



# Changes in soil microbial community structure and function after afforestation depend on species and age: Case study in a subtropical alluvial island

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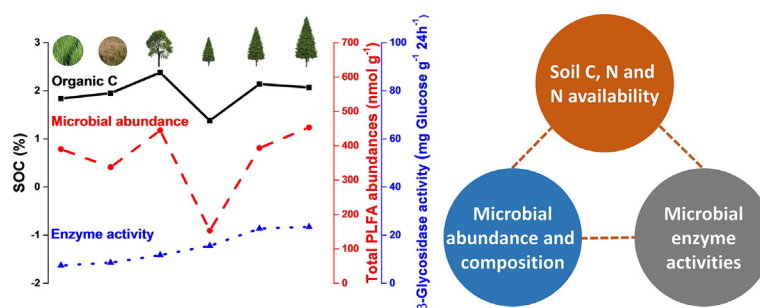
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## HIGHLIGHTS

- Microbial community structure and function significantly varied among land use types.
- Afforestation improved soil quality, but this effect varied with tree age and species.
- Dawn redwood soils had lower fungal-to-bacterial ratio compared to camphor soils.
- Significant relationships were found between soil chemical and biological variables.
- Microbial communities share a common pattern of functional organization.

## GRAPHICAL ABSTRACT



**Long-term afforestation is needed for improving soil quality**

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## ABSTRACT

It is well established that land use change can have a profound impact on soil physicochemical properties but the associated changes in soil microbial communities are poorly understood. We used long-term research sites in a subtropical alluvial island of eastern China to measure changes in soil physicochemical properties and microbial community abundance and composition (via phospholipid fatty acid (PLFA) analysis) and function (via extracellular enzyme activity) across different land use types developed on the same soil matrix, including a camphor (*Cinnamomum camphora*) plantation, a chronosequence of differently aged dawn redwood (*Metasequoia glyptostroboides*) plantings, a deforested land and a rice paddy. We hypothesized that afforestation could improve soil quality by enhancing carbon (C) and nitrogen (N) contents, microbial biomass and enzyme activities, but that this effect would vary depending on forest age and tree species. Soil C and N concentrations, PLFA abundances and activities of decomposition enzymes ( $\beta$ -glucosidase, urease, alkaline phosphatase and catalase) in older plantations all increased significantly compared to cropland. These variables changed little or decreased in deforested land compared to cropland. These variables also increased with planting age in the dawn redwood plantings. Soils under camphor plantations had higher soil nutrient contents, microbial biomass and lower enzyme activities than dawn redwood soils with similar age. We also found some significant relationships between soil chemical and biological properties: PLFA abundances were positively related to soil organic matter (SOM) contents; the fungal-to-bacterial ratio and fungal relative abundance were correlated positively with SOM contents and negatively with C/N ratio; both soil PLFA abundances and enzyme activities were positively linked with soil inorganic N content and potential net N mineralization rate; ratio of specific C, N and P (phosphorus)

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acquisition activities was limited to 10: 1: 10 across land use types. Our study underscores the fact that land use type can have a profound impact on soil microbial communities; in addition, tree species and planting age also play significant roles in afforestation.

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## 1. Introduction

Land-use change drives variations in soil environmental conditions and soil microbial communities (Guo et al., 2016). In China and many other countries, the area of tree plantations is rapidly increasing, much of it on former-agricultural lands (Deng et al., 2014). Many studies have documented the influence of afforestation on soil physicochemical properties (Paul et al., 2002; Georgiadis et al., 2017), but fewer studies have followed changes in microbial communities, especially in subtropical ecosystems, despite the important role of microorganisms in biogeochemical cycling (Smith et al., 2015).

Land use affects the form of plant inputs to the soil, resulting in a profound effect on soil microbial communities utilizing this resource. Microbial biomass can differ between forested and agricultural lands (Huygens et al., 2011), between coniferous and broadleaved trees (Xiao et al., 2016), and change with tree age (Chatterjee et al., 2009). Furthermore, forested lands can support distinctly different microbial communities compared with agricultural lands (Bossio et al., 2005). For example, following reforestation (Cavagnaro et al., 2016) or afforestation (Deng et al., 2016) of agricultural lands, more low-quality litter is introduced and soil environments are changed, which are likely to cause a shift from bacterial to fungal dominance in soil communities. Higher fungal-to-bacterial (F/B) ratio may also be observed in coniferous forests with lower-quality litter than broadleaved forests with higher-quality litter (Chang et al., 2016).

Enzymes play key roles in soil organic matter formation and degradation, nutrient cycling, and decomposition of xenobiotics. Enzyme activities can be used to indicate changes in soil quality due to land use change and for understanding soil ecosystem functioning (Acosta-Martínez et al., 2007). A wide range of microbial enzymes are involved in soil organic matter (SOM) decomposition. For example,  $\beta$ -glucosidase is involved in C cycling and catalyzes the final steps of degradation of cellulose into glucose; urease is involved in N cycling and hydrolyses nitrogen compounds to ammonium; phosphatase is involved in phosphorus (P) cycling and plays an essential role in the mineralization of organic P; catalase is an oxidoreductase associated with aerobic microbial activity (García-Gil et al., 2000). Although many studies have compared enzyme activities across land use types (Acosta-Martínez et al., 2007; Tosi et al., 2016), among tree species (Chang et al., 2016) or following stand age (Tian et al., 2015), few studies have taken all three aspects into account.

