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Short communication

Variations in the soil microbial community composition of a tropical montane forest ecosystem: Does tree species matter?

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ABSTRACT

We investigated tree species effects on the soil microbial community in the tropical montane forest on Mt. Kinabalu, in Malaysian Borneo. We investigated microbial composition (lipid profile) and soil physicochemical parameters (pH, moisture, total C, N and phenolics concentration) in top 5-cm soils underneath two conifers (*Dacrycarpus imbricatus* and *Dacrydium gracilis*) and three broad-leaves (*Lithocarpus clementianus*, *Palaquium rioence* and *Tristaniopsis clementis*). We found that the primary difference in microbial composition was between conifer versus broad-leaves. The abundance of specific microbial biomarker lipids correlated with soil pH, total C and N. We conclude that tree species have significant impacts on the soil microbial community through their effects on soil pH, total C and N.

Soil microbial communities play a central role in terrestrial ecosystem nutrient cycling. For example, soil community composition and activity drive decomposition, and nitrogen cycling. Growing evidence suggests that microbial composition and function can fundamentally alter microbial processes independent of environmental drivers such as water or temperature, and thus researchers have become increasingly interested in the factors which determine microbial composition and function (Zogg et al., 1997; Balser and Firestone, 2005). Previous studies indicate that microbial composition is influenced by factors such as pH (Fierer and Jackson, 2006; Högberg et al., 2007), phenolic compounds (Kuiters, 1990; Hättenschwiler and Vitousek, 2000), or nutrient and carbon availability (Fierer et al., 2007; Högberg et al., 2007). Furthermore, several studies report that the composition of the soil microbial community can be altered by plant species, plant diversity, vegetation or forest type (Waldrop et al., 2000; Porazinska et al., 2003; Balser and Firestone, 2005; Bartelt-Ryser et al., 2005). Through their effects not only on litter chemistry but also on the microbial community, plant species may thus be an important mediator of microbial process (Kao-Kniffin and Balser, 2007; Liang et al., 2007).

Accordingly, the objective of this study was to investigate tree species effects on the composition of the soil microbial community. The work was conducted in a tropical montane forest on the south slope of Mt. Kinabalu in Sabah, Malaysia (6°05'N, 116°33'E).

The research site was at 1560 m asl., with mean annual air temperature of 18 °C and mean annual rainfall of 3080 mm (Aiba and Kitayama, 1999). Tree cover is evergreen broad-leaved trees interspersed with conifers (relative basal area of conifer species is about 15%), and the number of tree species is 109 per hectare (Aiba and Kitayama, 1999). The soil, derived from sedimentary rocks folded in the tertiary period, is late in pedogenesis and its low phosphorus content is thought to limit plant growth (Kitayama et al., 2004). Soil properties (pH, %C and N, phenolics) are given in Table 1 (Kitayama and Aiba, 2002; Kitayama et al., 2004). We selected replicate trees from five tree species which are dominant in the plot: two conifer species, *Dacrycarpus imbricatus* (Podocarpaceae) ($n = 4$) and *Dacrydium gracilis* (Podocarpaceae) ($n = 6$); and three broad-leaved species, *Lithocarpus clementianus* (Fagaceae) ($n = 5$), *Palaquium rioence* (Sapotaceae) ($n = 4$) and *Tristaniopsis clementis* (Myrtaceae) ($n = 5$). Leaf chemistry differs among these tree species, especially in phenolics concentration (S. Suzuki, unpublished data). We collected the top 5-cm soil of organic layer with a 3.7-cm diameter core. Soil cores were taken at four locations approximately 1.5 m from the tree trunk and subsequently composited for analysis. Samples were taken immediately to the laboratory. Roots were removed by hand and samples stored in a refrigerator for up to one week during analysis of moisture, pH in water and 0.01 N KCl (soil: water = 1:5), total C and N content and the concentration of water-soluble phenolics (Table 1). Total C and N

