Factors Controlling the Composition of Soil Microbial Communities in Tropical Forest Ecosystems

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Introduction

Soil microbial community plays a fundamental role in ecosystem nutrient cycling through the mineralization of detritus organic matter (Chapin III *et al.*, 2002). In ecosystem models, the microbial mineralization process has been described as a function of environmental factors, and microbial community is regarded as a "black box". The role of different taxonomic or functional groups of microbes in ecosystem is therefore virtually unknown. Soil microbes appear to play particularly vital role in the tropics due to its unique geochemical condition. Tropical ecosystems are often maintained on highly weathered soils due to warm, humid climate regime and stability of land surface. Such tropical soils are dominated by reactive, small mineral particles down to tens of nano-meter in size (Birkeland, 1999). These minerals strongly influence biological processes at least by two geochemical reactions: (1) physical/chemical blocking of extracellular-enzyme activity and thus reduction in decomposition (nutrient mineralization) in consequence, and (2) sorption of phosphate, an essential nutrient for biota, and subsequent phosphorus limitation to plants and presumably to soil microorganisms. Observed high productivity and biomass of tropical forests, however, suggest that soil microbes adapted to these tropical soils efficiently mineralize nutrients by overcoming the geochemical constraints. We therefore tested the hypothesis that microbial community composition is controlled by soil mineral factors (e.g., geochemical condition and phosphorus limitation) as well as the availability of substrate (organic resource) using a series of tropical forest soils in Mt. Kinabalu. Based on the identified environmental factors that control microbial community in Mt. Kinabalu, we also speculated on the possible impact of logging on soil microbial community composition.

Methods

We studied soils on the eastern and southern slopes of Mt. Kinabalu (4095 m, $6^{o}05$'N, $160^{o}33$' E), developed on both acidic sedimentary and ultrabasic igneous parent materials, under the primary rain forests protected as the Kinabalu Park, Sabah, Malaysia. The six selected sites (at ca. 700, 1700, and 2700 m above sea level on the two rock types) are part of a long-term ecological study (Kitayama and Aiba, 2002). Briefly, the climate is humid tropical with weak influences of the Asiatic monsoon. A strong temperature gradient is present along the slope: mean annual air temperature (MAT) decreases with altitude at a mean lapse rate of 0.0055 $^{o}C\ m^{-1}$, with <2 ^{o}C intra-annual variations. Mean annual rainfall is relatively constant (2300-2400 mm yr^{-1}) with elevation. Air and soil moisture generally increase with elevation due to more frequent cloud cover and less evapotranspiration at upper elevations.

We used phospholipid fatty acid (PLFA) biomarkers to assess shifts in microbial community composition. PLFA is a microbial cell membrane constituent and its chemical composition is unique for different broad taxonomic groups. At each site, soil samples were collected from O-horizon, 0-5, and 5-15

cm from three transects. Four to six cores were taken along each transect to make a composite. Soil profiles down to 1 meter depth (or to BC horizon) were described and samples are taken along the profiles at each site. All samples were brought back in cooler box and frozen within 6 hours after the sampling, followed by freeze-drying. The dried samples were sieved (2 mm), ground, and extracted for PLFA following Balser *et al*. (2005). Dried sample masses of 0.3-4.0 g were used for the extraction depending on the total organic carbon content of the sample. Fatty acids were extracted with 10:5:4 volumes of methanol, chloroform, and 0.1 M phosphate buffer (pH 7.0). Following purification, polar-lipid fraction (phospholipids) were isolated by silicic acid columns and subjected to saponification and methylation. Gas chromatograph was used to identify and quantify individual fatty acid methyl esters.

Results and Discussion

General trends

Total microbial PLFA concentration, a sensitive indicator of active microbial biomass, ranged 2.0-2.7 nmol g^{-1} in organic horizon (O-horizon) and 0.2-0.8 nmol g^{-1} in the surface mineral horizon (Fig. 1). These ranges are comparable to those in temperate forest and grassland soils. Corresponding to the variations in the concentration of soil organic matter (i.e. microbial substrate), the PLFA concentration of each broad taxonomic group as well as that of total microbial community decreased in the order: O-horizon > 0-5 cm > 5-15 cm. At 700 m and 2700 m sites where deeper soil samples were analyzed, bacteria showed clearer decline with increasing depth than fungi.