As the world's largest alluvial island, Chongming Island is situated at the mouth of the Yangtze River in eastern China. Dongping National Forest Park on the island has not been planted or cultivated until 1959, thus providing an ideally uniform soil matrix. Based on these considerations, in the park, we selected six land use types (one rice paddy, one deforested land, one camphor plantation and three dawn redwood plantings of different ages) to quantify changes in soil C and N contents, N cycling, and biomass, structure and activity of the soil microbial community. We hypothesized that afforestation could improve soil quality by enhancing elemental contents, microbial biomass and enzyme activities while deforestation could have an opposite impact, and this effect would increase with tree age in the dawn redwood plantations. We expected higher SOM contents, microbial biomass and function and lower F/B ratio in soils under broadleaved camphor trees compared to those under similarly-aged coniferous dawn redwood plantings. We also anticipated some close links between soil physicochemical properties and microbial communities, e.g., positive relationships between SOM

contents and PLFA abundances as well as between soil C/N ratio and F/B ratio, and associations among soil N availability and microbial communities.

## 2. Materials and methods

### 2.1. Study area description

In April 2016, this study was conducted at the Dongping National Forest Park (31°41'N, 121°28'E) located on Chongming Island of Shanghai City in eastern China. Developed from mud flat, the island is 3–4 m above sea level and characterized by a subtropical humid climate, with mean annual temperature of 16.6 °C and mean annual rainfall of 1072 mm between 2009 and 2013 (Yin et al., 2016). As of 2015, the island covers 1185 km<sup>2</sup> and has a population of 696,400 (Shanghai bureau of statistics, 2016). Although close to a densely populated area, the park has not been planted or cultivated before 1959; in 1959 the park was planted with paddy rice to improve soil quality and most of the area has been converted to tree plantations since 1970 (Xiao, 2010). Within the park, we selected six land use types: one cropland for paddy cultivating since 1959, one 20-year camphor forest, three dawn redwood forests ~16, 25, and 35-years prior to sampling, and one deforested land covered with weeds where a 25-year *Cryptomeria fortunei* plantation was clear-cut in 2010. Both broadleaved camphor and coniferous dawn redwood trees are native species and widely planted in the city.

### 2.2. Field sampling

Three independent sites were selected for every land use type, and one 20 m × 50 m plot was established in the middle of each site. In each of these plots, after removing litter layer, nine soils cores at approximately 10 m spacing on a S-shape transect were collected using a 2.5 cm corer at 0–10 cm and composited to create one soil sample per plot. Soil samples were placed in polyethylene bags, stored on ice and transferred to the laboratory within 24 h. Soils were sieved (2 mm) with any remaining visible plant material and stone removed by hand. Then, soils were subsampled and processed separately for physicochemical properties analysis, PLFA analysis and enzyme analysis.

### 2.3. Soil physicochemical properties analysis

Soil organic carbon (SOC) and total N were determined by a Vario EL III elemental analyzer (Elementar Analysensysteme GmbH, Germany); calcium carbonate was removed from the samples prior to SOC analysis using 1 M hydrochloric acid.  $\text{NH}_4^+$ -N and  $\text{NO}_3^-$ -N of filtered soil extracts (10 g fresh soil in 50 mL of 1 M KCl on a shaker for 1 h) were determined colorimetrically using a phenolate-hypochlorite method and a cadmium reduction method, respectively (Ministry of Environmental Protection of the People's Republic of China, 2012). Net N mineralization rate was calculated as the difference in  $\text{NH}_4^+$ -N and  $\text{NO}_3^-$ -N concentrations between the lab-incubated (30 days) and immediately extracted soil samples. Soil moisture was decided by comparing the weights of soils before and after oven-drying (dried at 105 °C until constant weight). Soil pH was measured at a soil-to-water ratio of 1: 2.5 (m/v).

#### 2.4. Phospholipid fatty acid (PLFA) analysis

Phospholipid fatty acids (PLFAs) were extracted from the soil to determine total PLFA, fungal PLFA, bacterial PLFA and detailed microbial community composition. Based on (Bligh and Dyer, 1959) and (White et al., 1979) and adapted from (Frostegård et al., 1991), lipids were extracted from the soil using a single-phase mixture of chloroform, methanol, and phosphate buffer (pH 9) in a ratio of 1: 2: 0.8. Lipid classes were separated using solid phase extraction (SPE) chromatography and phospholipids were methylated using a mild-alkaline solution.

