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Soil and tree species traits both shape soil microbial communities during early growth of Chinese subtropical forests

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ABSTRACT

A better understanding of the linkages between aboveground and belowground biotic communities is needed for more accurate predictions about how ecosystems may be altered by climate change, land management, or biodiversity loss. Soil microbes are strongly affected by multiple factors including local abiotic environmental conditions and plant characteristics. To find out how soil microbial communities respond to multiple facets of the local soil and plant environment, we analysed soil lipid profiles associated with three-year-old monocultures of 29 tree species. These species are native of the diverse subtropical forests of southeast China and greatly vary in functional traits, growth or biomass characteristics, and phylogenetic relatedness. Along with the traits of each tree species, we also determined the soil and plot characteristics in each monoculture plot and tested for phylogenetic signals in tree species-specific microbial indicators. Microbial community structure and biomass were influenced by both soil properties and plant functional traits, but were not related to the phylogenetic distances of tree species. Specifically, total microbial biomass, indicators for fungi, bacteria, and actinomycetes were positively correlated with soil pH, soil organic nitrogen, and soil moisture. Our results also indicate that leaf dry matter content and the leaf carbon to nitrogen ratio influence multivariate soil microbial community structure, and that these factors and tree growth traits (height, crown or basal diameter) positively promote the abundances of specific microbial functional groups. At the same time, a negative correlation between leaf nitrogen content and Gram positive bacterial abundance was detected, indicating plant–microbial competition for nitrogen in our system. In conclusion, even at early stages of tree growth, soil microbial community abundance and structure can be significantly influenced by plant traits, in combination with local soil characteristics.

1. Introduction

Numerous studies have pointed out that environmental properties such as soil pH, soil texture, soil moisture, and soil nutrient availability influence the dynamics of soil microbial communities (Fierer and Jackson, 2006; Wakelin et al., 2008; Brockett et al., 2012; Docherty et al., 2015). Soil microbial communities also have close interactions with plants, and can be affected by plant productivity, community composition, and functional traits (Bauhus et al., 1998; Buckley and Schmidt, 2002; Kao-Kniffin and Balser, 2008; de Vries et al., 2012).

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Plant functional traits have increasingly been studied as determinants of ecosystem properties, especially for soil biogeochemical processes (Chapin, 2003; Díaz et al., 2007; de Vries et al., 2012). Plant functional traits related to growth may determine a tree species' ability to contribute to the soil carbon pool via the volume or quality of leaf litter inputs (De Deyn et al., 2008). Likewise, plant traits determining leaf litter quality and quantity influence leaf decomposition rates and other soil biogeochemical process (Cornelissen and Thompson, 1997; Bardgett and Wardle, 2010; Eichenberg et al., 2014a). Traits associated with the leaf economic spectrum such as specific leaf area (SLA), leaf nitrogen content (LNC), or leaf dry matter content (LDMC) can also be related to belowground soil properties (Lavorel and Garnier, 2002; Garnier et al., 2004). For example, Laughlin (2011) reported that communities dominated by plant species with high LNC, SLA and low LDMC tend to have high soil nitrification potential.

There are also many ways in which plant functional traits influence the soil microbial community as drivers of these biogeochemical processes. Forest growth and canopy structure, resulting from the collective contribution of individual trees, alter microsite albedo, temperature, light penetration, humidity, and raindrop velocity; variables all known to alter microbial communities (Anderson et al., 2011; Forrester et al., 2012; Wu et al., 2012; Geißler et al., 2013). Plant functional traits have also been reported to modify the soil microbial habitat by altering resource availability (Orwin et al., 2010), pH (Wang et al., 2001; Thoms et al., 2010) and soil moisture (Brussaard et al., 2007). At a more specific level, the amount or quality of plant resource inputs, both aboveground (litter) and belowground (dead roots and rhizodeposits), influence the abundance of different fungal and bacterial groups (Wilkinson and Anderson, 2001; Scherer-Lorenzen et al., 2007; De Deyn et al., 2008; Orwin et al., 2010). For example, plant species with high SLA, high LNC, and low LDMC can result in bacterial-dominated soil microbial communities in grasslands (Orwin et al., 2010). Leaf polyphenolic compounds are another example trait that can affect the activity and composition of soil microbial communities (Hättenschwiler and Vitousek, 2000; Fierer et al., 2001).

From a broader perspective than individual traits, related tree species could possibly cultivate similar microbial communities through plant–microbial coevolution. This idea of plant–microbial coevolution, and plant selection of a specific rhizosphere microbial community, has been demonstrated for some pathogenic fungi (Liu et al., 2012) and perhaps the most widespread, or well known, association is the symbiosis between plants and mycorrhizal fungi (Hoeksema, 2010; Buscot, 2015). Evidence of complementary traits coevolving between plants and arbuscular mycorrhizal fungi are well documented, such as the development of different functional roles across different arbuscular mycorrhizal fungal (AMF) lineages that colonize different hosts (Maherali and Klironomos, 2007). In turn host plants have evolved to provide a more hospitable environment for fungi (Brundrett, 2002). Despite this established knowledge regarding specific plant–microbe interactions based on plant species relatedness, few studies have investigated whether plant phylogenetic relatedness affects the soil microbial community in a more general way. Moreover, because most studies that have explored the effects of plant functional traits on microbial communities have only incorporated a low number of tree species or few plant species traits, the relative importance of different traits or of phylogenetic conservatism are less well understood.

The influence of plant characteristics on soil microbial communities may be modified by local soil characteristics, and soil and plant characteristics could interact in unexpected ways to mediate soil microbial communities (De Deyn et al., 2008; Orwin et al., 2010). Recent studies of plant–soil interactions have demonstrated that soil properties play a crucial role on the interactions between plant species and soil microbial communities (Innes et al., 2004; De Deyn et al., 2009; Harrison and Bardgett, 2010). In light of this interplay in influence of soil and plant characteristics, it's likely that in early successional systems where plants are just becoming established, soil characteristics will play a dominant role in driving soil community dynamics with plant traits playing a larger role as plant communities become developed. In general, little is known about the relative importance of traits across a wide range of plant species during plant development, their related plant functional traits, or how local soil characteristics alter the development of soil microbial communities.

In this study we examined the effects of plant traits, specific tree species, tree phylogeny and soil conditions on microbial community structure in the context of highly diverse subtropical forests. We conducted this study across 29 tree species grown in monocultures, for a broad examination of aboveground-belowground relationships during early tree growth. Specifically, we tested the following four hypotheses: (1) Soil properties have a strong impact on soil microbial community composition, and predominantly explain the variation in microbial structure and abundance in the early stage of tree reforestation. (2) Plant traits influence soil microbial community composition and biomass, but to a lesser extent than soil properties. (3) Soil microbial communities differ in association with different tree species through identity effects (the collective sum of their traits). (4) Soil microbial communities are more similar in association with more closely related tree species. In other words there will be a phylogenetic signal present in tree-associated soil microbial communities.

