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Cedar and bamboo plantations alter structure and diversity of the soil bacterial community from a hardwood forest in subtropical mountain

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ABSTRACT

Afforestation results in changes of vegetation and soil properties, which in turn could affect the soil bacteria that play critical roles in the biogeochemical cycles in forest ecosystems. Therefore, the objective of this study was to elucidate the impacts of afforestation on soil bacterial communities. Using the barcoded pyrosequencing technique, the phylogenetic structure and diversity of the soil bacterial communities in moso bamboo and Japanese cedar plantations were compared with that of an adjacent natural hardwood forest. A total of 43,097 bacterial pyrosequences were obtained from soil samples. The majority of these sequences were classified as either *Acidobacteria* or α-*Proteobacteria*, but the relative abundance of *Acidobacteria* in bamboo soils was much lower than that in the other two communities. The sequences related to the α-*Proteobacteria* were dominated by the orders *Rhizobiales* and *Rhodospirillales* and were more abundant in the bamboo community. Nonmetric multidimensional scaling analysis of the operational taxonomy units (OTUs) and the distribution of some of the most abundant OTUs revealed distinct bacterial community structures among the three sites. These differences were correlated to soil acidity and C/N ratio, with soil acidity affecting the distribution of *Acidobacteria*, *Actinobacteria*, α- and β-*Proteobacteria*. Based on Shannon diversity indices and richness and rarefaction analyses, the diversity of soil communities decreased in the order bamboo plantation > cedar plantation > hardwood forest. These findings suggest that afforestation and subsequent management of a natural hardwood forest causes differences in soil bacterial community structure and increases in diversity.

1. Introduction

Soils are among the most diverse microbial habitats on earth, and the indigenous soil microbial communities are important components of the biogeochemical cycles in terrestrial ecosystems (Doran and Zeiss, 2000; Burton et al., 2010). It is widely accepted that the soil microbial community is influenced by soil properties and type of vegetation (Wakelin et al., 2008; Curlevski et al., 2010; Shen et al., 2013). The disturbances caused by forest management often alters soil characteristics (Hartmann et al., 2014) and in turn influences microbial structure and diversity (Hartmann et al., 2014). For instance, land use changes from rainforests to crop lands in South America altered the community composition and the abundance of some bacterial phyla (Marcela et al., 2015). Our previous study showed that the disturbance of afforestation resulted in shifts of the bacterial community between natural hardwood and secondary coniferous forests (Lin et al., 2011). *Proteobacteria* predominated in the natural hardwood forest soils, but *Acidobacteria* was the most abundant phylum in the secondary coniferous forest soil communities (Lin et al., 2011). However, coniferous forests tend to lower the pH of soils, and it wasn't clear if these results would generalize to other types of forest management.

Because of its utility, bamboo is a valuable forest resource in East Asia. Young bamboo shoots are used as a healthy food, and the stalk can be used as construction or furniture material. Bamboo can grow and spread laterally by below-ground rhizomes. With the rhizome system and fast-growing properties, bamboo can invade adjacent forests (Touyama et al., 1998; Wu et al., 2010). The invasion of bamboo into the Japanese cedar plantation could accelerate the degradation of SOM (Wang et al., 2016). Bamboo also releases allelochemicals to reduce seedling abundance and species

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richness under bamboo canopies (Chou and Yang, 1982) and alters composition and diversity of the other members of the plant community (Larpkern et al., 2011).

In the present study, soil samples were collected in a subtropical montane ecosystem which was subjected to afforestation with either moso bamboo or Japanese cedar plantations. The study site was located at about 1800 m above sea level (a.s.l.), which is close to the upper limit of bamboo growth in Taiwan. The objective of this study was to elucidate the influence of vegetation succession on the soil bacterial community from the original natural broad-leaved forest at high elevation. The relationships between soil bacterial communities and environmental properties were also characterized. Thus, this study provided insights into the structure and diversity of the bamboo soil bacterial community and about afforestation and silvicultural practices in this ecosystem.

2. Materials and methods

2.1. Site description and soil sampling

This study was conducted in Mt. Fenghuang (23°39′ N, 120°48′ E) at the Experimental Forest, College of Bio-Resources and Agriculture, National Taiwan University (NTU), located in Nantou County, central Taiwan. The study was permitted by Dr. Y. N. Wang, the department head of the Experimental Forest, NTU. The field studies did not involve endangered or protected species. The elevation was about 1800 m a.s.l., the mean annual precipitation was about 2600 mm, and the mean annual temperature was 16.6 °C. The hardwood forest was nearly undisturbed and dominated by natural broadleaved forest species, in particular *Schima superba*, *Cyclobalanopsis glauca*, *Castanopsis carlesii* and *Cinnamomum reticulatum*. The adjacent moso bamboo (*Phyllostachys edulis*) and Japanese cedar (*Cryptomeria japonica*) plantations had been converted from natural hardwood forest more than three decades ago (Supplementary Fig. 1). Bamboo and cedar plantations were selected for comparison because they are the major species for plantations and widely cover mountainous areas in Taiwan.