PLFA analysis was performed on a 7890A gas chromatograph coupled with a 5975C mass spectrometer (Agilent, USA). The MSD ChemStation software was used to analyze data. PLFA peaks were identified by comparing their retention times and mass spectra with those from reference compounds (a 26-component bacterial acid methyl ester mix (Supelco, USA), a 37-component fatty acid methyl ester mix (Nu-chekprep, USA), and a C18: 1 $\omega$ 7c methyl ester standard (Nu-chekprep, USA)) and from the NIST 2011 spectral database. Concentrations of PLFAs were calculated from peak areas using an internal standard methyl nonadecanoate and reported as nmol g<sup>-1</sup> dry soil.

40 PLFAs were included in the analysis of total PLFA abundance. Specific fatty acids were used to represent fungal and bacterial groups. Saprotrophic fungal biomass was represented by 18: 2 $\omega$ 6,9 and 18: 1 $\omega$ 9 (Bardgett et al., 1996). Nine PLFAs were summed to represent bacterial biomass: i15: 0, a15: 0, i16: 0, i17:0, a17: 0, 16: 1 $\omega$ 9, 16: 1 $\omega$ 7, cy17: 0 and cy19: 0, with the first five and the last four also used as biomarkers for gram-positive and gram-negative bacteria, respectively (Frostegård and Bååth, 1996; Zelles, 1997, 1999). The fatty acid C16: 1 $\omega$ 5 was used as the biomarker for arbuscular mycorrhizal fungi (AMF) (Olsson, 1999). The fungal-to-bacterial ratio (F/B ratio) was calculated by dividing the sum of two saprotrophic fungal PLFA markers through all the bacterial PLFA markers; the ratio of gram-positive to gram-negative bacteria (G +/G - ratio) was calculated by dividing the gram-positive sum by the gram-negative sum. The relative abundance of a specific group was calculated by dividing the sum of its biomarkers by all the 40 PLFA biomarkers.

#### 2.5. Enzyme activity analysis

Activities of four enzymes, namely  $\beta$ -glucosidase, urease, alkaline phosphatase and catalase, were evaluated as described by (Guan, 1986), with the first three enzymes indicating C-cycling, N-cycling and P-cycling, respectively. 5 g of air-dried soils were incubated for enzyme activity at 37 °C for 2 h (alkaline phosphatase) or 24 h ( $\beta$ -glucosidase and urease). Using sucrose, urea, and disodium phenyl phosphate as substrates and based on the releases and detections of glucose, NH<sub>4</sub><sup>+</sup>, and P<sub>2</sub>O<sub>5</sub>, activities of  $\beta$ -glucosidase, urease, and alkaline phosphatase were measured photometrically (absorbance 508 nm, 578 nm, 510 nm), respectively. Catalase activity was determined by measuring the O<sub>2</sub> absorbed by KMnO<sub>4</sub> after addition of H<sub>2</sub>O<sub>2</sub> to the samples.

#### 2.6. Statistical analysis

Statistical tests were performed using the SPSS 19.0 software (SPSS Inc., Chicago, Illinois). One-way analysis of variance (ANOVA) followed by a Tukey's multiple comparison test was used to determine the statistical significance of land use type on soil physicochemical properties, microbial community structure and enzyme activity. All assumptions for statistical tests were met and  $P < 0.05$  was considered statistically significant. Pearson correlations were used to evaluate the relationships between soil physicochemical and biological variables. Principal Component Analysis (PCA) was carried out using the SIMCA-P 12.0 software package (Umetrics, Sweden) and performed on both the absolute and relative abundances of all PLFA biomarkers. Ratios of  $\beta$ -glucosidase: alkaline phosphatase and  $\beta$ -glucosidase: urease activities were calculated for all sites, indicating potential C: P and C: N acquisition activities,

respectively. These indices, measures of the enzymatic resources directed toward acquisition of organic P and organic N relative to C, were used to test for functional stoichiometry in soil enzyme activity distributions across land use types (Sinsabaugh et al., 2008).

### 3. Results

#### 3.1. Soil physicochemical properties

Except for pH levels which ranged from 7.8 to 7.9, soil physicochemical properties differed significantly across land use types (Table A.1). SOC contents were 1.38%–2.38%, total N contents were 0.10%–0.20%, and C/N ratios were 12–15 (Table 1). SOC concentration was strongly correlated with soil N ( $R = 0.92$ ,  $P < 0.01$ ). Both SOC and N contents were highest in the 20-year camphor forest. Compared to cropland, SOC and total N contents were lower in the young (16-year) dawn redwood forests but higher in the older (25- and 35-year) ones. There were significant ( $P < 0.05$ ) but not consistent differences in soil NH<sub>4</sub><sup>+</sup>-N, NO<sub>3</sub><sup>-</sup>-N, and net N mineralization rate across land use types, with the highest values in the oldest dawn redwood plantations and lowest levels in the recently deforested sites.

