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Research Article

RESEARCH ARTICLE

Rhizosphere effects on soil microbial community structure and enzyme activity in a successional subtropical forest

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One sentence summary: Rhizosphere-induced changes in soil microbial community and enzyme activity facilitate forest succession.

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ABSTRACT

Forest succession is a central ecological topic due to the importance of its dynamic process for terrestrial ecosystems. However, we have limited knowledge of the relationship between forest succession and belowground microbiota, particularly regarding interactions in the rhizosphere. Here, we determined microbial community structure and biomass using phospholipid fatty acid (PLFA) biomarkers and microbial activity using extracellular enzyme activity in bulk and rhizosphere soils from three successional stages of subtropical forests in eastern China. Principal component analysis of PLFAs indicated distinct soil microbial communities among different successional stages and habitat locations. Specifically for the topsoil, we found the total microbial biomass, bacterial biomass and enzyme activities showed higher levels in the late than early stage, with a significant succession-induced accentuated rhizosphere effect. The increase in total microbial biomass and activity coincided with a net growth in bacterial rather than fungal biomass, indicating a model in which microbial biomass carrying capacity and activity could be affected by the creation or expansion of niches for certain functional group rather than by a rebalancing of competitive interactions among these groups. Furthermore, we demonstrated that forest succession significantly influenced enzyme activity via the changes in microbial biomass, as driven by edaphic factors. Overall, our study deepens the mechanistic understanding of forest recovery by linking soil microbial community and activity along successional chronosequences.

Keywords: microbial community structure; microbial biomass; enzyme activity; forest succession; rhizosphere; subtropical forest

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INTRODUCTION

Forest ecosystems are a substantial and important carbon (C) pool and play a considerable role in global geochemical cycles (Mackey *et al.* 2013; Allen *et al.* 2016). Unfortunately, natural disturbances and human activities are destroying an increasing area of forests (Swanson *et al.* 2011; Ulery *et al.* 2017), including subtropical regions of China (Wang, Kent and Fang 2007). Forest succession is important for sustainable forest management because it affects a wide array of terrestrial ecosystem processes and services (Anderson-Teixeira *et al.* 2013). To date, most researches on forest succession have focused on aboveground plant characteristics, including dominant tree species, plant community assembly (Bruelheide *et al.* 2011), tree architecture variability (Yan *et al.* 2013), plant nutrient use strategies (Yan, Wang and Huang 2006) and plant biodiversity and biomass (Lasky *et al.* 2014). In contrast, fewer studies investigated the changes in microbial communities during the forest succession in subtropical regions (Gao *et al.* 2015), so the knowledge combining soil microbial communities and their functions (enzyme activity for example) along subtropical forest successional process is limited, despite that soil microorganisms play an important role in ecosystem services, particularly in the regulation of C and nutrient cycles (Lucas *et al.* 2007). Thus, understanding the ecological linkages between aboveground and belowground biota during forest succession is essential and urgent to improve our understanding of the processes of ecosystem, development and recovery.

Soil microorganisms are central to fundamental ecosystem processes and their associated ecosystem services. Most of these processes and services are directly catalyzed or indirectly modulated by the microbial communities, e.g. microbial communities are both drivers of and participants in soil organic matter (SOM) decomposition and formation through their metabolic processes (Schimel and Schaeffer 2012; Liang, Schimel and Jastrow 2017). Different microbial consortia produce different extracellular enzymes, which play an essential role in decomposing organic matter to acquire the C and nutrients that are indispensable for soil microbial growth, in turn, soil microbial communities will secrete degradative enzymes to moderate the decomposition and accumulation of SOM (Allison *et al.* 2010). In addition, soil microbial communities are sensitive to changes in external environments (Balser and Firestone 2005). For example, plant species can strongly affect the soil microbial community structure and functions via litter and root deposition through the allocation of plant C and other nutrients (Cotrufo *et al.* 2013; Khlifa *et al.* 2017). Identifying soil microbiota under environmental change together with the knowledge of community-specific functions will advance understanding of fundamental controls on the communities and their processes. Hence, one of the opportunities provided by subtropical forest succession studies is to identify those characteristics of soil microbial communities that are central to mediating soil ecosystem processes and services.

The rhizosphere is a highly dynamic soil environment, with much of the activity involving exchanges of energy and nutrients (Kuzyakov 2002; Hinsinger *et al.* 2009), and therefore has an important role in interacting with global nutrient cycles (Toberman, Chen and Xu 2011). Although most previous researchers speculated that SOM accumulation is regulated primarily by the saprotrophic decomposition of aboveground litter, there is a growing body of evidence supporting that organic layers develop from belowground through the continuous addition of recently fixed C from roots and root litter (Clemmensen *et al.* 2013). Thus, investigations into the role of the rhizosphere are essential. Root exudation and rhizodeposition provide labile C and nutrients (Koranda *et al.* 2011; Meier, Finzi and Phillips 2017), stimulating the decomposition and turnover of relatively recalcitrant compounds in the soil (Kuzyakov 2010; Huo, Luo and Cheng 2017), increasing microbial biomass (Malik *et al.* 2015), affecting microbial community structure and enzyme activity (Welc *et al.* 2014) and consequently changing the magnitude and direction of SOM storage (Miltner *et al.* 2009; Liang and Balser 2012). Accordingly, rhizosphere microbes may serve as primary contributors to forest development; however, the underlying mechanisms remain elusive due to a scarcity of data linking the microbial community, enzyme activity and environmental change during forest succession.