2. Material and methods

2.1. Study sites

The study sites are located in a subtropical forest in south-east China, Jiangxi province, near the town of Xingangshan (29.08–29.11° N, 117.90–117.93° E). The sites are part of a large scale forest biodiversity and ecosystem function (BEF) experiment, Biodiversity Ecosystem Function-China (BEF-China, Bruelheide et al., 2014). The full experiment includes a total of 566 plots allocated to two study sites (site A and site B). On both experimental sites, the original forests were clear-cut in 2009. Site A plots were planted and established in March 2009, and site B plots were planted in March 2010. We focused on a subset of 29 intensively studied monoculture plots in order to compare our microbial data to tree species-specific datasets on plant and soil characteristics.

The experimental region is characterized by a subtropical climate with a warm, rainy summer and a cool, dry winter. Mean annual rainfall in this area is 1821 mm (peaking in May to June) and mean temperature is 16.7 °C (Yang et al., 2013). Soils are Cambisols and Cambisol derivatives, with Regosols on ridges and crests (Geißler et al., 2012). The natural regional vegetation is dominated by broad-leaved forests that are extremely species-rich. Dominant plant species are *Quercus glauca* (previously *Cyclobalanopsis glauca*, adapted to the taxonomy given by Zanne et al., 2014), *Castanopsis eyrei*, *Daphniphyllum oldhamii*, and *Lithocarpus glaber* (Bruelheide et al., 2011, 2014). The 29 plant species included in this study are listed in Table 3 and Table S1.

2.2. Soil sampling and soil properties

Soil samples were taken from monoculture plots of the listed 29 tree species between October and November in 2011 (site A) and October in 2012 (site B), respectively. This was done to ensure that

all trees were three years old at the time of sampling, and we observed only minor differences in ambient conditions between the two years (Table S2). Within each monoculture plot, three random trees were selected for sampling. At each selected tree, four soil cores (0–10 cm depth) were taken at a circumference of approximately 30 cm from the base of each target tree. The subsamples were pooled to one final sample per target tree, and sieved (2 mm mesh) to exclude large roots and stones. Soil subsamples were frozen in liquid nitrogen directly in the field and transported to the nearby field lab (less than 5 km) where samples were freeze-dried to prevent changes in microbial communities until further analysis. All samples were stored at −80 °C freezer until and after freeze-drying. Samples were then transported dry in sealed plastic containers to Halle, Germany, where lipid extractions and data analysis were performed.

Soil moisture was determined gravimetrically for each fresh soil sample by drying 10 g subsamples at 105 °C for 48 h in the field lab to calculate the percentage of soil water content (SWC). The following plot level soil characteristics were determined in each plot between 2009 and 2013: soil organic nitrogen content (SON), soil organic carbon content (SOC), and soil pH_{H2O}. To determine these plot-level variables, two soil depths were collected (0–5 cm and 5–10 cm) with 9 subsamples per plot which were then pooled to one sample per depth increment. These soil samples were air-dried, sieved with a 2 mm mesh sieve, and transported to the University of Tübingen, Germany for grinding and further analysis. SON and SOC were determined by heat combustion (1150 °C), thermal conductivity analysis was determined on a CN-element analyzer (Elementar Vario EL III, Tübingen, DE) and soil pH_{H2O} was determined potentiometrically in a 1:2.5 soil water solution. In order to relate soil abiotic characteristics to soil microbial data, we averaged all soil parameters from the 0–5 and 5–10 cm increments to give one 0–10 cm value per sample.

2.3. Microbial community analysis

Extracting phospholipids has been validated as an effective approach for investigating soil microbial community composition (Frostegård et al., 2011). Microbial lipids were extracted according to a modified high-throughput lipid extraction protocol (Bligh and Dyer, 1959; Gutknecht et al., 2012). Briefly, 2 g of freeze-dried soil were extracted three times with chloroform, methanol, and citrate buffer (1:1:0.9, by volume). Fatty acids contained in the lower chloroform phase were then saponified and converted into fatty acid methyl esters using a strong acid methylation. The extracted fatty acid methyl esters were identified and quantified on an Agilent GC–MS, equipped with a HP DB5 column. Peak areas were converted to nmol lipid g^{-1} dry soil using known concentrations of an internal standard (13:0 tridecanoic methyl ester). We used the total nmol lipid g^{-1} dry soil with less than or equal to 20 carbons in length to calculate the soil microbial biomass (Frostegård and Bååth, 1996). Individual fatty acids were used to indicate broad functional groups of microorganisms: i15:0 for Gram positive bacteria (Gram+, Wilkinson et al., 2002), 18:1ω7c for Gram negative bacteria (Gram−, Wilkinson et al., 2002), and 10Me16:0 for actinomycetes (Fierer et al., 2003; McKinley et al., 2005). The biomarker 16:1ω5c was used to indicate AMF and 18:2ω6, 9c was used for indicating general fungi (GF) excluding AMF (Balser et al., 2005). The fungal-to-bacterial ratio (FB-ratio) was indicated by the ratio of fungal lipids (16:1ω5c, 18:1ω9c, and 18:2ω6, 9c) to bacterial lipids (i15:0, ai15:0, 16:02OH, i16:0, 16:1ω7c, 10Me16:0, i17:0, ai17:0, cy17:0, 18:1ω5c, and 18:1ω7c) (Frostegård and Bååth, 1996). For more detailed information about the modified hybrid lipid extraction method see Gutknecht et al. (2012).

2.4. Leaf functional traits and tree biomass characteristics

In the field, the leaves were sampled according to the protocol of Cornelissen et al. (2003), in which only fully developed, sun-exposed leaves from the present vegetation period without visible herbivore or pathogen damage were collected. Four leaves of a single tree in each plot were sampled. The following leaf parameters were determined for each species and each plot: leaf area (LA, mm), specific leaf area (SLA, mm^2/mg), leaf dry matter content (LDMC, mg/g), leaf nitrogen content (LNC, mg/g), carbon content (mg/g), carbon-to-nitrogen ratio (CN), leaf K, Mg, Na, P, S, Al, Fe, and Mn contents (μg/g), phenolics (mg/g), and tannin (mg/g). All leaf traits were determined according to the standard procedures for trait measurements described by Cornelissen et al. (2003), except for phenolics and tannin concentration. For further details on polyphenol determination see Kröber et al. (2015) and Eichenberg et al. (2014b).