#### 3.2. Soil microbial community composition

Overall soil microbial community structures represented by the absolute (Fig. 1A) and relative (Fig. 1B) abundances of 40 PLFA biomarkers both varied with land use changes. The first principal component (PC1) and the second principal component (PC2) of the absolute PLFA abundances accounted for 75% and 11% of the variance (Fig. 1A). The 16-year dawn redwood and deforested sites were separated from the other soils along PC1; paddy fields were separated from the other sites along PC2. Five PLFAs, namely 18: 2 $\omega$ 6,9, br19: 0, C20: 4, C20: 5, and 18: 2 $\omega$ 9, were most important for site separation. PC1 and PC2 of the relative PLFA abundances represented 33% and 22% of the total variation (Fig. 1B). The paddy sites were isolated from the others along PC1. Br19: 0, 18: 1 $\omega$ 7, C20: 4, 10me18: 0, 18: 2 $\omega$ 6,9 were the five major variables in separating different sites. In both figures, all sites were within the 95% confidence ellipses.

Total and fungal PLFAs were more abundant in the 20-year camphor plantations and the 35-year dawn redwood forests compared to agricultural and deforested sites (Fig. 2). Deforested land had less abundant bacteria than cropland and woodlands (except in the 16-year forest). PLFA abundances in dawn redwood forest soils increased significantly with age. The relative abundance of fungi and the fungal-to-bacterial ratio were significantly lower in the dawn redwood forest soils compared to those in the deforested land and camphor forest, while the relative abundance of gram-positive bacteria showed an opposite pattern (Fig. 3). The relative abundance of gram-negative bacteria was greater in the cropland and deforested land than in woodland.

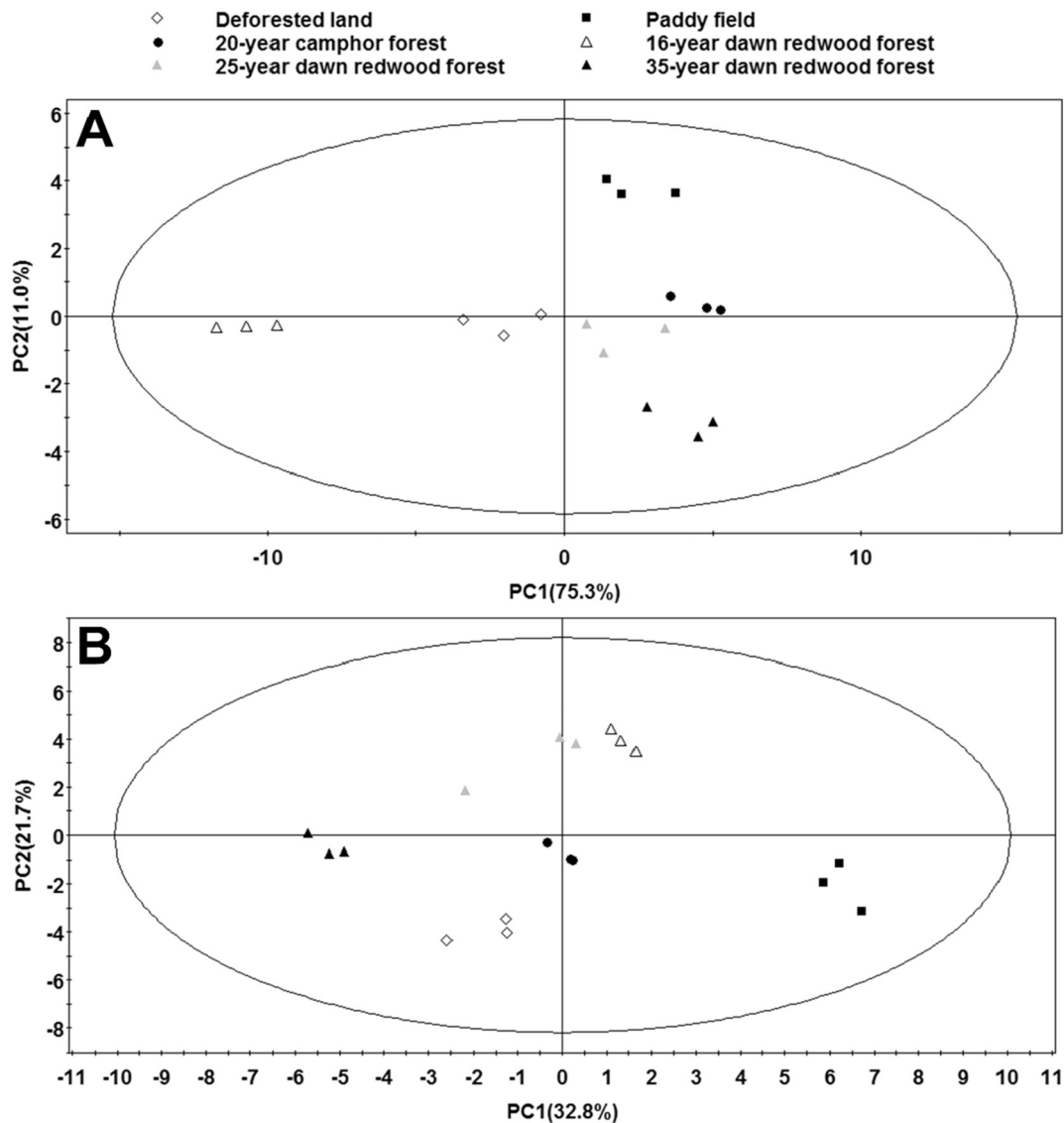
#### 3.3. Soil microbial enzyme activity