Elevation gradient

We considered two geochemical gradients (elevation and soil depth) to test above hypothesis as the abundance and chemistry of soil minerals predictably change along these gradients. Along the elevation gradient from 2700 m to 700 m, the ratio of gram-positive bacteria to gram-negative bacteria (G+:G- ratio) progressively increased in 0-5 cm mineral soils (Fig. 2) as well as O horizon (data not shown) in the sedimentary soils, while the ratio changed little in the ultrabasic soils. Along the same gradient, the ratio of fungi plus actinomycetes to bacteria consistently decreased at all three depths on both rock types. Fungi:bacteria ratio showed a similar yet less clear trend.

Relatively small standard deviations in these ratios (Fig. 2b) than those in each taxonomic group (Fig. 2a), together with above trends, suggest a significant shift in microbial community composition along the elevation gradient. Bacteria (esp. G+ ones) appeared to increase their relative abundance over fungi and actinomycetes in the surface soils under warmer climate regime where soil organic resources are lower in concentration and poorer in quality due to the protection by active mineral phases compared to the soils under cooler climate.

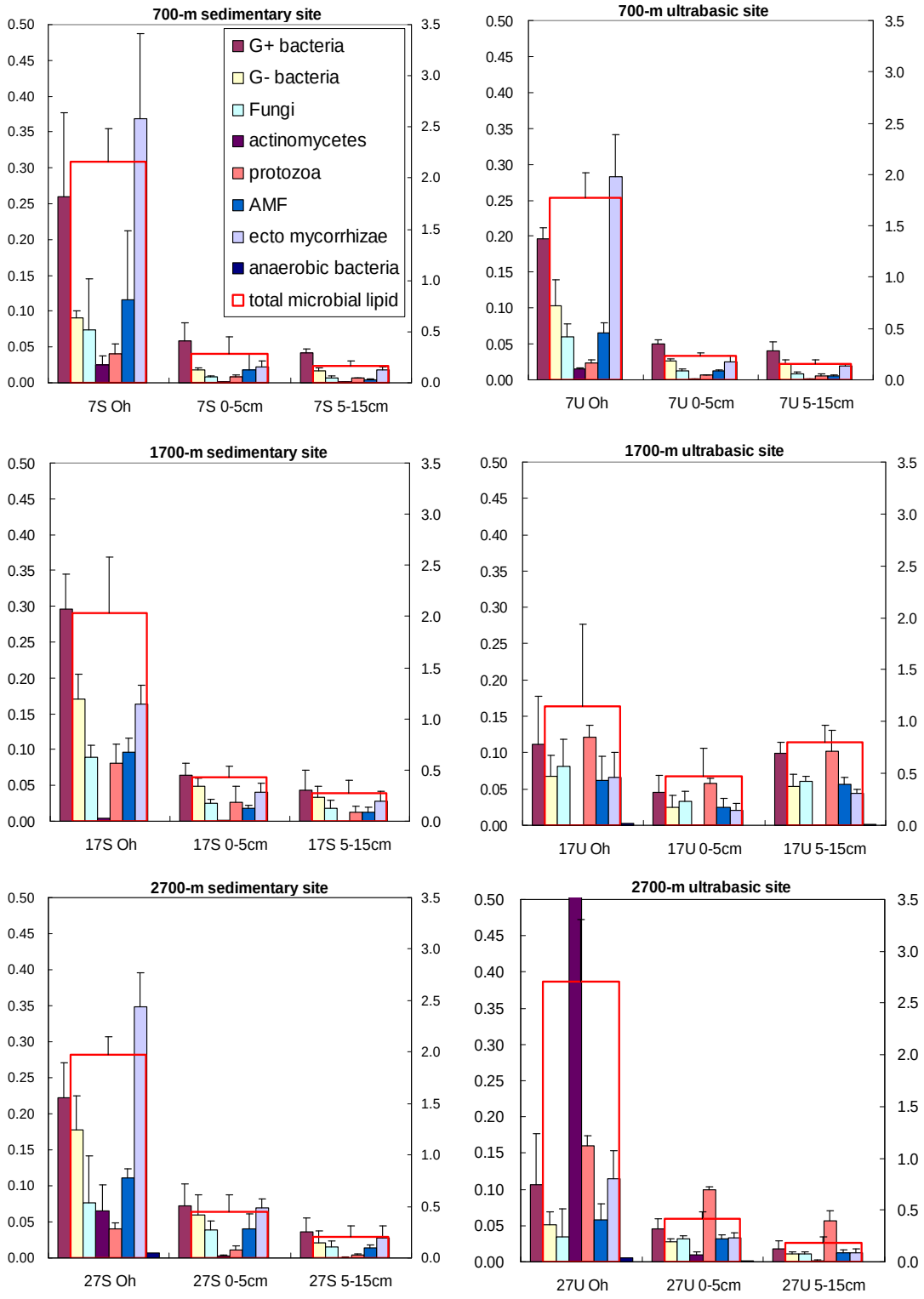

Figure. 1. PLFA concentrations of total active microbial biomass and broad taxonomic groups at each site and depth. N = 3 (error bar = SD). Left axis is PLFA concentrations of individual broad taxonomic group (nmol g^{-1}) and right axis is total microbial PLFA (nmol g^{-1}).

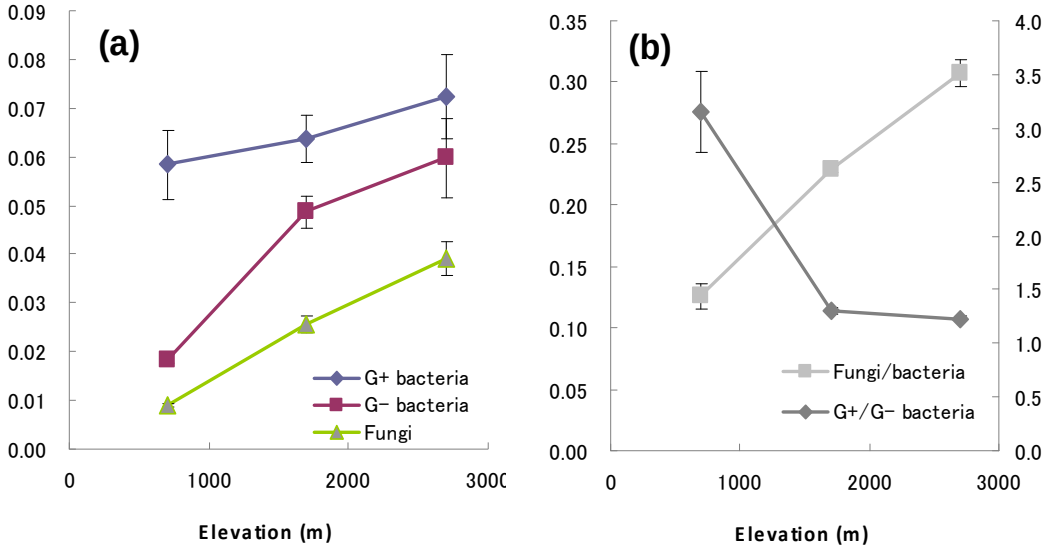

Figure 2. (a) PLFA concentrations of selected broad taxonomic group with elevation (nmol g^{-1}) in sedimentary soils (0-5cm). (b) Ratio of fungi to bacteria (left axis) and gram-positive to gram-negative (right axis) based on (a).