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Table 1
Soil moisture, pH (measured with H_2O and 0.01 N KCl), percentages of C and N, C/N ratio and phenolics for soil sampled beneath each tree species

	Dacrycarpus	*Dacrydium*	*Lithocarpus*	*Palaquium*	*Tristaniopsis*	*P* value
Soil moisture (%)	204 (37)	288 (24)	196 (31)	253 (29)	248 (53)	0.249
pH (H_2O)	3.84 (0.16)	3.83 (0.11)	4.08 (0.08)	4.02 (0.10)	4.13 (0.06)	0.153
pH (KCl)	3.21 (0.16)	3.00 (0.03)	3.20 (0.08)	3.28 (0.08)	3.27 (0.08)	0.121
Total C (%)	35.0 (5.7)	45.6 (0.3)	35.8 (5.5)	37.9 (5.3)	37.0 (3.6)	0.347
Total N (%)	1.58 (0.20)	1.81 (0.05)	1.52 (0.22)	1.72 (0.20)	1.53 (0.13)	0.617
C/N ratio	21.8^{b} (1.11)	25.4^{a} (0.76)	23.4^{ab} (0.65)	21.8^{b} (0.65)	24.1^{ab} (0.81)	0.020
Water-soluble phenolics ($\mu g\,g^{-1}$)	429 (92)	490 (114)	682 (129)	447 (75)	441 (81)	0.434

Values are means of replicate sample parentheses indicate SEM. Different letters indicate significant difference by Tukey–Kramer's HSD (for equal variance data) or Games-Howell (for unequal variance data) ($P < 0.05$). *P* values are calculated by ANOVA. *Dacrycarpus* and *Dacrydium* are conifer, and *Lithocarpus*, *Palaquium* and *Tristaniopsis* are broad-leaved speceis Soil moisture (%) = 100(wet weight − dry weight)/dry weight The primary soil characteristics of top 15 cm were as followings: soil pH (H_2O) = 3.95, Organic-C = 4.35%, Total *N* = 0.31% and C/N ratio = 13.65 (see more detail in Kitayama and Aiba (2002) and Kitayama et al. (2004)).

contents were measured on a macro corder JM 1000CN (J-SCIENCE LAB Co, Ltd, Japan.). The concentration of water-soluble phenolics was determined with Folin-Ciocalteau methods (Waterman and Mole, 1994).

Immediately after soil sampling and root removal, a soil subsample was lyophilized for lipid analysis. These samples were shipped to the Balser laboratory in Madison Wisconsin, USA. We used microbial lipid analysis (extraction of signature lipid biomarkers from the cell membrane and wall of microorganisms (White and Ringelberg, 1998)) to assess microbial community composition. We extracted, purified and identified PLFAs from microbial cell membranes in freeze-dried soil samples using a hybrid lipid extraction based on a modified Bligh and Dyer (1959) technique (see Kao-Kniffin and Balser, 2007). Fatty acids were quantified by comparison of peak areas from the sample compared with peak areas of two internal standards, 9:0 (nonanoic methyl ester) and 19:0 (nonadeconoioc methyl ester), of known concentration.

We found that total lipid abundance (a proxy for microbial biomass) was higher in the soils underneath *Dacrydium* than the soils underneath *Tristaniopsis* ($P < 0.05$). The abundance of specific indicator lipids also differed among some combinations of tree species (Table 2). The abundances of 15:0, *i*16:0, 17:0 (generally thought to indicate Gram+ bacteria), 18:2ω6,9 (a saprophytic fungal biomarker), 19:0 10Me (thought to indicate actinomyces), 16:1ω5 (indicating arbuscular mycorrhizal fungi (AMF)) and 18:1ω9 (indicating saprophytic fungi, and possibly a biomarker for ectomycorrhizae) were higher in the soils underneath *Dacrydium* than in the soils underneath *Tristaniopsis* ($P < 0.05$). The ratio of fungi to bacteria differed significantly between *Dacrycarpus* and *Lithocarpus*, *Dacrydium* and *Lithocarpus*, *Dacrydium* and *Tristaniopsis* ($P < 0.05$). The ratio of Gram+ to Gram− was significantly higher in the soils underneath *Dacrydium* than in the soils underneath *Tristaniopsis* ($P < 0.05$). We used principal components analysis to determine differences among microbial community fingerprints. The principal components analysis also indicated tree