Tree biomass characteristics were recorded in 2011 on site A and in 2012 on site B, in the same years in which the soil samples were collected. Because all trees were the same age when sampled, we used these biomass traits as a proxy for growth traits. This assumes that biomass change over time is equal to growth, where time was held constant (all trees were three years old when sampled). In each plot, all the trees in the central area of 6 × 6 planting positions (36 trees in total) were investigated for tree height (cm), ground diameter (GD, cm) and crown diameter from north to south (NS crown, cm) and east to west (EW crown, cm) (see Li et al., 2014). Tree growth traits were averaged for each tree species (corresponding to a monoculture plot) for the purpose of this study.

2.5. Statistical analyses

To determine the main factors affecting multivariate microbial community composition, we used non-metric multidimensional scaling (NMDS) to analyse the dissimilarity between microbial communities of different plant species. NMDS has been shown to be an effective tool in community composition analysis (Minchin, 1987; Wu et al., 2012) and does not assume linear distribution of the data (Clarke, 1993; McCune and Grace, 2002; Bach et al., 2010). The abundance of individual lipids (nmol g^{-1} soil) was used in the NMDS analysis. We used Bray–Curtis dissimilarity to construct distance matrices because of its powerful ecological ranking measurement (Clarke, 1993). We also assessed the significance of correlations between the NMDS scores and the soil and plant traits by using post-hoc permutational correlations ($n = 999$, envfit function in vegan package). Variance partitioning analysis was used (Legendre and Legendre, 1998, R vegan package) to address the relative importance of soil characteristics versus plant traits as drivers of multivariate microbial community composition. For variance components analysis we used the same lipid indicator matrix as used for NMDS analysis. We additionally used three explanatory matrices including soil characteristics, leaf trait variables, and plant growth traits. The exact variables composing the three explanatory matrices are the same as those used for post-hoc correlations with NMDS axes, and are presented in Table 1.

We further analysed the correlations between the abundance of individual lipid indicators (nmol g^{-1} soil), total biomass, the FB-ratio, and soil and plant variables with Pearson correlations. In addition to relationships between the microbial community and either soil or plant traits, we analysed correlations between plant and soil variables to discern possible confounding factors (each species is only present on one plot). To test for general differences in microbial lipid indicators and specific tree species, an ANOVA with a post-hoc Tukey HSD was applied to test for pairwise differences across the 29 tree species.

To assess whether closely related tree species share similar soil microbial communities, we tested for a tree species specific phylogenetic signal in seven indicators of the microbial community (biomass, FB-ratio, Gram positive, Gram negative, actinomycetes, GF, and AMF). A phylogenetic tree for the tree species in our study was obtained from the most recent dated and ultrametric phylogeny of global angiosperms (Zanne et al., 2014). Missing species (*Elaeocarpus chinensis*, *Phoebe bournei*) were added manually into the phylogeny based on taxonomy. To assess the phylogenetic signals, we used three metrics: Blomberg's *K* (Blomberg et al., 2003), Pagel's λ (Pagel, 1999), and Abouheif/Moran's *I* (Abouheif, 1999), because phylogenetic signal statistics have been demonstrated to have low power with small phylogenies (Godoy et al., 2014) (e.g. 29 species in our study). Values of Blomberg's *K* smaller than one indicate that microbial biomarker values are randomly distributed on the phylogenetic tree, while *K*-values equal to or greater than 1 would indicate a stronger phylogenetic signal in a microbial lipid indicator than expected by a Brownian motion model of trait evolution. That is that closely related tree species share similar microbial communities. The observed *K*-values were compared to a null distribution obtained by shuffling species names across the tips of the phylogeny. A significant phylogenetic signal is indicated by observed values of *K* in the upper 2.5% of randomized *K*-values. Likelihood ratio tests were used to test whether Pagel's λ values were greater than zero. The observed values of Abouheif/Moran's *I* were tested using the two-sided test proposed by Gittleman and Kot (1990) based on 999 permutations. All statistical analyses were carried out in R (version 3.0.1, R Development Core Team, 2013) using the packages 'vegan' (Oksanen et al., 2013), 'agricolae' (de Mendiburu, 2014), 'phytools' (Revell, 2012) and 'adephylo' (Jombart and Dray, 2013).

3. Results

Multiple analyses of data in this study demonstrate that both soil characteristics and plant traits explain variance in the soil microbial community of our subtropical ecosystem. Variance partitioning analysis showed that soil variables alone explained 29% of the variation in lipid data, while leaf trait variables and plant growth variables explained 18% and 11% of variation, respectively, and with combined effects between soil and plant characteristics as well (Fig. 1). There were also both soil and plant trait variables that correlated significantly with the NMDS matrix (Fig. 2, Table 1). The optimal NMDS configuration was based on two dimensions (stress = 0.15 after three runs). As shown in Table 1, soil pH and SWC were significantly correlated with both axes 1 and 2 ($P < 0.001$). SON also had a significant ($P < 0.05$) influence on the microbial community structure (Fig. 2, Table 1). With regard to leaf traits and tree growth characteristics, LDMC and leaf CN were both significantly negatively related to axis 1 and axis 2 ($P < 0.01$ and $P < 0.05$, respectively, Table 1).

We also observed significant correlations between individual microbial indicators and associated soil or plant characteristics (Table 2). With the exception of the FB ratio, all other bacterial and fungal lipid indicators and total microbial biomass showed an intermediate to strong positive correlation with soil pH (all $P < 0.05$). Total biomass of soil microorganisms, bacterial indicators (both, Gram+ and Gram−) and fungal indicators (AMF and actinomycetes) were all significantly correlated with SWC ($P < 0.05$), while only total lipid biomass and the abundance of Gram+ and actinomycetes indicators were significantly positively correlated with SON. In addition, we found soil CN to be negatively related to the abundance of Gram− lipid indicator ($P < 0.05$). With regard to plant characteristics, we found that different lipid indicators were significantly related to different plant characteristics, with few

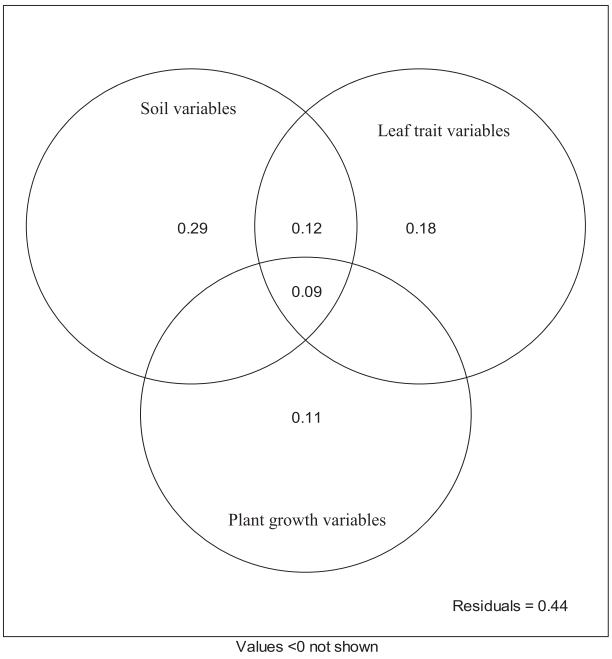

Fig. 1. Variance partitioning analysis to determine how tree species traits and plot-level soil characteristics explain variance in lipid abundance data. Three explanatory matrices were used, including soil characteristics of the given plot, plant leaf traits, and plant growth variables (see Methods section). Each circle represents the portion of variation accounted for by an explanatory matrix. Shared variance or shared effects are shown in the intersecting portions of the circles.

