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Tree species effects on soil enzyme activities through effects on soil physicochemical and microbial properties in a tropical montane forest on Mt. Kinabalu, Borneo

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ABSTRACT

Tree species influence on the soil mineralization process can regulate overall nutrient cycling in a forest ecosystem, which may occur through their effects on substrate quality, soil physicochemical properties and soil microbial community. We investigated tree species effects on soil enzyme activities in a tropical montane forest on Mt. Kinabalu, Borneo. Specifically, we analyzed C- and P-degrading enzyme activities, as well as the relationships among the enzyme activities, soil physicochemical properties, substrate quality (C, N, and P concentrations), and microbial composition in the top 5 cm soils beneath conifers (*Dacrycarpus imbricatus* and *Dacrydium gracilis*) and broadleaves (*Lithocarpus clementianus*, *Palaquium rioence*, and *Tristaniopsis clementis*). Activities of acid phosphatase and β-D-glucosidase were significantly different among the tree species. Soil moisture, total C and N content and microbial lipid abundance (a proxy for microbial composition) could influence the enzyme activities although the relative contributions of microbial composition to the enzyme activities might be smaller. A higher acid phosphatase activity beneath *Dacrydium* than those beneath the other tree species can compensate for a lower concentration of P in available fractions beneath *Dacrydium*. This localized mineralization activity could subsequently influence soil nutrient availability in this forest.

Introduction

The potential effects of tree species on soil properties have been a focus of study for a long time, and the associated plant–soil interactions provide important feedbacks that regulate ecosystem processes (Binkley and Menyailo, 2005; France et al., 1989; Kulmatiski et al., 2008; Pallant and Riha, 1990; Porazinska et al., 2003; Zinke, 1962). Past research on plant–soil interactions has revealed that plant species can have significant impacts on soil physicochemical properties (e.g., soil water content and pH) and on the quality of substrate for soil microbes (e.g., total carbon (C), nitrogen (N), and phosphorus (P) concentrations, C/N ratio, and the phenolic concentrations in soil) beneath the plant species through their litter quality and quantity (Binkley and Giardina, 1998; Binkley and Valentine, 1991). Soil physicochemical properties and substrate quality have been used to explain differences in decomposition processes (i.e., mineralization of soil organic matter) beneath different plant species.

However, growing evidence suggests that plant species have significant impacts not only on soil physicochemical properties and substrate quality but also more directly on the composition of soil microbial community (Bartelt-Ryser et al., 2005; Porazinska et al., 2003; Ushio et al., 2008). Soil microbial communities play a key role in nutrient cycling, and recent studies suggest that microbial composition and function can fundamentally alter soil decomposition processes (e.g., mineralization of soil organic matter) independent of environmental drivers such as water content or soil temperature (Balser and Firestone, 2005; Zogg et al., 1997). However, relationships among plant species, soil microbial community, and mineralization processes have not been well evaluated yet.

The tropical montane forest on Mt. Kinabalu, in Malaysian Borneo is an ideal site to investigate the effects of plant species on microbial mineralization processes. First, in the tropical montane forest, decomposition of soil organic matter is relatively slow due to low air temperatures compared to those at lowland sites (Kitayama et al., 2000), and thus organic matter specific to a tree species (e.g., in terms of nutrient concentration and plant secondary metabolites) accumulates beneath the tree, and it can influence the soil microbial communities. Second, the slow rate of decomposition results in a slow rate of supply of inorganic nutrients to the soil, and the soil of the montane forest of Kinabalu contains inherently low concentrations of inorganic nutrients, especially P (Kitayama et al., 2004, 2000). Therefore, tree litter containing varying amounts of P can

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critically influence the microbial activities because the low concentration of available P in soils often limits not only plant productivity (Vitousek, 1984) but also microbial activities (e.g., soil respiration rate; Cleveland et al., 2002). Finally, conifers (e.g., *Dacrycarpus imbricatus* (Podocarpaceae) and *Dacrydium gracilis* (Podocarpaceae)) and broadleaf trees (e.g., *Lithocarpus clementianus* (Fagaceae), *Palaquium rioence* (Sapotaceae), and *Tristaniopsis clementis* (Myrtaceae)) locally coexist within this tropical montane forest (Aiba et al., 2002), and their leaf chemistries are quite different, especially the concentration of total phenolics and condensed tannins (Suzuki S., unpublished data). Phenolic compounds have been shown to strongly impact the soil microbial community (Kraus et al., 2003). In accordance to Kraus et al. (2003), Ushio et al. (2008) have found that the composition of soil microbial communities can converge under canopies of the same species within a forest.