Forest ecosystems at the 700-m elevation are characterized by high rates in plant detritus input (litterfall), rapid decomposition of the detritus in O-horizon, and severe reduction in microbial substrate in mineral horizons due to physical/chemical protection of organic matter by abundant, reactive mineral phases. Our previous work showed that the 700-m soils have the specific surface area of 40-100 m^2 g^{-1} with clay mineralogy of kaolinite and gibbsite at sedimentary site and that of goethite and hematite at ultrabasic site. These mineral phases have significantly greater capacity to adsorb organic substrate and anionic nutrients such as phosphate than upper-elevation soils. Thus microbes in lowland forest soils likely experience rapid changes in physical environment (temperature/moisture) and stochastic pulses of substrate and nutrient input (as litterfall and root death) that are quickly consumed and/or stabilized by soil minerals into unavailable forms. Bacteria appear to be more adapted to these conditions than fungi as the former is capable of rapid growth and dormancy when facing stress (e.g., limitation in substrate and nutrients, draught). Compared to G- bacteria, G+ bacteria hold thicker cellwall, are generally more capable of degrading complex organic substrate and tolerating to stress, and may be more effective in the attachment to soil mineral surfaces. These traits may account for the greater G+ abundance in the 700-m soils.

Depth gradient

Soil samples with depth at each soil pit provide another geochemical gradient in which soil mineralogical characteristics and substrate quality (amounts and chemistry of detritus organic matter) progressively change while macro climate remains constant. With increasing soil depth from surface, the G+:G- ratio among bacteria also increased with depth (Fig. 3a). The ratios for the ultrabasic soils at >60 cm were very high as G+ bacteria was detectable (0.004 nmol g^{-1}) while G- was not detected at all. The fungi+actinomycetes to bacteria ratio showed the lowest values at 10-30 cm and then increased down to 80-100 cm in both sedimentary and ultrabasic soils (Fig. 3b). In a given forest, soils at deeper horizons

contain smaller amounts of substrate that are increasingly more stabilized by soil mineral particles and are sparsely located in soil matrix. Fungi and actinomycetes who can extend filaments/hyphae at great extents may thus be more successful utilizing the substrate at depth. Furthermore, fungi (and, to a less extent, G+ bacteria) are capable of producing wider ranges of enzymes to degrade more recalcitrant substrate, which may also account for their relative abundance at depth.

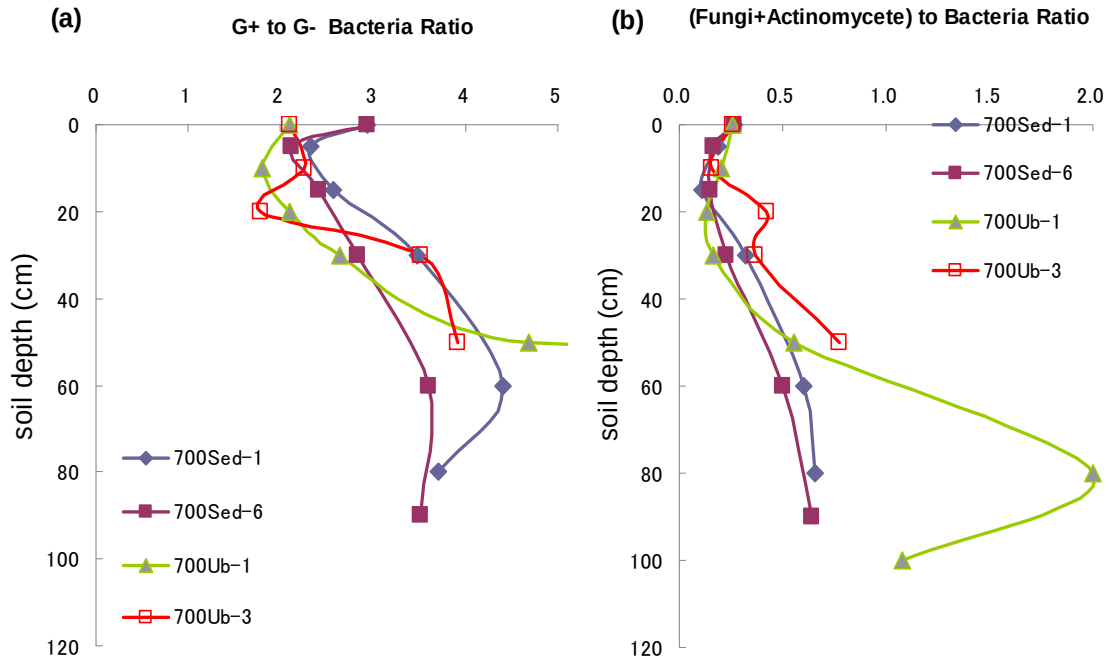

Figure 3. The ratio of gram-positive to gram-negative bacteria (a) and that of fungi plus actinomycete to bacteria (b) along soil profile depth gradients at two 700-m sedimentary soils and two 700-m ultrabasic soils.