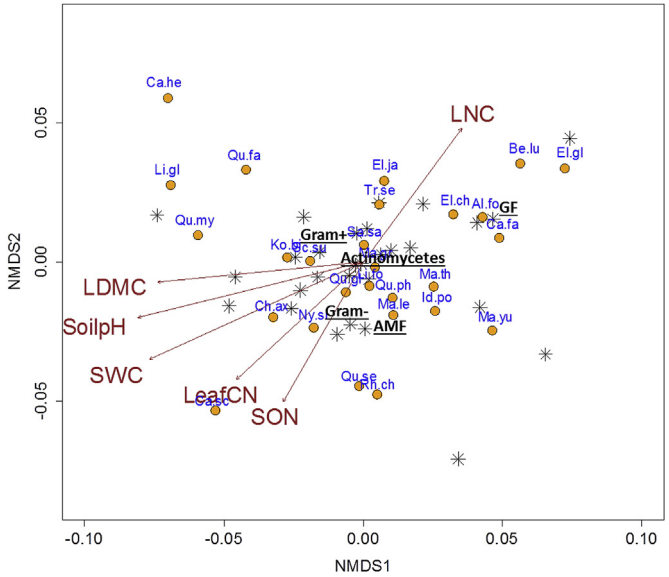

Fig. 2. NMDS results of microbial community composition across soil and plant variables on lipid abundance data (nmol lipids g dry soil^{-1}). Axes are arbitrary and scaled in units of Bray–Curtis dissimilarity. The solution was reached after 3 runs (stress = 0.15). Asterisks represent individual lipids and round orange circles represent different plant species. Circles are accompanied by tree species names that are abbreviated using the first two letters of the genus name followed by the first two letters of the species name (example: *Ailanthus altissima* would be abbreviated as Ai.al, for a complete list see Table S1). Arrow length refers to the strength of correlation with microbial lipid composition, and only those factors significantly related to NMDS axes at $P < 0.05$ are shown in this figure (for a full list of factors included, see Table 1).

Table 1
Non-metric multidimensional scaling (NMDS) analysis and soil, plant variables correlated with microbial abundance.

	NMDS1	NMDS2	r^2	P
Soil variables				
Soil pH	**−0.970**	**−0.241**	**0.497**	**0.001*****
SON (%)	**−0.495**	**−0.869**	**0.239**	**0.041***
SOC (%)	−0.199	−0.980	0.147	0.146
SWC (%)	**−0.909**	**−0.417**	**0.508**	**0.001*****
Soil CN	0.907	−0.421	0.144	0.135
Leaf variables				
LA (mm)	−0.515	−0.857	0.058	0.470
LDMC (mg/g)	**−0.995**	**−0.099**	**0.392**	**0.003****
SLA (mm^2/mg)	0.647	0.762	0.125	0.178
LNC (mg/g)	**0.592**	**0.806**	**0.254**	**0.031***
Leaf C (mg/g)	−0.990	0.135	0.036	0.632
Leaf CN	**−0.733**	**−0.680**	**0.273**	**0.021***
Leaf K (μg/g)	0.855	0.517	0.061	0.481
Leaf Mg (μg/g)	0.752	−0.658	0.095	0.307
Leaf Na (μg/g)	−0.157	0.987	0.135	0.179
Leaf P (μg/g)	0.325	−0.945	0.041	0.623
Leaf S (μg/g)	0.902	−0.431	0.048	0.557
Leaf Fe (μg/g)	−0.894	0.447	0.027	0.741
Leaf Mn (μg/g)	0.045	0.998	0.029	0.694
Leaf Al (μg/g)	−0.702	0.711	0.096	0.304
Phenolics (mg/g)	0.235	0.972	0.092	0.315
Tannin (mg/g)	0.182	0.983	0.100	0.289
Tree growth variables				
Height (cm)	−0.568	0.822	0.026	0.728
GD (cm)	−0.475	0.879	0.030	0.691
NS.crown (cm)	−0.187	0.982	0.038	0.617
EW.crown (cm)	−0.196	0.980	0.032	0.658

r^2 shows the proportion of variance explained and asterisks refer to the significance of correlation, based on a post-hoc permutation test (n = 999). ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$. Significant correlations in bold.

overarching commonalities. The Gram− indicator was significantly positively related to LDMC ($P < 0.05$). The Gram+ indicator was positively related to leaf CN, but negatively correlated to LNC ($P < 0.05$). In addition to traits related to the leaf economics spectrum (Wright et al., 2004), lipid indicators were positively correlated with leaf elemental contents including Mn, Na, and Al ($P < 0.05$, see Table 2). Tree growth traits correlated mainly with FB-ratio, GF, AMF as well as the actinomycetes lipid indicator ($P < 0.05$, Table 2). Based on correlations between plant and soil variables, to explore possibly confounding effects, we found that LA was positively correlated with soil SOC ($P < 0.05$) and that LDMC was positively correlated with pH ($P < 0.01$) and soil CN ($P < 0.05$, Table S3).

Analysis of the effects of tree species identity showed that all specific lipid indicators except GF, the FB-ratio, and the total lipid biomass varied significantly in association with different tree species (Table 3). In detail, the highest observed total microbial biomass and abundance of actinomycetes was associated with *Castanea henryi* (Table 3); soil near *Koelreuteria bipinnata* was associated with significantly higher abundance of both Gram+ and Gram− indicators. Soils near the two species *Betula luminifera* and *Castanopsis fargesii*, however, had significantly lower bacterial and fungal abundance when compared with other species. *B. luminifera*-associated soil had the lowest Gram+ and AMF abundance and soils around *C. fargesii* were associated with the lowest actinomycetes abundance.

We found no phylogenetic signal in any of the seven microbial indicators (biomass and abundance) using any of the three metrics Blomberg's K, Pagel's λ and Abouheif/Moran's I (Table 4, Fig. 3), indicating that closely related tree species did not share similar microbial communities.