The soils in the study site were derived from sandstones and shales (Ho, 1988) and could be classified as Inceptisols (Soil Survey Staff, 2014). Based on our soil survey, the soils in bamboo forest are Typic Dystrudepts, which are characterized by ~50 cm in depth, loamy, and a shallow (~10 cm) A horizon with a very dark brown soil color (10 YR 2/2). The soils in the broad-leaved forest were similar with those in the bamboo forest and were also classified as Typic Dystrudepts, with loamy, and a dark brown A horizon. Under the cedar forest, the soils were loamy, deeper and had a lithic contact at about 70 cm. These soils were classified as Humic Dystrudepts because of a thicker (~20 cm) A horizon with a very dark grayish and brown soil color (10 YR 3/2). The soil pedons surveyed in each forest type were triplicates with an interval of 250 m. For the classification level in soil series, the soils under bamboo and broad-leaved forests were classified as loamy skeleton, mixed, acidic, mesic Typic Dystrudepts. The soils under the cedar forest were loamy skeleton, mixed, acidic, mesic Humic Dystrudepts. Thus, the soils in the three forests were loamy, either silty loam or clay loam in texture. The soils were well-drained, with pH values from 3.3 to 4.4. Other properties of soils are reported in Table 1.

At each vegetation type, three 25 m × 25 m plots were established 50 m apart along transect lines in February 10, 2014. The soil samples were all collected in winter to avoid differences caused by seasonal changes and to minimize disturbances from farmers, such as fertilization, thinning and insecticide application during the growing season for bamboo. The sampling plot of each vegetation was set at least 100 m from the boundary of each plantation. Within each plot, three subsamples were collected with a soil auger 8 cm in diameter and 10 cm deep, and were combined to serve as one replicate. For each vegetation, three plots (replicates) were collected. Visible detritus, such as roots and litter, were manually removed prior to passing soil through a 2-mm sieve. Soils were then stored at −20 °C, and extraction of soil community DNA was performed within two weeks.

2.2. Barcoded pyrosequencing of the 16S rRNA genes

Soil community DNA was extracted using the PowerSoil® Soil DNA Isolation kit (MoBio Industries, Carlsbad, CA, USA) in accordance with the manufacturer's instructions. The V1–V2 regions of the bacterial 16S rRNA gene were amplified using 27F and 338R primers (Lane, 1991). Polymerase chain reactions (PCR) were performed as described previously (Lin et al., 2015). Secondary PCR (3 cycles rather than 20) was carried out to add the bar-codes for each sample (Lin et al., 2015). The unique and error-correcting bar codes facilitated sorting of sequences from a single pyrosequencing run (Hamady et al., 2008). The barcoded PCR products were then purified with a column filter using the PCR cleanup system (Viogene Biotek Corp., New Taipei City, Taiwan). The qualities and concentrations of the purified barcoded PCR products were determined using a NanoDrop Spectrophotometer (Thermo Scientific). Amplicon pyrosequencing was performed by Mission Biotech (Taipei, Taiwan) using the 454/Roche GS-FLX Titanium Instrument (Roche, NJ, USA). All sequences have been submitted to the Short Read Archives with the accession number of SRS1046023.

2.3. Sequence analysis

The pyrosequences were processed through the RDP pyrosequencing pipeline (http://pyro.cme.msu.edu; RDP Release 11.3; release date: 2014.09.17). The sequences were assigned to the samples by recognition of the bar code from a tag file, followed by trimming to remove the bar codes, primers and linker. The pyrosequences were filtered, and sequences where the maximum number of N's = 0, more than 200 bp in length, and quality scores >25 were selected for further analyses. Taxonomic information was also analyzed using the naïve Bayesian rRNA classifier of the RDP (Wang et al., 2007). Rarefaction curves were calculated by using the programs Aligner, Complete Linkage Clustering, and

Table 1
Soil properties of study sites. Values represent mean ± SD of three replicate samples.[a]

Vegetation	pH	Organic C (g kg^{-1})	Total N (g kg^{-1})	C/N	SOC[b] (mg kg^{-1})	SON[b] (mg kg^{-1})	Sand (%)	Silt (%)	Clay (%)
Bamboo	4.7 ± 0.2a	130.2 ± 22.2a	9.2 ± 1.6a	14.1 ± 0.8a	626.4 ± 115.9a	25.0 ± 9.5a	27.4 ± 7.5a	37.2 ± 11.8a	35.3 ± 4.3a
Cedar	3.8 ± 0.1b	156.3 ± 57.2a	10.0 ± 2.6a	15.4 ± 2.0a	478.3 ± 74.9a	19.8 ± 4.4a	25.0 ± 2.6a	28.5 ± 5.7a	46.5 ± 6.1ab
Hardwood	3.4 ± 0.1b	213.7 ± 47.2a	12.2 ± 3.5a	17.9 ± 1.4a	576.3 ± 53.4a	23.8 ± 3.9a	24.3 ± 2.5a	36.6 ± 4.3ab	39.0 ± 2.9a

[a] Values with the different letter within a column indicates that the differences were significant among the sites.
[b] SOC, soluble organic carbon; SON, soluble organic nitrogen.