The potential activity of all the four extracellular enzymes differed significantly across land use types (Fig. 4). The four enzyme activities were strongly ( $R > 0.82$ ) and positively correlated with each other (data not shown). All the enzyme activities were significantly lower in the cropland and deforested land compared to the woodlands, and the highest enzyme activities were all found in the oldest dawn redwood forest. In the dawn redwood forests, all the activities of four enzymes increased with stand age. The specific activities of urease, phosphatase and catalase in the camphor forest, which was 20 years old, were comparable to those in the 16- and 25-year dawn redwood forests. Ratio of  $\beta$ -glucosidase: urease activities varied between 5.79 and 17.70 and ratio of  $\beta$ -glucosidase: alkaline phosphatase activities ranged from 0.64 to 1.48 (Fig. 5). These two ratios, respectively indicating potential C: N and C: P acquisition activities, showed a good linear relationship ( $R^2$

**Table 1**  
Environmental and soil properties of the six land use types on Chongming Island.

	Paddy field	Deforested land	20-year camphor forest	16-year dawn redwood forest	25-year dawn redwood forest	35-year dawn redwood forest
Age (year)*	57	6	20	16	25	35
DBH (cm)	–	–	19	16.7	20.9	28.1
Canopy height (m)	–	–	6.7	11.4	17.1	27.3
Tree density (trees ha <sup>-1</sup> )	–	–	300	740	613	463
pH (in water)	7.89 ± 0.01a	7.80 ± 0.07a	7.78 ± 0.03a	7.80 ± 0.04a	7.77 ± 0.01a	7.81 ± 0.03a
Moisture (%)	27.0 ± 1.4b	22.7 ± 0.5a	25.0 ± 0.8ab	24.1 ± 0.4ab	23.8 ± 0.4ab	23.6 ± 0.6ab
SOC (%)	1.84 ± 0.02b	1.95 ± 0.03bc	2.38 ± 0.06e	1.38 ± 0.01a	2.14 ± 0.03d	2.07 ± 0.02 cd
Total N (%)	0.12 ± 0.00b	0.17 ± 0.00c	0.20 ± 0.01d	0.10 ± 0.00a	0.16 ± 0.00c	0.17 ± 0.00c
C/N ratio	14.9 ± 0.4b	11.7 ± 0.2a	11.9 ± 0.3a	13.8 ± 0.1b	13.7 ± 0.4b	12.2 ± 0.1a
NH <sub>4</sub> <sup>+</sup> -N (μg g <sup>-1</sup> )	2.38 ± 0.07bc	1.76 ± 0.17ab	1.50 ± 0.07a	1.89 ± 0.07ab	2.73 ± 0.06c	3.48 ± 0.25d
NO <sub>3</sub> <sup>-</sup> -N (μg g <sup>-1</sup> )	10.3 ± 1.0bc	5.8 ± 0.9a	11.7 ± 1.1 cd	6.7 ± 0.2ab	13.7 ± 0.7 cd	15.3 ± 1.3d
Net N mineralization (μg g <sup>-1</sup> d <sup>-1</sup> )	1.13 ± 0.03bc	0.77 ± 0.05a	1.20 ± 0.05 cd	0.88 ± 0.01ab	1.14 ± 0.10bcd	1.44 ± 0.09d

\* Age means years since clear cutting for the second land use type, and years since planting for the others; DBH, stem diameter at breast height. Values are given as mean ± standard error (n = 3). Letters not shared across columns represent significantly different means via Tukey's multiple comparison test.



**Fig. 1.** Principal component analysis of soil microbial community structure using (A) the absolute abundances of 40 PLFA biomarkers and (B) the relative abundances of 40 PLFA biomarkers across the survey sites on Chongming Island.