4. Discussion

As hypothesized in this study, we found that predominantly soil characteristics, but also tree species traits and identity, act in concert to shape soil microbial communities, even at early stages of tree growth. In the following sections we discuss each of these factors (soil, plant traits, and plant identity) in more detail and then consider them together in a final conclusions paragraph.

4.1. Soil factors and microbial communities

The results obtained from this study confirm that in the context of a highly biodiverse subtropical ecosystem, as has thoroughly demonstrated elsewhere (e.g. Van Gestel et al., 1992; Högberg et al., 2007; de Vries et al., 2012), soil characteristics play a significant role in determining the composition and growth of soil microbial communities. Soil pH was one of the most influential factors for both community composition and microbial abundance. This is consistent with evidence from biomes other than the subtropics that soil pH is a strong driver of microbial growth and community composition (Bååth and Anderson, 2003; Fierer and Jackson, 2006; Högberg et al., 2007). For example, microbial diversity may be highest in near-neutral pH soils (de Vries et al., 2012). Although our experimental site has a relatively narrow acidic pH range (4.2–5.2, data not shown), microbial abundances increased with rising pH. This indicates that subtropical ecosystems may have similar soil-based constraints on microbial communities as other ecosystems, even at relatively smaller changes in ionic concentration. Soil moisture is also well known to play an important role in influencing micro-faunal and microbial community dynamics because of its influence on soil enzyme activities (Stark and Firestone, 1995) and fluxes of soil nutrients, oxygen, and other gases (Wagener and Schimel, 1998; Brockett et al., 2012). Similar to our findings, a positive relationship between soil moisture and microbial biomass has been reported elsewhere (Van Gestel et al., 1992).

We did not find indications of a confounding effect between tree species growth and soil water content in our system, in this early stage of tree growth (no correlations found, see Table S3). It is widely recognized that soil nutrient availability also shapes microbial communities (Fierer et al., 2009) and high soil fertility has been shown to support microbial growth in forests (Mendham et al., 2002; Cao et al., 2010). It was therefore not surprizing that we would detect significant positive correlations between indicators of soil fertility, in this case SON, and the microbial community, as we observed with multivariate microbial community composition, total microbial biomass, and indicators for Gram+ bacteria and actinomycetes.

4.2. Plant traits and the soil microbial community

We found evidence that leaf dry matter content (LDMC) is an important driver for multivariate soil microbial community structure and Gram− bacterial abundance, possibly due to the relative amounts of leaf structural inputs between species. LDMC is the ratio of leaf dry weight to fresh weight and has been used as a proxy for the ratio of structural compounds to assimilatory tissue (mesophyll and epidermis, Van Arendonk and Poorter, 1994). High values of LDMC represent a high portion of vascular tissue, cellulose, insoluble sugars, and leaf lignin (Poorter and Bergkotte, 1992). The positive correlations we observed between Gram− bacterial abundance and LDMC indicates that leaf structural compounds can have afterlife implications for leaf litter quality and soil microbial communities (Garnier et al., 2007; Cornwell et al., 2008). Although the presence of chemically resistant elements such as lignin reduce decomposition rates (Hättenschwiler et al., 2005), the increased

Table 2
Pearson's correlation analyses of microbial variables (abundance of indicator lipids, total microbial biomass, and FB-ratio) with soil properties, leaf traits, and tree species growth traits.

	Total biomass (nmol g^{-1} soil)	Fungal to bacteria ratio	Gram+ (iso15:0) (nmol g^{-1} soil)	Gram− (18:1w7c) (nmol g^{-1} soil)	General fungi (18:2 w6,9) (nmol g^{-1} soil)	AMF (16:1w5c) (nmol g^{-1} soil)	Actinomycetes (16:0 10me) (nmol g^{-1} soil)
Soil variables							
pH_{H2O}	**0.644*****	−0.012	**0.634*****	**0.711*****	**0.454***	**0.758*****	**0.691*****
SON (%)	**0.410***	−0.342	**0.612*****	0.335	0.091	0.247	**0.388***
SOC (%)	0.173	−0.267	0.374	0.055	−0.030	0.090	0.199
SWC (%)	**0.489****	−0.143	**0.650*****	**0.564****	−0.006	**0.457***	**0.512****
Soil CN	−0.340	0.115	−0.300	**−0.447***	−0.147	−0.150	−0.256
Leaf variables							
LA (mm)	0.165	0.067	0.182	0.265	−0.080	0.256	0.290
SLA (mm^2/mg)	0.024	0.118	−0.163	−0.001	0.311	0.007	−0.032
LDMC (mg/g)	0.263	−0.275	0.351	**0.482***	−0.182	0.253	0.250
LNC (mg/g)	−0.234	0.056	**−0.409***	−0.247	0.086	−0.380	−0.295
C (mg/g)	0.130	−0.179	0.211	0.056	−0.121	−0.033	0.098
CN (g/g)	0.243	−0.081	**0.436***	0.250	−0.075	0.367	0.337
K (μg/g)	−0.049	−0.170	0.067	−0.208	−0.109	−0.088	−0.012
Mg (μg/g)	−0.119	0.167	−0.140	−0.189	−0.103	−0.034	−0.205
Na (μg/g)	0.297	0.262	0.093	0.340	**0.419***	0.302	0.290
P (μg/g)	−0.095	−0.179	−0.108	−0.045	−0.255	−0.159	−0.097
S (μg/g)	−0.057	−0.260	0.107	−0.147	−0.154	−0.234	0.046
Al (μg/g)	**0.427***	0.197	0.347	**0.381***	**0.565****	0.177	0.283
Fe (μg/g)	0.075	−0.113	0.137	0.214	0.055	0.006	−0.032
Mn (μg/g)	**0.387***	0.146	0.262	0.239	**0.559****	0.197	0.260
Phenolics (mg/g)	0.083	0.103	−0.023	0.165	0.062	0.125	0.080
Tannin (mg/g)	0.098	0.125	0.004	0.195	0.152	0.124	0.061
Tree growth variables							
Height (cm)	0.179	**0.413***	0.026	0.235	0.313	**0.383***	**0.392***
GD (cm)	0.241	**0.494****	0.078	0.258	0.371	0.378.	**0.393***
NS crown (cm)	0.257	**0.397***	0.057	0.207	**0.423***	0.301	0.345
EW crown (cm)	0.241	0.377	0.044	0.196	**0.405***	0.295	0.324

The values presented are Pearson's correlation coefficients. $^{***}P < 0.001$, $^{**}P < 0.01$, $^{*}P < 0.05$.

amount of cellulose from leaves with high LDMC represents an additional carbon source that may enhance bacterial growth. This trend may have been emphasized in our field sites because of the recent disturbance for establishment. The original forest was clear-cut before the plots were replanted, which both physically disturbed the soil and acted to remove available carbon (through removal of plant material and through increased mineralization due to aeration of surface soil from physical disturbance). Thus, soil carbon availability may have become more limiting and consequently the soil microorganisms could have become more reliant on new carbon inputs such as those from trees with a higher LDMC. This assumption is further supported by the observation of a positive relationship between Gram− bacteria and LDMC, which indicates the importance of leaves as a carbon source for microbial growth during forest regeneration.

