a
i15:0	5.0a	4.9a	5.3a	4.7a	5.0a	5.7a
a15:0	3.2a	2.7a	3.0a	3.0b	3.4b	4.8a
15:0 3OH	0.0b	2.0a	0.0b	0.0b	2.1a	0.0b
16:0	19.3b	17.5b	22.9a	21.9a	18.3a	20.0a
i16:0	5.0a	4.6a	5.1a	4.3a	5.4a	4.8a
a16:0	0.0c	0.6b	1.1a	2.2a	0.0b	0.0b
16:1 2OH	0.0c	3.4a	2.7b	0.0b	3.9a	4.6a
16:1ω5c	2.3b	2.6b	3.5a	3.1a	3.8a	3.6a
10Me 16:0	3.7a	3.9a	2.9b	2.9a	3.5a	3.2a
16:1ω7c	1.6a	0.7a	1.4a	0.0a	0.6a	0.0a
i17:0	3.8a	3.3ab	2.7b	3.7a	4.0a	4.0a
a17:0	2.6a	2.3a	2.2a	2.5a	2.1a	3.3a
10Me 17:0	0.0b	0.8a	0.0b	0.0a	0.0a	0.0a
18:0	5.1a	4.2a	4.4a	5.7a	4.9a	6.1a
18:0 2OH	0.0a	0.0a	0.0a	1.9a	0.0a	2.6a
10Me 18:0	3.0a	3.2a	3.2a	0.0a	0.0a	0.0a
18:1ω7c	3.2a	3.4a	1.9b	3.1b	4.2a	0.0c
18:1ω9c	23.3a	17.8b	18.7b	24.7a	20.7b	22.2ab
18:2ω6,9c	5.0a	4.0a	5.0a	4.8a	4.9a	5.4a
18:3ω6c (6,9,12)	2.4a	2.4a	1.2b	3.6a	0.5b	0.8b
19:1ω6c	0.5b	2.3ab	3.4a	0.0a	1.1a	0.0a
cy19:0 ω 8c	7.9a	8.8a	8.2a	5.4b	9.4a	7.7ab
20:0	1.1ab	2.5a	0.0b	0.7a	0.9a	0.0a

Data in the row are mean values（n=3）, which are compared among forest types within each depth and are not different at the 5% level of significance if followed by the same letter.

This indicated that the soils under the mixed species of forest plantations contained more diverse PLFAs than those under mono-species forest plantations. In the 0-20 cm layer, amounts of PLFAs 16:0, a16:0, 16:1ω5c and 19:1ω6c under the Broadleaf Phoebe were significantly higher than those under the Fir and Mixed Species, while 15:0 3OH, 16:1 2OH, 10Me17:0 and 20:0 under the Mixed Species were remarkably higher than those under the Fir and Broadleaf Phoebe (Table 1). Amounts of the PLFAs 10Me16:0, i17:0, 18:1ω7c and 18:3ω6c (6,9,12) in the 0-20 cm soils were significantly higher under the Fir than those under the Broadleaf Phoebe, while those under the Mixed Species were intermediate. The PLFA 18:1ω9c in the 0-20 cm soils under the Fir was significantly higher than that under both Mixed Species and Broadleaf Phoebe (Table 1). In the 20-40 cm layer, the amounts of PLFAs a16:0, 18:1ω9c and 18:3ω6c (6,9,12) under the Fir were significantly higher than those under the Mixed Species and Broadleaf Phoebe, while amounts of 15:0 3OH, 18:1ω7c and cy19:0 ω 8c under the Mixed Species were higher than those under the Fir and Broadleaf Phoebe (Table 1). The amount of 16:1 2OH was significantly higher at both depths under the Mixed Species and Broadleaf Phoebe than those under the Fir (Table 1).

Results from the PCA have clearly showed the separation among the forest types and the soil depths along both PC1 and PC2 (Figure 1a). Three forest types, Fir, Mixed Species and Broadleaf Phoebe, had distinct PLFA compositions from each other. In addition, distinct PLFA patterns were also found between the 0-20 cm and 20-40 cm soils (Table 1, Figure 1a). The sum of PC1 and PC2 accounted for 44.5% of the variation in the PLFA composition. From the loading values of individual PLFA (Figure 1b), it is clear that the PLFAs 18:1ω9c, 18:1ω7c, 19:1ω6c, 18:00, a15:0, 10Me17:0, 10Me18:0 have made most important contributions to the separation of the 0-20 cm soils of different forest types (total contributions to the PC1: 55.8%), while 10Me16:0, cy19:0ω8c, i16:0, 16:1 2OH, a16:0 and 18:3ω6c (6, 9, 12) were most important in separating the 20-40 cm soils of different forest types (total contributions to the PC2: 65.5%). In addition, one of most abundant PLFAs, 16:0, contributed to 5% of variation in PC2, but 13% to the PC3 (data not shown).

Bacteria were the major microbial group in both 0-20 cm and 20-40 cm layers in the three plantation forests, accounting from 34% to 44% of total microbial community, followed by fungi with comprising 24%-34 %, and actinomycetes with up to 3.2%. Actinomycetes were only detected in the 0-20 cm layer (Table 2).