Here we used well-replicated successional chronosequences to evaluate the effects of rhizosphere on microbial community and enzyme activity during subtropical forest succession. Specifically, the objectives were as follows: (i) to examine the changes of microbial community and enzyme activity along three successional stages in subtropical forests, (ii) to evaluate the correlations among forest succession, microbial community biomass, enzyme activity and edaphic factors, and (iii) to determine the impacts of the rhizosphere on microbial community biomass and enzyme activity under forest succession. We hypothesized that: (i) forest succession will increase microbial community biomass and enzyme activity in soil, (ii) rhizosphere effects on microbial community biomass and enzyme activity become stronger during the progression of succession.

MATERIALS AND METHODS

Site description and sampling

The study area was located within the Tiantong Nature Reserve, along the Ningbo coast (Zhejiang Province, eastern China; 29°48′N, 121°47′E). The region is characterized by a monsoonal climate with hot, humid summers and cold, dry winters (Yan, Wang and Huang 2006). The area has a mean annual temperature of 16.2°C; the warmest month is July, with a mean temperature of 28.1°C, and the coldest is January, with a mean temperature of 4.2°C. The annual average precipitation is 1374.7 mm (Song and Wang 1995). Shrubs and broad-leaved evergreen trees are the dominant vegetations. Soils were classified as ferralsols with the parental material consisting mostly of is mesozoic sediments, some acidic igneous rocks and residual granite weathering material. The vegetation type is subtropical evergreen broadleaved forests (EBLFs). The vegetation has been severely disturbed, and the secondary shrubs are mostly formed after the cessation of repeated harvesting. After 20–25 years in the shrub stage the succession proceeds to sub-climax EBLFs (50–55 years) because of the dense stem density. At the mature stage of succession, the climax EBLFs form 105–120 years after the cessation of forest harvest (Yan *et al.* 2009).

We sampled the soils from these three successional stages from two replicated secondary forest succession sequences. One was located to the west of Tiantong Temple and included three plots in secondary shrubs, three plots in sub-climax *Schima superba* forest and three plots in climax *Castanopsis fargesii* forest. The other secondary forest succession sequence was located to the east of Tiantong Temple and included one plot in secondary

shrubs (two other plots were destroyed by human disturbance), three plots in sub-climax *Schima superba* forest and three plots in climax *Castanopsis carlesii* forest. Forests representing each successional stage were located on similar slopes and had similar natural histories and mineral soils developed from quartzite bedrock (Yan, Wang and Huang 2006). Each plot was 20 × 20 m and located at least 100 m from the forest edge.

We collected soil cores at five random locations from each plot. To minimize the topographic disturbance on soil sampling, all selected plots were located in similar slope and distance areas. At each plot, after the removal of litters and organic horizon (no organic horizon for early stage) from the soil surface, two 10 cm diameter cores were collected, one representing the topsoil (0–15 cm) and the other representing the subsoil (15–30 cm). Each soil sample was placed in a plastic bag and samples were transported to the laboratory on ice. Within 48 h, rhizosphere soil was separated from bulk soil by hand. First, fine root and adhering soil were carefully picked out with forceps and shaken gently to remove loose soil; then rhizosphere soil was collected with forceps for the part adhering to roots after shaking by hand (Phillips and Fahey 2008; Angst *et al.* 2016). Carefully collected, root-free soil was defined as the bulk soil fraction (Brzostek *et al.* 2012). Bulk soil fractions were collected from the same cores and transect to rhizosphere soil in order to ensure that the paired soils were processed similarly. After root hairs and gravel were removed from each soil fraction by hand, the five soil samples from each plot were combined to create a single composite top-rhizosphere, top-bulk, sub-rhizosphere and sub-bulk sample, totally 64 samples for all plots. Three subsamples were collected from each pooled soil fraction: one was air dried for chemical analyses; a second was stored at 4°C for determining enzyme activities, soil moisture, dissolved organic C (DOC) and available nitrogen (N); and a third was freeze dried for phospholipid fatty acid (PLFA) analysis.

Soil analyses

Soil properties analyses

Total carbon (TC) and total nitrogen (TN) were determined in dry soil using combustion gas chromatography (EL III Elemental Analyzer; Elementar Analysensysteme GmbH, Hanau, Germany). We interpreted TC as soil organic C (SOC) because all soil samples had pH < 7.0. DOC was extracted from fresh soil by shaking in 0.5 M K_2SO_4 (1:5 w/v) for 0.5 h, filtered using a 0.45 μm Millipore filter and then detected with a Multi N/C 3000 (Analytik Jena, Jena, Germany). Available N included nitrate and ammonium nitrogen extracted from 10 g of fresh soil with 2 M KCl (soil: extract = 1:5) and was analyzed using a flow-injection auto analyzer (SEAL, AA3, Germany). Soil pH was determined by the slurry method and using a glass electrode (soil: H_2O = 1:2.5). Soil moisture was measured according to the weight loss after drying at 105°C for 48 h (Zhang *et al.* 2013).