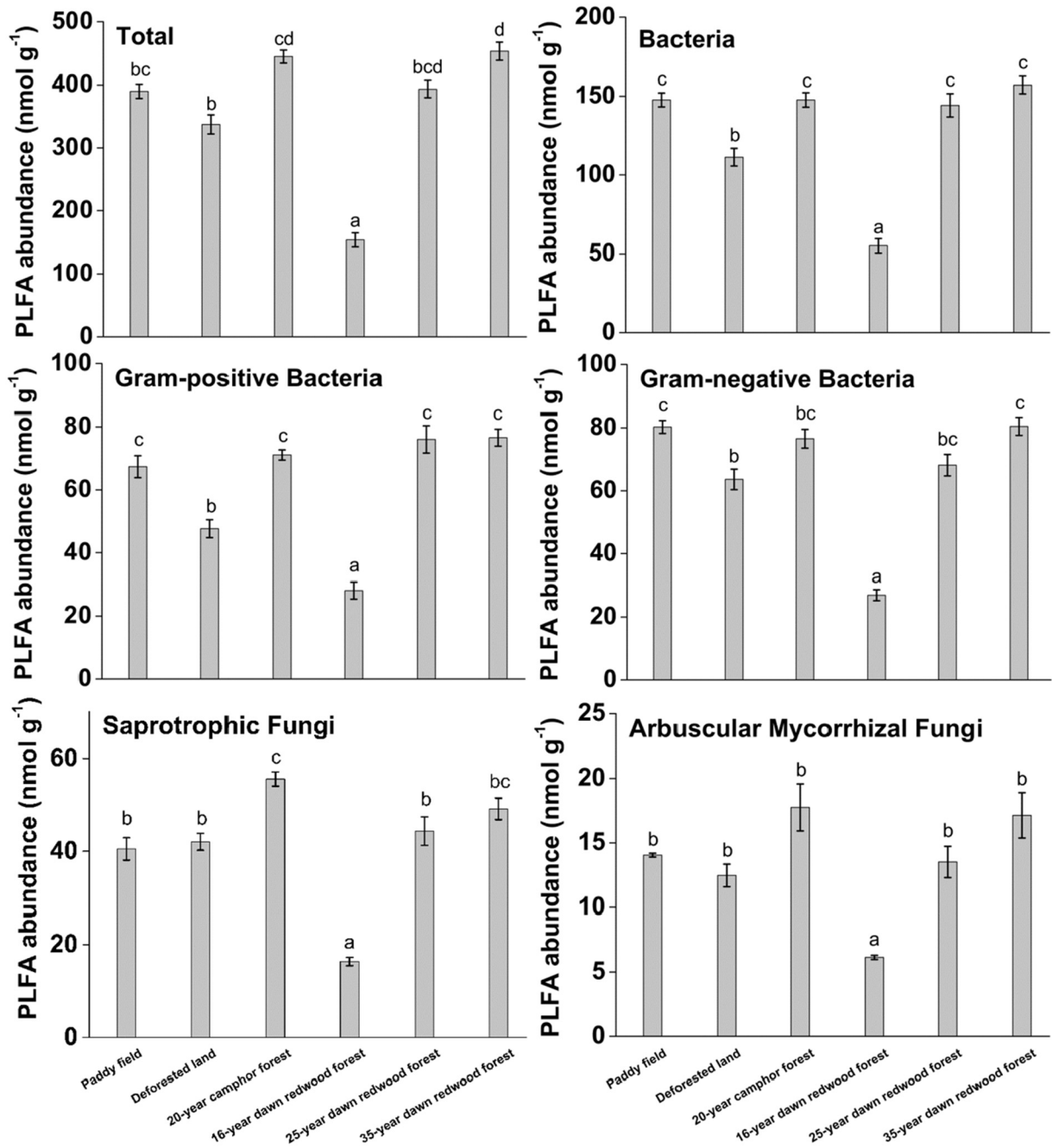


Fig. 2. Abundance of soil indicator PLFAs under different land use types on Chongming Island. Values are mean ± standard error (n = 3). Letters not shared across columns represent significantly different means via Tukey's multiple comparison test.

= 0.75). By these indicators, the ratio of C: N: P acquisition activity is 10: 1: 10.

### 3.4. Relationship between soil physicochemical and biological variables

Abundances of main microbial groups all had strong relationships with elemental concentrations ( $0.76 < R < 0.93$  for SOC content and  $0.62 < R < 0.87$  for total N content) (Table 2). Both the fungal-to-bacterial ratio and the relative abundance of fungi were positively correlated with SOC and N concentrations and negatively correlated with C/N ratio. Interestingly, significantly positive relationships were observed between soil net N mineralization and  $\text{NO}_3^-$ -N and microbial communities ( $0.54 < R < 0.83$  for PLFA abundance and  $0.50 < R < 0.76$  for enzyme activity). There were no significant correlations between soil microbial communities and pH, which varied little among sites, or moisture.

Some relationships were also observed among the biological variables. For example, although enzyme activities were not significantly linked with PLFA abundances, they were correlated negatively with the relative abundance of gram-negative bacteria ( $0.59 < R < 0.74$ ) and positively with ratio of gram-positive to gram-negative bacteria ( $0.68 < R < 0.77$ ) (data not shown).

## 4. Discussion

### 4.1. Soil elemental contents, microbial biomass and activity and their links

On this subtropical alluvial island, we observed higher SOC and N contents in the three older plantations than in the paddy field, and we also found that SOC and N contents in the three dawn redwood stands increased with age (Table 1). These findings supported our overall