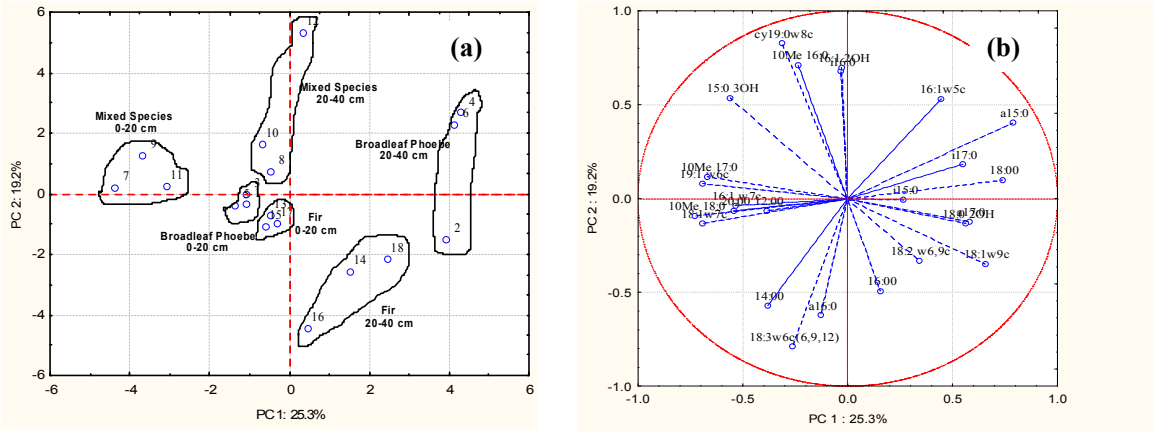

Figure 1. (a) Scores plot of PCA showing the separation of different forest types and soil depths along principal components (PC) 1 and 2; and (b) Loading values of the individual PLFA for PC1 and PC2.

Table 2. Mole percentages (%) of soil microbes under adjacent coniferous Chinese fir (Fir), coniferous-broadleaf mixed species of broadleaf Nagai Podocarpus and coniferous Pond cypress (Mixed species), and broadleaf Phoebe Nanmu (Broadleaf Phoebe) plantation forests in subtropical China.

Microbial community	0-20 cm			20-40 cm		
	Fir	Mixed species	Broadleaf Phoebe	Fir	Mixed species	Broadleaf Phoebe
Bacteria	38.3b	43.9a	40.0b	34.9b	46.3a	41.7ab
Gram positive bacteria	23.3a	22.5a	21.2a	21.1b	22.3b	25.8a
Gram negative bacteria	15.0a	15.4a	15.0a	11.6b	17.9a	11.3b
Fungus	30.7a	24.3b	24.9b	33.7a	26.2b	28.5b
Actinomycetes	3.0a	3.2a	3.2a	0.0a	0.0a	0.0a
Fungal-to-bacterial ratio	0.80a	0.57b	0.63b	0.97a	0.60b	0.67b

Data in the row are mean values（n=3）, which are compared among forest types within each depth and are not different at the 5% level of significance if followed by the same letter.

Amounts of total bacteria were higher in the soils at both depths under the Mixed Species than under the Fir and Broadleaf Phoebe, while there were no significant differences in the amount of bacteria under the Fir and Broadleaf Phoebe (Table 2). There were no significant differences in Gram positive and Gram negative bacteria in the soils under three plantation forests. On the other hand, amounts of fungi were higher in the soils under the Fir than under the Broadleaf Phoebe and Mixed Specie. The fungal-to-bacterial ratio was generally greater in the soils under the Fir than under the Broadleaf Phoebe and Mixed Species.

Conclusion

Different plantation forests had significant impacts on soil microbial community composition as revealed by the PLFA profiling. Amounts of total bacteria in the soils under the Mixed Species were higher than under the Fir and Broadleaf Phoebe, while amounts of fungi in the soils under the Broadleaf Phoebe and Mixed Species were lower than under the Fir. The PCA results showed that PC1 and PC2 explained 44.5% of variations in the PLFAs, and different forest types could be clearly separated along PCs 1 and 2, which were mainly attributed to 14 major PLFAs. The different organic inputs and availability of soil C substrates may be responsible for the discrimination of the soil microbial communities among different forest types.

References

Chen CR, Xu ZH (2008) Analysis and behavior of soluble organic nitrogen in forest soils: a review. *Journal of Soils and Sediments* **8**, 363-378.

Frostegärd A, Bääth E (1996) The use of phosphoslipid fatty acid analysis to estimate bacterial and fungal biomass in soil. *Biology and Fertility of Soils* **22**, 59-65.

Frostegärd A, Bääth E, Tunlid A (1993) Shifts in the structure of soil microbial community in limed forests as revealed by phospholipid fatty acid analysis. *Soil Biology and Biochemistry* **25**, 723-730.

Grayston S, Prescott CE (2005) Microbial communities in forest floors under four tree species in coastal British Columbia. *Soil Biology and Biochemistry* **37**, 1157-1167.

Karlinski L, Ravnskov S, Kieliszewska-Rokicka B, Larsen J (2007) Fatty acid composition of various ectomycorrhizal fungi and ectomycorrhizas of Norway spruce. *Soil Biology and Biochemistry* **39**, 854-866.

Kourtev PS, Ehrenfeld JG, Haggelom M (2002) Exotic plant species alter the Microbial community structure and function in the soil. *Ecology* **83**, 3152-3166.

Ramsey PW, Rillig MC, Feris KP, Holben WE, Gannon JE (2006) Choice of methods for soil microbial community analysis: PLFA maximizes power compared to CLPP and PCE-based approaches. *Pedobiologia* **50**, 275-280.