PLFA analysis

Microbial community composition was assessed using PLFA analysis (Bligh and Dyer 1959), with modifications described by Certini, Campbell and Edwards (2004). Briefly, PLFAs were extracted from freeze-dried, homogenized top-rhizosphere, top-bulk, sub-rhizosphere and sub-bulk soils (3g for top-rhizosphere soils and 6g for top-bulksoils and subsoils, respectively) using a chloroform–methanol–citrate buffer (1:2:0.8 v/v/v). Phospholipids were separated from neutral lipids and glycolipids on a solid phase extraction (SPE) column (Supelco Inc., Bellefonte, PA). The phospholipids were trans-esterified using methanolysis under mildly alkaline conditions (Guckert *et al.* 1985; Bossio *et al.* 1998), and the resulting fatty acid methyl esters were extracted into hexane and dried under a stream of nitrogen gas. The methyl esters were prepared for GC analysis by dissolution into 150 μl of hexane and spiking with 50 μl of nonadecanoic acid methyl ester (19:0; Sigma-Aldrich) as an internal standard. These 200 μl solutions were introduced to an Agilent 7890 series gas chromatograph (Germany) with MIDI peak identification software (version 4.5; MIDI Inc., Newark, DE). Thirty-one different PLFAs, including saturated, monounsaturated, polyunsaturated, cyclopropyl and methylates were identified. We focused on sixteen PLFAs (i14:0, i15:0, a15:0, 15:0, i16:0, 16:1ω5c, 16:1ω7c, 16:0, i17:0, a17:0, cy17:0, 17:0, 18:2ω6c, 18:1ω9c, 18:1ω7t, cy19:0) that consistently appeared in the samples. Fatty acids signifying bacterial PLFAs included i14:0, i15:0, a15:0, 15:0, i16:0, 16:1ω7c, 16:0, i17:0, a17:0, cy17:0, 17:0, 18:1ω7t and cy19:0 (Smyth, Macey and Trofymow 2015). Within this collection, i14:0, i15:0, a15:0, i16:0, a17:0 and i17:0 represented Gram-positive (Gm^+) bacteria and cy17:0, 16:1ω7c, 18:1ω7c and cy17:0 represented Gram-negative (Gm^-) bacteria (Bååth and Anderson 2003; Mitchell *et al.* 2015). Fatty acids signifying fungal biomass included 18:2ω6c, 18:1ω9c and 16:1ω5c (Frostegård and Bååth 1996; Smith, Marín-Spiotta and Balser 2015). The sum of all fatty acids reflected the overall abundance of bacteria and fungi. We admit there are somewhat limitations on the PFLA method to fingerprint microbial community structure due to non-specific origin and extremely-high microbial diversity in soil, but the method is considered sensitive and powerful to detect changes in microbial community in some soils (Frostegård, Tunlid and Bååth 2011; Liang *et al.* 2016).

Enzyme analysis

Important enzymes involved in C and N cycling processes include β-glucosidase (BG) and cellobiohydrolase (CB) (involved in the decomposition of cellulose and C polymers) (Jing *et al.* 2017), and β-N-acetylglucosaminidase (NAG) and leucine aminopeptidase (LAP) (catalyze the decomposition of chitin, leucine and other hydrophobic amino acids from the N terminus of polypeptides in SOM) (Peng and Wang 2016; Feng *et al.* 2018). These potential soil extracellular enzyme activities were measured using the protocol by German *et al.* (2011). Enzyme activity was determined using 96-well microtiter plates. The analysis involved 8 replicate wells for each blank, a negative control and a standard for one sample. Sample suspensions were prepared by adding 1.5 g of soil to 125 ml of 50 mM, pH 5.0, sodium acetate buffer and homogenizing for 1 min. The supernatant was continuously stirred using a magnetic stir plate, and 200 μl aliquots were dispensed into 96-well microplates (each sample included three rows). Blank wells received 50 μl of sodium acetate buffer and 200 μl of sample suspension in the first row; the second row, in addition to add 200 μl suspension, received amounts of 4-Methylumbelliferone (MUB), 7-Amino-4-methylcoumarin (AMC) was used instead for the LAP assay; and the third row received an additional 50 μl of substrate solution and 200 μl suspension. The microplates were incubated in the dark at 25°C for 2.5 h. The fluorescence of the plates was read at 365 nm excitation and 450 nm emission with a Microplate Reader (BioTek, Synergy2, USA). The enzyme activities were expressed in units of nmol h^{-1} g^{-1}.

Statistical analyses

A linear mixed model analysis was used to test for effects of succession, depth and rhizosphere on microbiological (microbial community structure and enzyme activities) and soil variables (the concentrations of SOC, TN, soil moisture, DOC, available N and pH), with site replicate as the random factor, using

the 'nlme' package in R. The *P*-values were referred to analysis of variance (ANOVA). Soil and microbial community structure and enzyme activity traits were compared between topsoil and subsoil, rhizosphere and bulk soil, and for differences among the three successional stages using multiple comparisons based on the least significant difference (LSD) test. Data analysis was conducted in the R statistical environment (version 3.0.3).

We conducted principal components analysis (PCA) to investigate the shift in microbial communities. All data requires a percent analysis before performing PCA. PLFAs of the soil samples were subjected to PCA using CANOCO software 4.5 (Braak 1988). A redundancy analysis (RDA) was performed between microbial community structure and environmental variances (rhizosphere, succession, soil depth, pH, SOC, TN and soil moisture). The significance of each explanatory variable included in the models was evaluated using 999 permutations. We also compared the respective correlation (adjusted R^2) of each environmental variable alone with the species data using variation partitioning (Peresneto *et al*. 2006). Statistical tests were considered significant at $P < 0.05$. The ordination diagram showing the relationships between enzyme activity and the significant factors was based on the RDA results. We also conducted structural equation modeling (SEM) analysis, a multivariate statistical method that allows for hypothesis testing of complex path-relation networks (Grace *et al*. 2007), to gain a mechanistic understanding of how forest succession affects edaphic factors, microbial community biomass and enzyme activity. We constructed a priori model based on our knowledge of the relative contributions and interactions of binding agents to forest succession. The model-implied variance–covariance matrix was compared to the against the observed variance–covariance matrix with maximum likelihood estimation. We used the χ^2 and its associated *P*-value to judge the model fitness and conducted SEM analysis using the AMOS 7.0 software.

RESULTS

Soil physicochemical properties

Late stage soils had significantly higher SOC, TN, DOC, available N and soil moisture than the early stage soils for the rhizosphere and bulk topsoil ($P < 0.05$), with intermediate values in the middle successional stage. In subsoil, late stage soils also had significantly higher SOC, TN, DOC and soil moisture than the early stage soils in the rhizosphere, but SOC, TN and available N were not significantly different in bulk soil. All soils were acidic (pH 4.17 to 4.52), with pH values lower in rhizosphere soil than in bulk soil and lower in topsoil than in subsoil (Supplementary Table 1, Supporting Information).