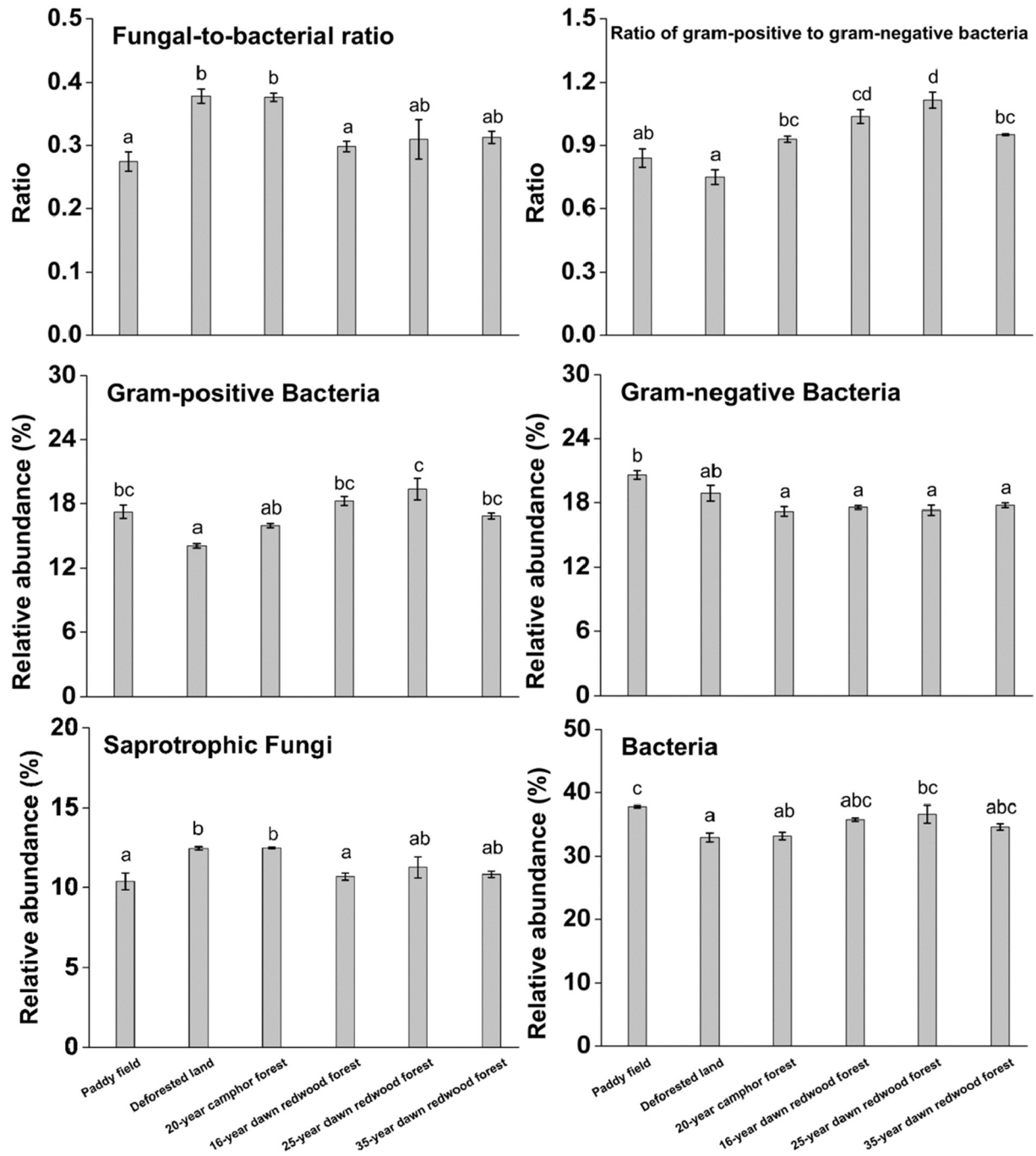


Fig. 3. Relative abundance of soil indicator PLFAs under different land use types on Chongming Island. Values are mean  $\pm$  standard error ( $n = 3$ ). Letters not shared across columns represent significantly different means via Tukey's multiple comparison test.

hypothesis: afforestation increased soil C and N concentrations and this effect strengthened with stand age. With increased litter input and in the absence of soil cultivation, conversion from cropland to woodland could result in increased SOM stocks (Georgiadis et al., 2017). However, we found the 16-year dawn redwood stand had lower SOC and N contents than the adjacent paddy field. These decreases might be explained by the exposure of organic matter to microbes when planting trees (Mackay et al., 2016). Another possible reason is that enhanced SOM contents as a result of increased litter input could be counteracted by higher oxygen availability in the young forest soils, as low oxygen concentration caused by periodic flooding in rice paddies could accumulate SOM (Liu et al., 2016). In fact, a synthesis of 135 publications also reported a temporal pattern for soil C stock changes after cropland

conversion showing an initial decrease in soil C during the early stage, followed by a gradual return of C stocks to cropland levels and then an increase to net C gains (Deng et al., 2016). The decreases of soil nitrogen following afforestation could be further attributed to cessation of fertilizer inputs and the greater demand for N of growing trees compared with crops (Berthrong et al., 2009).

Similar to SOM quantity, both microbial biomass (indicated by total, bacterial, and fungal PLFAs) and activities of the four enzymes increased with tree age in the dawn redwood stands (Fig. 2 and Fig. 4). This is consistent with our prediction and with earlier studies showing higher PLFA concentrations under a 135-year lodgepole pine stand than a 45-year one (Chatterjee et al., 2009) and higher enzyme activities under a 43-year locust stand compared to a 10-year one (Tian et al., 2015).