Another major pattern we observed was a negative correlation between the relative abundance of Gram+ bacteria and leaf nitrogen content (LNC), in keeping with the concept that LNC is an index of plant resource requirements. LNC is regarded as an indicator of plant growth and resource uptake and as such reflects the so-called leaf economics spectrum (Wright et al., 2004). At one end of the spectrum, it has been recognized that leaf litter from communities with N rich leaves and fast growing species leads to bacterially dominated soil microbial communities (de Vries et al., 2012). By contrast, long-lived, slowly decomposing and low N leaves promote fungal domination (Orwin et al., 2010; de Vries et al., 2012). Based on our results, we conclude that in this subtropical ecosystem, high LNC actually restrained the growth of bacterial communities and is an indicator of plant competition for soil N. It is possible that soil microbes and nearby plants were in competitive relationships for acquiring N from the soil, as suggested in other ecosystems (Kaye and Hart, 1997). Although our sites are relatively N rich, where plant–microbial competition is most likely minimal (Schimel and Bennett, 2004), perhaps as with microbial C limitation, N dynamics were less stable in our system.

Our results also indicate a link between tree growth characteristics and soil microbial communities. We found that tree height and GD were positively correlated with the abundance of actinomycetes and with the FB-ratio. Tree morphological traits like tree height and lateral spread are thought to be linked to plant competitive ability (Grams and Andersen, 2007). Thus, the strong links between tree growth traits and soil microbial communities may be interpreted as a response to the influence of aboveground productivity on belowground microbes (Zak et al., 1994; Wardle et al., 2004), as was discussed with the correlation between bacterial abundance and LDMC. Numerous studies have connected belowground soil microbial communities to aboveground plant production. It is known that certain microorganisms, especially fungal communities, enhance plant nutrient acquisition (Hodge et al., 2001; van der Heijden et al., 2008). At broader spatial scales, plant aboveground productivity has been reported to influence microbial biomass and explain broad microbial community distribution patterns (Zak et al., 1994). This idea is supported by our results that demonstrate positive relationships between microbial abundance and tree growth. A possible confounding effect regarding our results was that we found some correlation between plant leaf traits and soil characteristics, and we also found through variance partitioning that plant leaf traits, plant growth traits, and soil characteristics all explain, and overlap in explaining, variance in the soil microbial community. Therefore relationships we have observed between leaf traits and the soil microbial communities could be a result of both the microbes and plant nutrient status being governed together by soil processes, and through the

Table 3
Mean effects of plant species on microbial total biomass, FB-ratio and abundance of fungal and bacterial indicators. SD is given in parentheses. *P* values at the top of each column are based on an ANOVA test for species differences. Different letters indicate significant differences among difference based on posthoc Tukey's tests after ANOVA analysis. Bold values assist in visualization of which species have higher or lower abundances of microbial indicators.