Soil microbial community biomass and pattern

The overall composition of the soil microbial community was analyzed with PCA. PC1 and PC2 explained 44.3% and 25.6% of the total variation, respectively, showing the effect of forest succession on PC1 and the effects of soil depth and rhizosphere on PC2. Forest succession formed a distinct cluster on the PCA ordination (Fig. 1).

Forest succession did not affect fungal biomass ($P > 0.05$); however, in the rhizosphere soil, the biomass of total PLFAs, bacterial, and Gm^+ and Gm^- bacteria was significantly higher in the late than in the early successional stage ($P < 0.05$), with intermediate values in the middle successional stage (Fig. 2). The differences ($P < 0.05$) in the biomass of total PLFAs, bacteria, Gm^+ and Gm^- bacteria, and fungi were significant between the rhizosphere soil and the associated bulk soil (except early stage in subsoil) (Supplementary Table 3, Supplementary Fig. 1 (Supporting Information)) and between the topsoil and the corresponding subsoil (Supplementary Table 4, Supporting Information). A significant interaction effect (Supplementary Table 2, Supporting Information) was detected for rhizosphere and depth with respect to overall microbial PLFA structure ($P < 0.001$).

Soil enzyme activity

The potential enzyme activity of C- and N-degrading extracellular enzymes in rhizosphere soil and N-degrading enzyme activity in bulk soil were significantly higher ($P < 0.05$) in middle and late forest successional stages than that in the early stage in surface topsoil, whereas the C-degrading enzyme activity of bulk soil was higher in the late stage than in early and middle stages (Fig. 3). No differences were detected in the deep soil layer ($P > 0.05$) (Fig. 3). The C-degrading and N-degrading enzyme activities were significantly higher in the rhizosphere soil than in bulk soil in topsoil, with the exception of the C-degrading in early stage; however, no differences were detected in subsoil with the exception of the N-degrading in early stage (Supplementary Table 5, Supporting Information). Additionally, soil extracellular enzyme activities were significantly higher ($P < 0.05$) in topsoil than in subsoil with the exception of both in early stage and C-degrading in bulk soil, in middle stage (Supplementary Table 6, Supporting Information). A significant interaction ($P < 0.001$) was detected among forest successional stage, rhizosphere and depth effects for both C- and N-degrading enzyme activities (Fig. 3; Supplementary Table 2, Supporting Information).

Correlations among forest succession, microbial community biomass, enzyme activity and edaphic factors

Edaphic and ecological grouping factors explained most of the variability in soil microbial community (Table 1). Within the ecological grouping factors, rhizosphere and succession had large individual contributions to the soil microbial community; within the edaphic factor, SOC, TN and soil moisture were the predominated to influence soil microbial community (Table 1). The SEM, informed by the variance partitioning analysis, showed that forest succession indirectly affected microbial community biomass and enzyme activity, primarily by impacting SOC, i.e. succession-induced SOC dynamics influenced microbial community biomass, then microbial community in turn drove the dynamics of enzyme activity ($\chi^2 = 2.513$; $df = 3$; $P = 0.473$). Those variables have a causal relationship with forest succession, which explained 59% of the variation in enzyme activity (Fig. 4).

Rhizosphere effects on microbial community biomass and enzyme activity

The rhizosphere effect (using rhizosphere minus bulk soil to represent rhizosphere effect) for C-degrading enzyme activity was significantly ($P < 0.05$) higher in the forest successional middle and late stages than in the early stage. The rhizosphere effect for N-degrading enzyme activity was significantly ($P < 0.05$) higher in the late stage than in the early and middle stages (Fig. 5). The linear correlations were detected between rhizosphere effect on

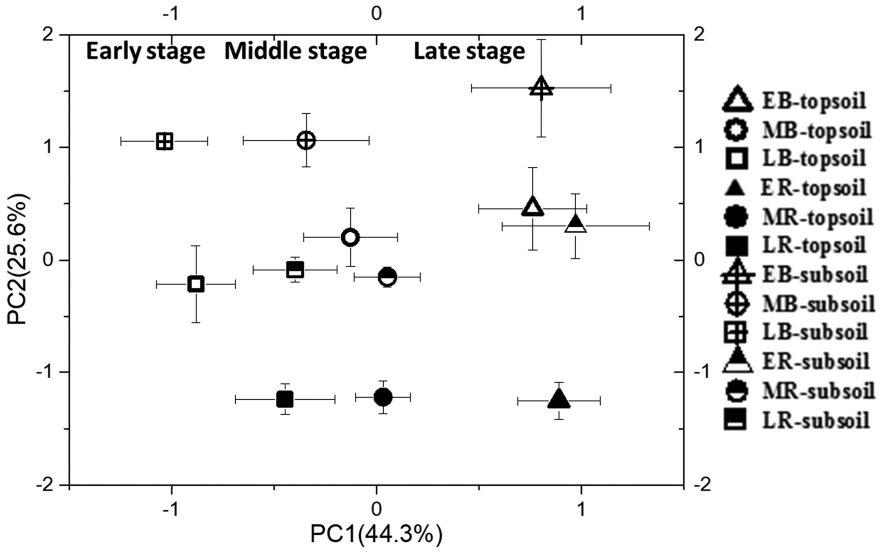

Figure 1. Principal component analysis (PCA) of soil microbial communities along forest succession in Tiantong National Forest Park, eastern China. Error bars represent standard errors of the means (n = 4 or 6). Note: Only the first two axes are displayed. Triangles, circles and squares represent successional early stage, middle stage and late stage, respectively. The difference in the style of symbols indicates whether the samples are topsoil or subsoil and rhizosphere or bulk soil.