Table 2
Mean abundance of indicator lipids from soil sampled beneath each tree species

Lipid abundance ($nmol\,g^{-1}$)	*Dacrycarpus*	*Dacrydium*	*Lithocarpus*	*Palaquium*	*Tristaniopsis*	*P* value
Total lipid	1617^{ab}	2309^{a}	1799^{ab}	1566^{ab}	1118^{b}	0.049
Iso-branched lipids (Gram + bacteria)						
*i*14:0	7.2	13.0	8.7	8.6	2.5	0.482
15:0	14.4^{ab}	23.9^{a}	19.6^{ab}	18.5^{ab}	11.8^{b}	0.048
*i*15:0	73.0	91.9	83.4	79.0	68.1	0.431
*a*15:0	24.2	30.4	30.7	26.8	20.9	0.334
*i*16:0	33.1^{ab}	43.0^{a}	47.6^{ab}	36.4^{ab}	27.3^{b}	0.166
17:0	3.5^{ab}	8.7^{a}	4.2^{ab}	2.1^{ab}	0.0^{b}	0.018
*i*17:0	12.2	13.5	13.9	10.6	8.3	0.405
*a*17:0	2.5	6.9	4.9	2.6	0.0	0.260
Methyl branched lipids (Actinomycetes)						
17:0 10Me	17.7^{ab}	27.2^{a}	26.6^{a}	22.0^{ab}	6.6^{b}	0.007
19:0 10Me	6.4^{ab}	32.4^{a}	26.4^{ab}	20.7^{ab}	0.9^{b}	0.023
Mono-unsaturated and cyclopropyl lipids (Gram−)						
16:1ω7	50.0	73.4	73.2	59.5	60.4	0.399
*cy*17:0	0.0	5.6	2.9	0.0	0.0	0.404
*cy*19:0	40.1	50.3	52.4	54.5	37.2	0.333
Saprophytic fungi						
18:2ω6,9	111.9^{ab}	156.5^{a}	99.5^{ab}	93.9^{ab}	76.6^{b}	0.008
Ectomycorrhisae/Saprophytic fungi						
18:1ω9	140.7^{ab}	180.9^{a}	143.8^{ab}	119.1^{ab}	101.0^{b}	0.022
Arbuscular Mycorrhizae						
16:1ω5	69.7^{ab}	97.2^{a}	69.9^{ab}	63.8^{ab}	47.5^{b}	0.028
Protozoa						
18:3ω6,9,12	107.2	52.8	42.9	46.6	27.3	0.005
Fungi/bacteria ratio	0.991^{ab}	0.933^{b}	0.735^{c}	0.749^{abc}	0.751^{ac}	<0.001
Gm+/Gm− ratio	2.111^{ab}	2.276^{a}	2.055^{ab}	1.957^{ab}	1.532^{b}	0.035

Values within a row having the same lowercase letter are not significantly different by Tukey–Kramer's HSD (for equal variance data) or Games-Howell test (for unequal variance data) ($P < 0.05$). *P* values are calculated by ANOVA. *Dacrycarpus* and *Dacrydium* are conifer, and *Lithocarpus*, *Palaquium* and *Tristaniopsis* are broad-leaved speceis. Fungi/bacteria ratio is the sum of abundance of fungal lipids (18:2ω6,9, 18:1ω9) to the sum of all bacterial lipids shown here. The Gm+/Gm− ratio is the ratio of the sum of iso and methyl branched lipids to the sum of mono-unsaturated and cyclopropyl lipids.