	Total biomass (nmol g^{-1} soil) $P = 0.001$		FB-ratio $P < 0.001$		Gram+ (15:0iso) (nmol g^{-1} soil) $P < 0.001$		Gram− (18:1w7c) (nmol g^{-1} soil) $P < 0.001$		AMF (16:1w5c) (nmol g^{-1} soil) $P < 0.001$		GF (18:2w69c) (nmol g^{-1} soil) $P = 0.02$		Actinomycetes (10Me16:0) (nmol g^{-1} soil) $P < 0.001$	
Ailanthus altissima	219.41	(47.70)ab	0.36	(0.11)cde	9.46	(2.64)bcde	**8.52**	**(1.07)c**	9.76	(4.57)bcd	8.36	(1.39)a	**3.28**	**(0.50)f**
Alniphyllum fortunei	285.85	(32.34)ab	0.41	(0.09)abcde	10.41	(1.50)abcde	10.82	(1.96)abc	11.01	(3.51)abcd	13.65	(2.08)a	5.06	(0.52)bcdef
Betula luminifera	202.41	(46.75)ab	0.38	(0.07)bcde	**6.39**	**(2.05)e**	**6.79**	**(1.24)c**	**5.15**	**(0.51)d**	10.30	(4.94)a	**3.16**	**(0.53)f**
Castanea henryi	**371.85**	**(78.03)a**	0.58	(0.01)ab	15.75	(3.22)abcd	20.81	(3.86)ab	21.85	(5.16)abc	18.47	(5.87)a	**8.82**	**(2.16)a**
Castanopsis fargesii	197.32	(19.66)ab	0.35	(0.11)bcde	7.94	(0.28)de	**7.14**	**(1.27)c**	**5.52**	**(2.62)d**	7.32	(3.03)a	**2.96**	**(0.31)f**
Castanopsis sclerophylla	275.10	(91.30)ab	**0.25**	**(0.05)e**	15.71	(6.08)abcd	10.66	(5.09)abc	13.04	(7.43)abcd	8.61	(4.24)a	5.12	(1.82)bcdef
Choerospondias axillaris	286.76	(60.39)ab	0.55	(0.06)abcd	13.25	(3.10)abcde	17.06	(5.18)abc	**24.68**	**(7.14)a**	12.16	(2.31)a	**8.14**	**(1.51)ab**
Elaeocarpus chinensis	230.87	(31.53)ab	0.36	(0.10)bcde	9.48	(1.11)bcde	**9.17**	**(1.13)c**	12.04	(2.25)abcd	10.05	(3.77)a	4.02	(1.00)def
Elaeocarpus glabripetalus	251.10	(9.75)ab	0.47	(0.02)abcde	7.47	(0.56)de	**9.17**	**(0.60)c**	10.77	(2.32)abcd	10.94	(1.50)a	4.53	(0.50)cdef
Elaeocarpus japonicus	**177.99**	**(70.35)b**	0.57	(0.20)abc	6.94	(4.28)de	**9.07**	**(3.75)c**	8.17	(2.53)cd	7.21	(2.22)a	**3.29**	**(1.57)f**
Idesia polycarpa	215.03	(17.37)ab	**0.3**	**(0.01)de**	10.98	(0.72)abcde	**8.06**	**(1.29)c**	8.38	(1.46)cd	6.98	(2.10)a	4.55	(0.75)cdef
Koelreuteria bipinnata	**371.74**	**(34.78)a**	0.35	(0.02)bcde	**19.35**	**(0.76)a**	**22.18**	**(4.98)a**	18.84	(4.50)abcd	12.20	(2.42)a	7.60	(0.27)abc
Liquidambar formosana	284.09	(16.31)ab	0.46	(0.05)abcde	11.8	(1.60)abcde	10.89	(1.05)abc	18.82	(4.02)abcd	10.40	(0.82)a	5.24	(0.45)bcdef
Lithocarpus glaber	292.17	(140.58)ab	0.41	(0.11)abcde	13.34	(7.20)abcde	18.03	(9.21)abc	11.98	(7.22)abcd	8.81	(3.96)a	5.57	(2.94)abcdef
Machilus grijsii	235.26	(24.01)ab	0.42	(0.06)abcde	11.68	(1.42)abcde	10.56	(1.09)bc	13.91	(3.69)abcd	10.64	(2.73)a	4.88	(0.62)bcdef
Machilus leptophylla	285.49	(50.95)ab	0.39	(0.07)bcde	13.55	(2.31)abcde	12.85	(2.56)abc	19.74	(7.90)abc	14.34	(6.00)a	5.72	(0.56)abcdef
Machilus thunbergii	223.74	(53.52)ab	0.44	(0.06)abcde	8.97	(3.04)cde	**9.06**	**(1.39)c**	11.17	(2.74)abcd	8.81	(3.55)a	**3.83**	**(0.90)ef**
Magnolia yuyuanensis	285.65	(61.13)ab	**0.67**	**(0.15)a**	11.32	(1.43)abcde	11.55	(3.58)abc	15.65	(6.83)abcd	15.46	(5.68)a	4.65	(1.08)bcdef
Nyssa sinensis	321.21	(26.29)ab	0.44	(0.03)abcde	14.03	(1.24)abcde	16.46	(5.09)abc	**23.21**	**(4.30)ab**	14.01	(3.18)a	6.04	(0.57)abcdef
Phoebe bournei	243.82	(18.36)ab	0.41	(0.11)abcde	10.6	(0.53)abcde	**9.08**	**(1.98)c**	13.22	(1.91)abcd	11.40	(3.27)a	5.33	(0.61)abcdef
Quercus fabri	270.77	(65.27)ab	0.42	(0.09)abcde	10.91	(1.91)abcde	15.7	(6.66)abc	13.53	(4.46)abcd	8.74	(2.80)a	4.92	(0.84)bcdef
Quercus glauca	243.75	(55.04)ab	0.31	(0.04)cde	13.31	(4.18)abcde	10.09	(2.74)bc	10.22	(2.91)bcd	8.67	(2.74)a	4.96	(1.48)bcdef
Quercus myrsinifolia	341.00	(97.57)ab	**0.29**	**(0.01)de**	17.46	(3.79)abc	17.47	(5.25)abc	10.67	(3.52)abcd	8.38	(2.02)a	7.48	(1.44)abcd
Quercus phillyreoides	227.66	(27.04)ab	0.38	(0.08)bcde	12.36	(2.82)abcde	10.23	(1.84)bc	14.28	(1.27)abcd	11.12	(5.40)a	4.41	(0.83)cdef
Quercus serrata	230.10	(66.21)ab	0.37	(0.08)bcde	11.53	(1.60)abcde	13.53	(5.85)abc	14.39	(6.89)abcd	7.90	(3.84)a	4.28	(0.69)cdef
Rhus chinensis	287.18	(44.05)ab	**0.29**	**(0.01)de**	14.38	(0.75)abcde	12.20	(1.33)abc	13.78	(5.02)abcd	7.92	(1.98)a	5.28	(0.64)bcdef
Sapindus saponaria	201.52	(17.61)ab	0.5	(0.10)abcde	**7.97**	**(0.37)de**	**8.70**	**(0.85)c**	9.32	(0.63)bcd	7.67	(1.47)a	3.99	(0.20)def
Schima superba	315.36	(22.36)ab	0.39	(0.04)bcde	**18.21**	**(2.30)ab**	15.88	(0.44)abc	14.98	(0.29)abcd	10.26	(0.43)a	7.29	(0.26)abcde
Triadica sebifera	295.04	(23.63)ab	0.54	(0.11)abcd	13.68	(2.94)abcde	13.52	(2.16)abc	16.05	(3.92)abcd	19.22	(12.25)a	6.01	(1.00)abcdef

interplay between plant and soil characteristics that both are associated with changes in the soil microbial community.

4.3. Tree species identity effects

It has been frequently reported that belowground microbial communities are associated with different plant species (Grayston and Campbell, 1996; Wardle et al., 2004; Boyle et al., 2008; Chaparro et al., 2012). Many plant taxa are colonized by specific microbial communities (Berg and Smalla, 2009), e.g. the Pinaceae family dominantly colonized by ectomycorrhizal fungi (Smith and Read, 1997). Considering specific tree species, Priha et al. (2001) reported distinct soil microbial communities among soils under three different tree species: *Betula pendula* stands that favoured the

Table 4
Phylogenetic signal (Blomberg's *K*, Pagel's λ, Abouheif/Moran's *I* and associated *P* values) for all seven microbial lipid indicators.

	Biomass	FB-ratio	Gram+	Gram−	Actinomycetes	AMF	GF
Blomberg's *K*	0.037	0.089	0.051	0.057	0.043	0.063	0.065
P	0.679	0.079	0.359	0.273	0.521	0.221	0.227
Pagel's λ	0	0	0	0	0	0	0
P	1	1	1	1	1	1	1
Abouheif/Moran's *I*	−0.112	−0.028	−0.039	−0.092	−0.115	−0.048	−0.133
P	0.388	0.819	0.802	0.49	0.377	0.672	0.274