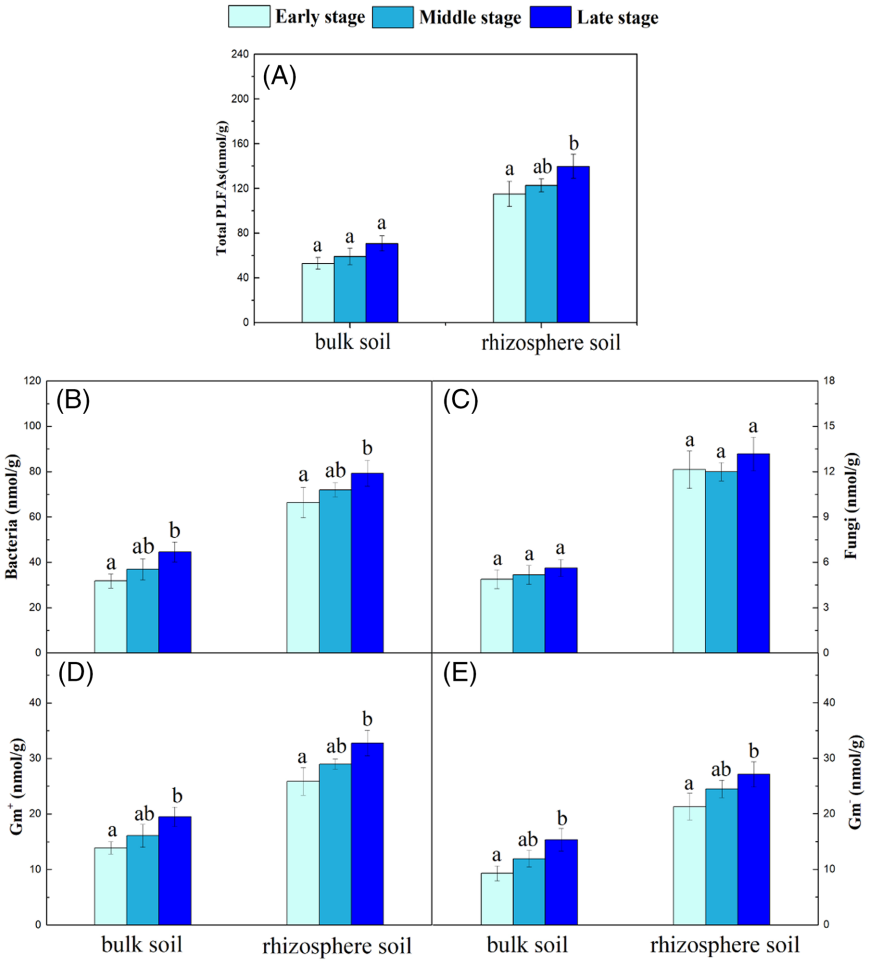

Figure 2. Microbial functional group abundances in topsoil (based on PLFA analysis) under subtropical forests in three successional stages in Tiantong National Forest Park, eastern China. **(A)** Total PLFAs; **(B)** bacterial PLFAs; **(C)** fungal PLFAs; **(D)** Gm^+ PLFAs; and **(E)** Gm^- PLFAs. Bars represent standard errors (n = 4 or 6). Bars sharing the same letter are not significantly different ($P = 0.05$, LSD test). Gm^+, Gram-positive bacteria; Gm^-, Gram-negative bacteria.