Implication for logging impacts

Above results were generally consistent with our hypothesis – the shifts in microbial community composition appeared to be controlled by climate and soil geochemical factors, specifically by temperature, substrate abundance and its interaction with soil mineral particles in the studied ecosystems. Furthermore, we found predictable shifts in microbial community composition corresponding to the changes in climatic and geochemical factors that affect availability of substrate and nutrient. In the environment where organic resources are limited and mineral protection of organic substrate and nutrients is strong, community shifted towards bacterial dominance (particularly G+ ones) over fungi and actinomycetes. Similar shifts in microbial community has been observed along soil depth gradient (down to 2 meter) in Californian grasslands (Fierer *et al.*, 2003).

Then how would soil microbial community respond to the logging of tropical forests? Logging likely causes initial increase and then long-term decline in organic resources due to reduced litter inputs, surface soil erosion, and enhanced decomposition due to disturbance. The prolonged decline in organic resources and enhanced fluctuation in temperature/moisture regime likely result in the community shift towards bacterial dominance. While microbial community hasn't been characterized with respect to logging intensity,

two lines of observations in microbially-driven processes suggest significant shifts in microbial community associated with logging activity.

We compared indicators of soil N and P availability between severely-logged site (Tunkulap) and reduced-logging site (Deramakot). Readily-available ammonium and nitrate in surface soils were roughly two-fold higher at Deramakot than Tunkulap when comparing both on skit trails and under forest canopy (Table 1). Similarly, acid phosphotase activity, microbe- and root-derived enzyme that mineralizes organic phosphorus, was about two-fold greater under reduced-logging sites: 4.04 ± 0.71 in Daramakot and 2.24±0.26 μmol hr^{-1} g^{-1} soil in Tunkulap. These results suggest that severe logging likely resulted in a reduction in nitrogen and phosphorus availability in soil, which was presumably accompanied by shifts in soil microbial community.

Table 1. Comparison of soil total C, N, C:N ratio, and readily-available ammonium and nitrate between skit trail and adjacent forested area. Soil samples (0-10cm deep) were taken from 20-meter long parallel transects along each skit trail (A to E).

Location	TOC	Total N	C:N	KCl–extr NH4+		KCl–extr NO3–	
	%	%		μg/g soil	mgN/gN	μg/g soil	mgN/gN
Deramakot							
Skit trail A	1.27	0.092	13.8	8.1	8.8	5.4	5.8
Forested A	1.99	0.122	16.4	15.0	12.3	5.4	4.4
Skit trail B	1.46	0.108	13.5	12.7	11.8	5.8	5.4
Forested B	2.29	0.161	14.2	9.4	5.8	4.3	2.7
Skit trail C	1.81	0.131	13.9	10.3	7.9	6.0	4.6
Forested C	2.05	0.141	14.5	9.0	6.3	4.7	3.3
Tunkup							
Skit trail D	1.28	0.096	13.3	4.9	5.1	3.4	3.6
Forested D	2.77	0.190	14.6	7.4	3.9	2.9	1.5
Skit trail E	0.98	0.085	11.6	2.5	3.0	-1.6	-1.9
Forested E	2.29	0.162	14.1	4.1	2.5	1.1	0.7

Three samples from each transect were mixed for the chemical analysis.
The inorganic N was extracted by 1.5M potassium chloride solution followed by paper filtration.

To understand logging effects on long-term productivity of tropical forests, it is important to establish direct linkage between microbial community and critical ecosystem processes such as mineralization of N and P. Further investigations on the direct controls on microbial community composition as well as the role of different microbial groups on ecosystem processes would help to substantiate the role of soil microbes in ecosystem functioning and maintenance of biodiversity in tropical forests.

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