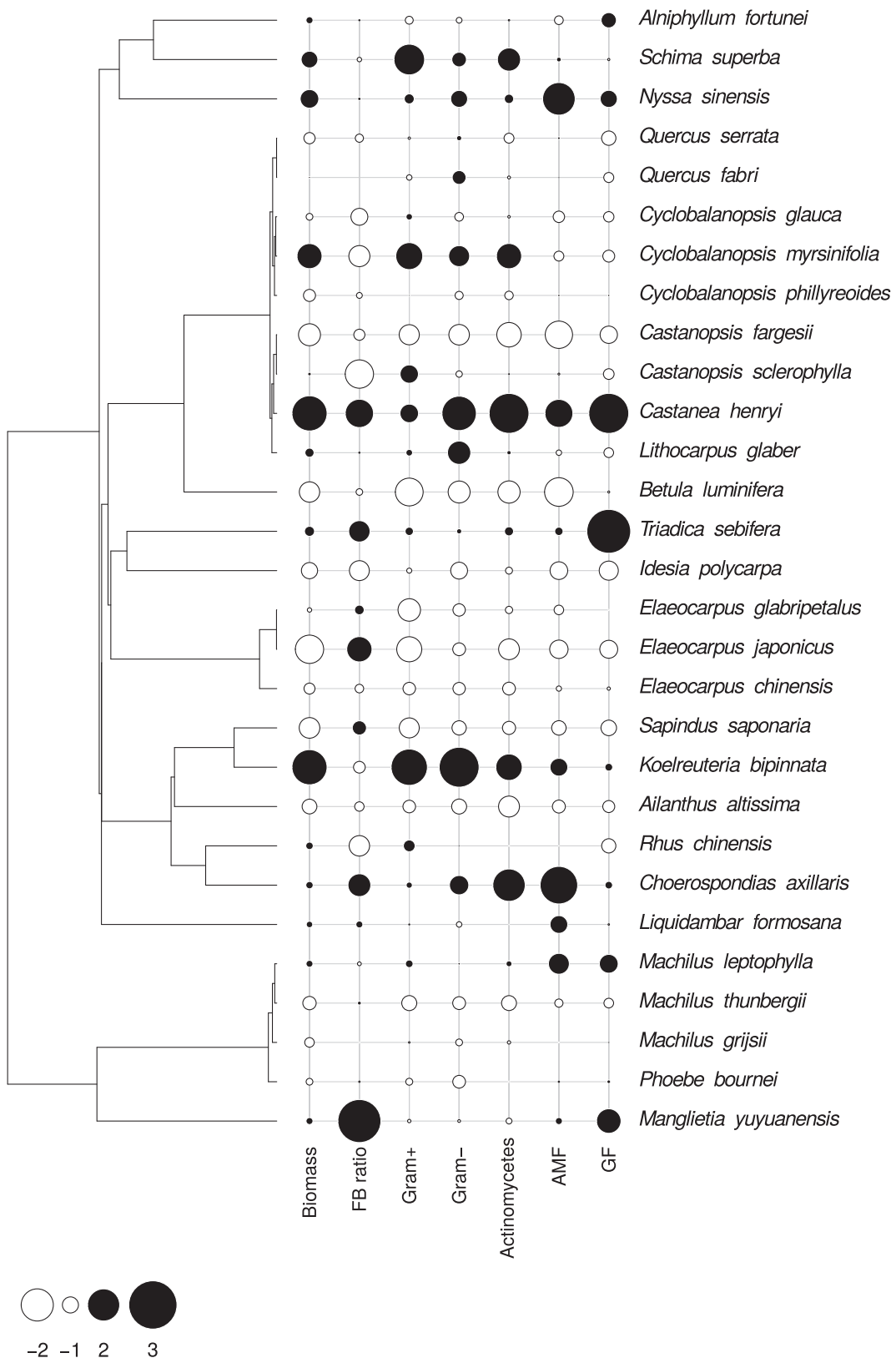

Fig. 3. Lipid total biomass, FB-ratio and biomarker abundances (standardized to mean = 0, SD = 1) mapped onto the phylogeny of the 29 tree species. Large circles correspond to high and small circles to low microbial indicator values, respectively. Open circles indicate negative and filled circles positive values.

growth of Gram− bacteria, while soil microbes associated with *Pinus sylvestris* and *Picea abies* stands had more Gram+ bacterial growth. We further investigated this observation of species effects by linking soil microbial communities to plant phylogeny. An earlier study by Reinhart et al. (2012) showed that AMF communities showed plant family (species) related conservatism along plant phylogeny. However, no such phylogenetic associations were observed in this study for either species-specific microbial biomass or abundance of specific lipid indicator groups. This indicates that closely related species do not necessarily share the similar microbial communities, even though the non-specificity in the use of lipid indicators (Frostegård et al., 2011; Ngosong et al., 2012) allows only a broad interpretation of microbial community composition. Previous studies have indicated that plant root exudates and litter inputs were the driving forces for the effects of plant species on microbial communities (Wardle et al., 2004; Prescott and Grayston, 2013), as opposed to tree relatedness. Plant species differ in quality and quantity of the resources they allocate belowground, and this in turn may trigger changes in the rhizodeposition (Grayston et al., 1996; Van der Krift et al., 2001). These inputs are a key source of nutrients available to microbes (Van Veen et al., 1989). In our study, *B. luminifera* had the highest LNC and the lowest leaf CN. Considering the negative influence of leaf N on Gram+ bacterial abundance and the importance of leaves as a carbon source, we propose this high leaf LNC and low leaf CN to be the reason that soils around *B. luminifera* have relatively lower bacterial and fungal abundances when compared to other tree species. Plant species also vary markedly in the amount and composition of root exudates and thus may alter the types of substrate available for soil microbe nutrition (Grayston et al., 1996; Somers et al., 2004). In summary, we conclude that plant species identity effects on soil microbial communities most likely reflect the sum of the influence of plant species specific root exudates and plant traits, while plant species phylogenetic relatedness may or may not closely map traits which have an important influence over soil microbial communities.

5. Conclusions

Our study examined a broad range of subtropical tree species (29 species in total) to assess the influence of local soil characteristics, specific plant traits, and tree species phylogenetic relatedness on characteristics of the soil microbial communities. Variance partitioning revealed that soil characteristics explained 28% of microbial community variance, while plant leaf traits and plant growth variables explained 18% and 11%, respectively. Thus, in addition to significant effects of soil characteristics, we found meaningful interdependencies between aboveground plant traits and belowground soil microbial community characteristics across our analyses but specifically demonstrated through variance components analysis. With regard to plant species traits, soil microbial abundances were significantly correlated with LDMC, leaf CN, tree height and GD, indicators of plant nutrition and growth, as well as to individual species, representing an influence of tree species as a sum of these individual traits. We did not find a relationship between tree species phylogenetic relationships and soil microbial communities, suggesting that tree species traits, but not evolutionary relationships, drive soil microbial community development. The close link between the soil microbial community and the plant functional traits and growth characteristics clearly demonstrates the importance of a comprehensive study on plant traits in order to explain the form and dynamics of soil microbial communities in this species-rich subtropical forest. However, our study was based on tree species grown in monocultures, which are simple communities without intraspecific tree–tree interactions. As a consequence, studies on mixed plant species community, and at later stages in tree growth, could further inform our understanding of aboveground belowground relationships in subtropical forests.

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Appendix A. Supplementary material

Supplementary data related to this article can be found at doi:10.1016/j.soilbio.2016.02.004.

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