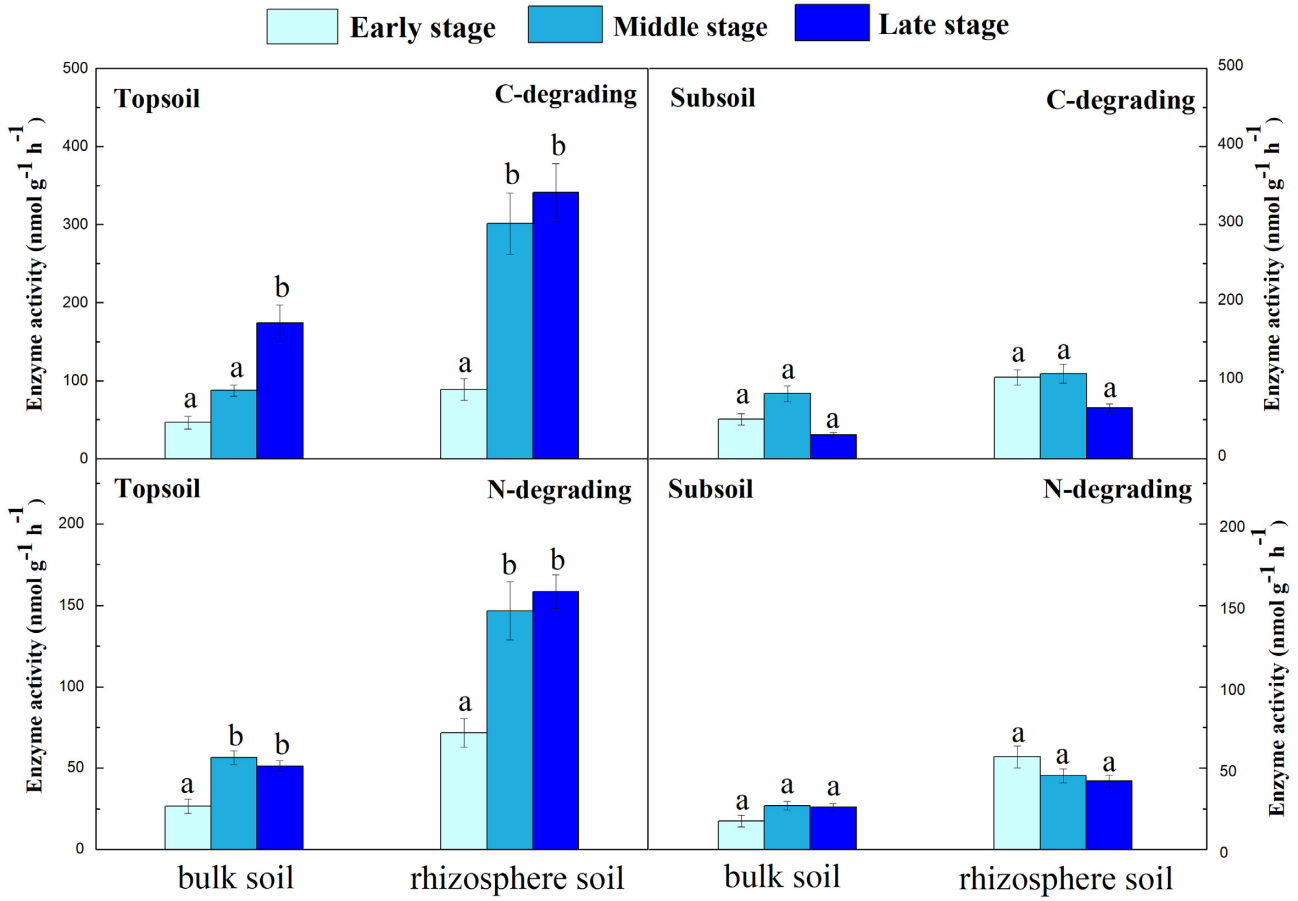

Figure 3. Enzyme activities in forest soil of C-degrading enzymes in topsoil and subsoil, N-degrading enzymes in topsoil and subsoil under subtropical forests in three successional stages in Tiantong National Forest Park, eastern China. Bars indicate standard errors (n = 4 or 6). Bars sharing the same letter are not significantly different (P = 0.05, LSD test). C-degrading enzyme activity was represented by potential enzyme activities of BG + CB; N-degrading enzyme activity was represented by potential enzyme activities of NAG + LAP.

Table 1. Percentage of variance of edaphic and ecological grouping variables in explaining variation in the soil microbial community of the three forest successional stages based on RDA analysis.

Variables	Microbial community	
	Adjust R^2	P-value
Edaphic factor		
SOC	14.77%***	0.001
TN	14.54%***	0.001
SM	14.53%***	0.001
pH	8.79%***	0.001
Ecological grouping factors		
Rhizosphere	13.67%***	0.001
Succession	13.62%***	0.001
Depth	8.31%***	0.001
Groups of variables		
Edaphic (SOC + TN + SM + pH)	25.04%***	0.001
Ecological grouping (Rhizosphere + Succession + Depth)	36.78%***	0.001

total microbial biomass and that on enzyme activities (including C- and N-degrading enzyme activities) (Fig. 5).

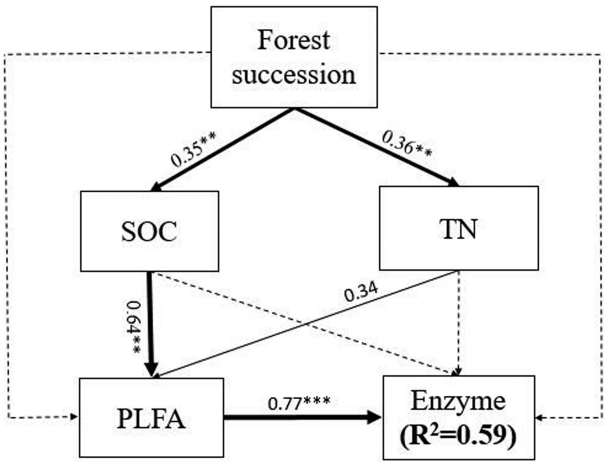

Figure 4. This diagram of the Structural Equation Model for forest succession effects on SOC and TN, microbial community biomass, and enzyme activity. Significant pathways are bold solid line in the model, non-significant pathways are not. Numbers on arrows are standardized path coefficients. Numbers in bold indicate the variance explained by the model (R^2). Arrow thickness represents the magnitude of the path coefficient. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

DISCUSSION

Changes in microbial community structure and biomass along forest succession

The PCA results showed that the microbial communities for the three forest successional stages were dissimilar (Fig. 1). The increase in Gm^+ and Gm^- bacterial abundance in the microbial community along the natural forest successional gradient (Fig. 2) might be due to the improved substrate supply as reflected in the increases in SOC and TN (Table 1) from early to climax stage, as different plant-based traits at different successional stages have strong effects on SOC and TN (Shao *et al.* 2017). This pattern is consistent with the more remarkable microbial abundances found in rhizosphere than in bulk soil (Fig. 2). In contrast to some studies that showed bacteria and fungi both increased during temperate forest succession (Shao *et al.* 2019), or bacteria predominated in the early forest succession stage but fungi in the late stage based on a global comprehensive database (Zhou *et al.* 2017), our study revealed a significant increase in total microbial biomass from early to late successional stages for the both rhizosphere ($P = 0.01$) and bulk soils ($P = 0.07$) coinciding with a net growth in bacterial rather than fungal biomass. These results suggest a model in which microbial community change and microbial biomass carrying capacity are affected by the creation or expansion of niches for certain functional group, rather than by a rebalancing of competitive interactions among these groups in subtropical forests.