In the tropical montane forest, microbial P mineralization is essential for supplying trees with inorganic P, which is thought to limit the net primary productivity (Kitayama et al., 2004). Microbial C mineralization is also important as a regulator of organic matter decomposition rate (Shi et al., 2006). Since the soil microbial community drives mineralization and decomposition by secreting extracellular enzymes, soil enzyme activity can be a measure of microbial mineralization processes (Allison et al., 2007). Therefore, the activities of P-degrading enzymes (e.g., acid phosphatase) and C-degrading enzymes (e.g., β-D-glucosidase, phenol oxidase) are important measures of microbial mineralization processes in a forest. Since C- and P-degradation processes are essential for plant nutrient acquisition in this forest, the spatial patchiness of C- and P-mineralization processes resulting from the tree-specific impacts on the mineralization processes is likely to influence overall nutrient cycling in the forest. Studies of macro-scale ecology has often treated a forest floor as relatively homogeneous in terms of substrate quality, microbial community and mineralization process, or if not, studies that simultaneously consider the spatial patchiness of soil physicochemical and microbial properties in a forest are rare except for a few cases (Saetre and Bååth, 2000). Thus, our examination of how tree species influence soil mineralization processes, and how soil physicochemical and microbial properties relate to the influence can give great insights into understanding nutrient cycling in a forest ecosystem.

In this study, we investigated the effects of individual tree species on enzyme activities in decomposing soil organic matter. Furthermore, by examining the soil physicochemical properties (soil moisture and pH), substrate quality (total C and N concentrations, C/N ratio, total phenolics, and P concentration in soil), and soil microbial community composition, we estimated the relative contributions of the variables on soil enzyme activity. We focused on the activity of β-D-glucosidase and phenol oxidase (C-degrading enzymes), and acid phosphatase (a P-degrading enzyme) as measures of microbial mineralization processes. We ask: (1) do enzyme activities and substrate quality differ among soils underneath different tree species?, (2) what are the relationships among soil enzyme activities, soil physicochemical properties, substrate quality, and soil microbial community? Then, we will discuss the ecological consequences of the impacts of tree species on soil mineralization process.

Materials and methods

Research site

This study was conducted within a permanent plot in primary montane forest on the south slope of Mt. Kinabalu in Sabah, Malaysia (summit height, 4095 m; 6°05′N, 116°33′E). The research plot is at 1560 m above sea level, near the Park Headquarters of the Kinabalu Park. The climate is humid and tropical with mean annual air temperature of 18 °C and mean annual precipitation of 2714 mm (Aiba and Kitayama, 1999). This area does not have marked seasonality but does vary slightly in monthly precipitation (Brief data of precipitation can be obtained from a website of the Sabah Forest Department: http://www.forest.sabah.gov.my/caims). The plot is covered with evergreen broadleaf trees interspersed with conifers (the relative basal area of conifers is approximately 15%). There are 109 tree species per hectare with >10 cm diameter at breast height in the plot (Aiba et al., 2002). The decomposition rate of standing litter is low compared to lowland sites because of the relatively low temperature and modest litter supply rate; therefore, standing litter in this plot is thick. The plot is thought to be in the last stage of pedogenesis and the soil contains a low concentration of available P, which is thought to limit plant growth (Kitayama et al., 2004).

Soil sampling

We selected replicate trees from five tree species that are dominant in the plot: two conifer species, *Dacrycarpus imbricatus* ($n=4$) (Podocarpaceae) and *Dacrydium gracilis* ($n=6$) (Podocarpaceae); and three broadleaf species, *Lithocarpus clementianus* ($n=5$) (Fagaceae), *Palaquium rioence* ($n=4$) (Sapotaceae), and *Tristaniopsis clementis* ($n=5$) (Myrtaceae). These tree species differ in leaf phenolic concentration (Suzuki S, unpublished data). The top 5 cm of soil from the organic horizon was collected in August 2005, using a 3.7-cm diameter corer, after removing undecomposed surface litter. We collected top 5 cm soils because litter chemistry of each tree species is most distinct in the surface organic layer, and because most fine roots occur in this surface organic layer. Cores were taken at four locations approximately 1.5 m from the stem to avoid the impacts of stem flow (the impacts of stem flow usually occur within 50 cm from tree stem; Naito, unpublished data), and to detect the effects of tree-specific litter on soil properties (tree-specific litter mostly shed beneath the tree crown, i.e., usually about 3–6 m from tree stem; Ushio et al., unpublished data). The four soil samples were subsequently combined for analysis. Samples were taken immediately to the laboratory, and roots were removed by hand. Samples were refrigerated at 4 °C for up to 1 week, and analyzed for moisture, pH in water and 0.01 N KCl, C/N ratio, and concentration of water-soluble phenolics. Organic C and total N 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).

Enzyme activities

We measured the activities of acid phosphatase, β-D-glucosidase, and phenol oxidase. We followed the modified method of Tabatabai (1969) for acid phosphatase and β-D-glucosidase analysis. Soil samples were kept in a refrigerator at 4 °C up to 1 week before the enzyme assay. Briefly, 7.5 g of wet soil per sample was mixed with 50 ml of 50 mM acetate buffer (pH 5.0), and shaken vigorously for 30 min. Subsequently, 500 μl of soil suspension was added to a 1.5-ml microtube, and 500 μl of 5 mM substrate solution was added to each microtube. Substrates were *p*-nitrophenyl phosphate for acid phosphatase and *p*-nitrophenyl β-D-glucopyranoside for β-D-glucosidase. The mixture was incubated for 2 h at 25 °C. Following incubation, tubes were centrifuged at 10,000 rev min^{-1} for 5 min and a 0.5 ml aliquot of clear supernatant was taken from each tube and transferred to a