Previous studies reported that dominant tree species mediated soil microbes through accumulated SOC from their corresponding litters (Urbanová, Šnajdr and Baldrian 2015; Winsome *et al.* 2017) and roots (Ladygina and Hedlund 2010). The litter of *C. fargesii* vegetation (late stage dominant species) provides a 'rich' substrate for microbial growth as reflected by the highest plant leaf nutrient and root N concentrations (Yan, Wang and Huang 2006), shortest leaf life span and fastest decay rate (Yan *et al.* 2009); all these species characteristics promote the movement of nutrient inputs into belowground soil by stimulating microbial growth, in associated with the changes in microbial community structure and biomass (Smyth, Macey and Trofymow 2015). Moreover, the uptake of nutrients by *C. fargesii* is a form of 'resource spending' when the nutrient supply increases (Yan, Wang and Huang 2006), which might also explain the highest soil microbial PLFAs in the late stage of forest succession. In addition to the expected strong contributions of SOC and TN to microbial community structure during the growth process from the early stage to late stage of forest succession, we found that the soil moisture was another influential edaphic factor, serving as a limiting factor for microbes to access and utilize a substrate (Schimel and Schaeffer 2012). An increase in soil moisture may increase the supply of substrates to microbes through diffusion (Manzoni *et al.* 2014).

Relationships among forest succession, microbial community biomass, enzyme activity and edaphic factors

As expected to our hypothesis, enzyme activity and microbial community biomass significantly increased along forest succession process, where the succession effects were more remarkable in topsoil than in subsoil and in rhizosphere soil than in bulk soil (Supplementary Table 4, Supporting Information; Figs 2 and 3).

SOC content was a good predictor of the major differences in soil enzyme activities (Wallenius *et al.* 2011). Simulation of SOC and nutrients on microbial growth was well documented (Morrissey *et al.* 2017; Shahbaz, Kuzyakov and Heitkamp 2017). One of the mechanisms that have been proposed regarding the influence of C and N on microbial community and activity is that 'the rich get richer, and the poor get poorer'. Under the stress of a physiological niche with low C and N resources, microbes utilize those resources only to maintain cell integrity and viability, and enzyme production is reduced to near zero with the beginning of substrate resource decomposition (Burns *et al.* 2013; Castle *et al.* 2017). Previous studies also indicated that differences in microbial communities were related to differences in soil nutrient availability, and soil nutrient availability has a positive relationship with microbial activity based on the 'Evolutionary-Economic Principles' model for enzyme production strategies, which minimize C and nutrient costs to the cell and maximize associated benefits (Allison *et al.* 2010; Burns *et al.* 2013). In our study, similar trends for soil microbial community biomass and enzyme activity during forest succession are expected, because soil enzymes are mainly secreted by soil microorganisms (Waring, Weintraub and Sinsabaugh 2013).

The SEM analysis showed a clear pathway that forest succession might have a strong causal relationship with enzyme activity through changing microbial community biomass, which was significantly regulated by SOC (Fig. 4). As enzymes are important agents for degrading SOM, the effect of the microbial community on enzyme activity occurs through the regulation of ecosystem processes such as C and N degradation. Consistent with this observation, recent studies found that the microbial community had consequences for the activity of enzymes directly for C degradation (Trivedi *et al.* 2016) and for the mediation of N cycling (Balser and Firestone 2005). So, we speculated that enzyme activity might in turn facilitate forest succession. Although a previous study showed that pH was an important soil factor affecting the soil microbial community (Rousk *et al.* 2010), this is not consistent with our results, most likely because all soils in our study were acidic soils with pH values that varied over a relatively narrow range.

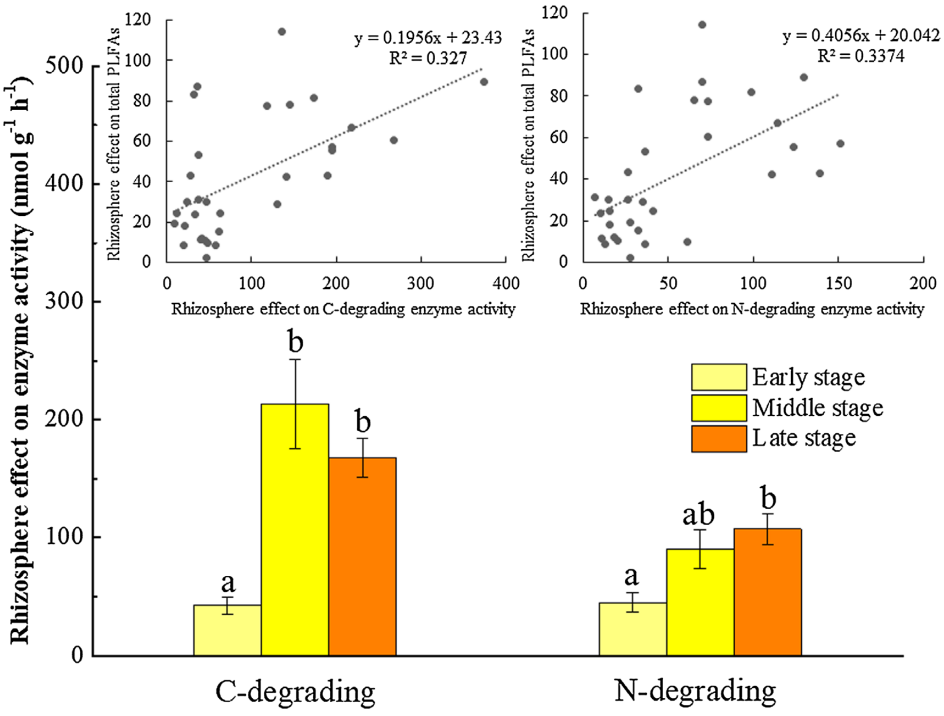

Figure 5. Rhizosphere effect (using rhizosphere minus bulk soil to represent rhizosphere effect) on enzyme activity analysis along forest succession. The inserted figures show linear correlation between rhizosphere effect on total PLFAs and that on enzyme activities under three successional stages in Tiantong National Forest Park, eastern China. Bars indicate standard errors (n = 4 or 6). Bars sharing the same letter are not significantly different ($P = 0.05$, LSD test).

Implications of rhizosphere along subtropical forest succession

The highly dynamic environment around plant roots affects soil microbial communities through exchanges of energy and nutrients within the soil. The rhizosphere strongly affected enzyme activity in each stage of forest succession (Fig. 1). Our results support that rhizosphere processes are more important than bulk soil processes for soil functioning in ecosystems, which is not in surprise because the use of organic components released from plant roots by soil microbial communities is a key process linking atmospheric and terrestrial C fluxes (Paterson *et al.* 2007). We found that the rhizosphere effects on microbial community biomass and enzyme activity became gradually stronger along forest succession (Fig. 5). The edaphic factors around roots are modified by a range of processes that occur during forest succession, including changes in C and other nutrients, which in turn affect the rhizosphere microbiota (Philippot *et al.* 2013). The mineral weathering ability of bacterial communities is strongly dependent on the nutrients available in soil (Uroz *et al.* 2011), and mineral weathering is more rapid in the rhizosphere than in bulk soil (Calvaruso, Turpault and Frey-Klett 2006). This difference might explain the larger rhizosphere effects on the microbial community biomass in the late successional stage. In our research site, fine root biomass was significantly higher in the early successional stage of forest than that in the late stage, while the C and N contents in fine root were higher in the late successional stage relative to the middle and early one (Liu *et al.* 2019). In addition, SOC and TN contents, microbial community biomass and enzyme activity were higher in rhizosphere in late successional stage relative to the early and middle one (Supplementary Table 1, Supporting Information; Figs 2 and 3). These jointly indicated that the effects of rhizosphere on soil SOC, TN, microbial community biomass and enzyme activity in soil were driven by the contents of C and N in root, not by the root biomass during forest succession. Consequently, we speculate that the rhizosphere effects may be the primary mechanism that drives forest development and succession, as controlled by C and N contents in root.

We developed a simplified conceptual model to demonstrate that forest succession affected enzyme activity under the influence of SOC, TN, soil moisture and the microbial community; furthermore, microbial enzyme activity drives forest succession by affecting mineralization of SOM (Fig. 6). Different forest successional stages have different tree species, and dominant tree species identity is a stronger driver to impact soil properties, especially SOC and TN (Dawud *et al.* 2016; Zederer *et al.* 2017). Cell-to-cell communication (quorum sensing) by bacteria releases chemical signal molecules called autoinducers, which microbes can use to sense when diffusion rates are favorable for enzyme production and extracellular foraging (Redfield 2002) to increase enzyme activity; when the microbial biomass is sufficient, the signal concentration reaches a threshold, and the 'quorum' is expected to be present (Miller and Bassler 2001). Extracellular enzyme activities further affect SOM decomposition and facilitate plant growth promotion (Burns *et al.* 2013).

CONCLUSION

Our study showed that microbial community and enzyme activity were significantly affected by forest succession, rhizosphere and soil depth, and significantly lower microbial biomass and enzyme activity were associated with the early stage, bulk soil and subsoil than that with the late stage, rhizosphere soil and topsoil, respectively. Forest succession had significant influence on enzyme activity through the changes in microbial community biomass, which was controlled by SOC. We speculated that the increases in enzyme activities might further facilitate tree growth since they enhanced the degradation of plant litter and

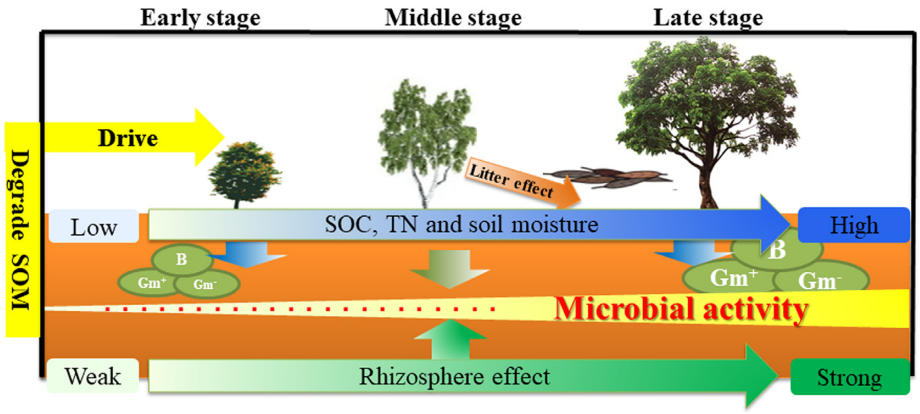

Figure 6. A simplified conceptual model to explain that forest succession is influenced by microbial activity as affected by edaphic properties, particularly in the rhizosphere. Forest succession is the most significant ecological factor contributing to microbial community biomass and activity, and microbial activity promotes tree growth through the degradation of soil organic matter, affecting the process of forest succession. B, bacteria; Gm^+, Gram-positive bacteria; Gm^-, Gram-negative bacteria.

SOM. Our findings also highlight how the effects of the rhizosphere on microbial communities and enzyme activity differed during the successional process, suggesting that the rhizosphere exerts a relatively systematic influence on microbial community and enzyme activity, and plays a significant role in the progress of forest succession through highly dynamic exchanges of nutrients. Our findings provide new insight into how forest succession affects microbial community and functions with respect to impacts of the rhizosphere along forest succession, which can provides the conceptual basis theory for modeling.

SUPPLEMENTARY DATA

Supplementary data are available at *FEMSEC* online.

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Conflict of interest. None declared.

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