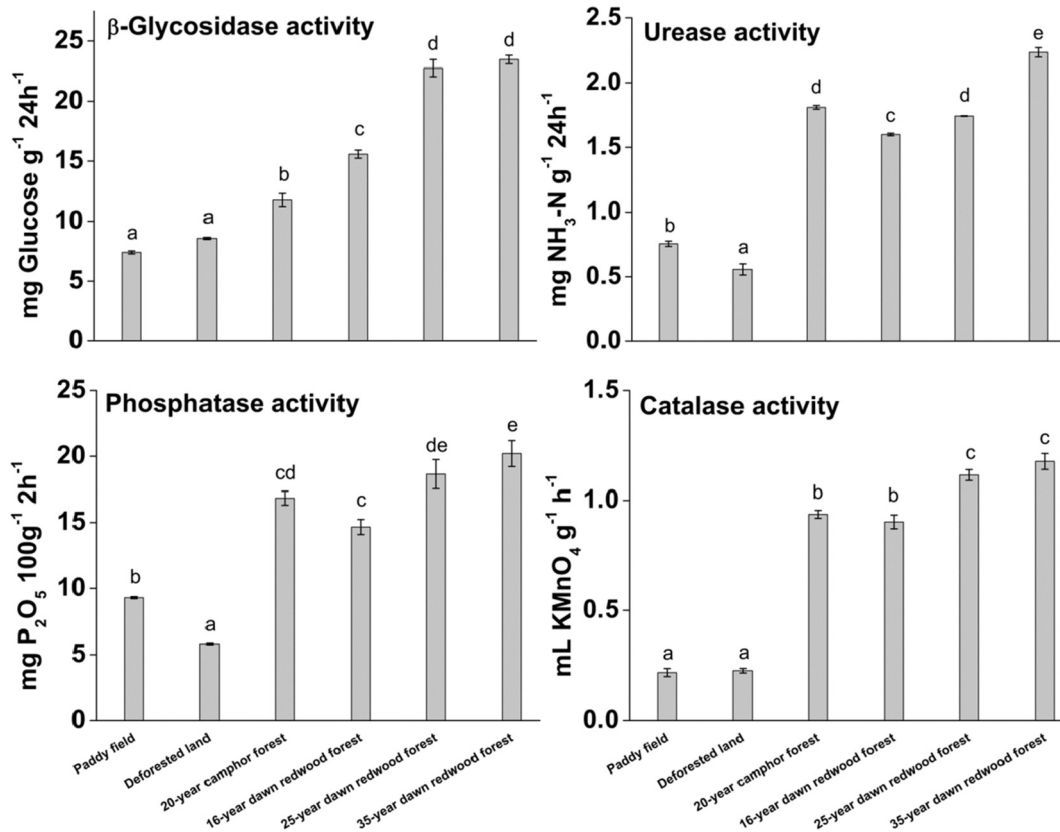


Fig. 4. Soil enzyme activities under different land use types on Chongming Island. Values are mean ± standard error (n = 3). Letters not shared across columns represent significantly different means via Tukey's multiple comparison test.

Also, as expected, we found that total and fungal PLFA abundances in the 20-year camphor forest and 35-year dawn redwood forest were higher compared to agricultural and deforested soils; this trend was more evident for microbial activity, as plantations, regardless of age and tree species, all had higher enzyme activities. Increases in microbial biomass or activity in woodlands compared to agricultural or deforested

soils were also found in some other studies (Bossio et al., 2005; Acosta-Martinez et al., 2007; Huygens et al., 2011; Ying et al., 2013; Tosi et al., 2016). The enhanced soil microbial biomass and activity could be linked to higher amounts of C inputs through litter fall which is a major energy input to soil microorganisms (Pietikainen et al., 2007; Fierer et al., 2009; Chen et al., 2013; Deng et al., 2016), as evidenced by our study showing a strong correlation between PLFA abundances and SOC contents (Table 2). This correlation may also help explain the initial decreases of PLFA abundances in the young (16-year) dawn redwood forest compared with cropland, as lower SOM contents were also observed there. The weaker correlation between enzyme patterns and SOM quantity suggests complex interactions among soil organic matter and microbial activities, and that SOM chemistry, spatial separation or physical stabilization of SOM might determine substrate availability for enzymatic breakdown (Schnecker et al., 2015).

Total, bacterial, and fungal PLFAs were comparable between the 20-year camphor plantation and the 35-year dawn redwood plantation (Fig. 2), which is consistent with our expectation and with findings of higher PLFA abundances in a 30-year broadleaf forest (*Robina pseudoacacia*) than in a nearby 30-year conifer forest (*Pinus tabuliformis*) (Xiao et al., 2016) and greater microbial biomass carbon in 20-year broadleaved forests (*Liquidambar formosana* and *Schima superba*) than in 20-year coniferous plantations (*P. massoniana* and *P. elliottii*) (Liu et al., 2012). However, contrary to our anticipation, the 25-year dawn redwood soils had higher or comparable enzyme activities in comparison to the 20-year camphor soils (Fig. 4). This finding was also inconsistent with a report of better catabolic function of soil microbial community under broadleaf tree species than under conifer tree species (Jiang et al., 2012). Given the limited sites and species in

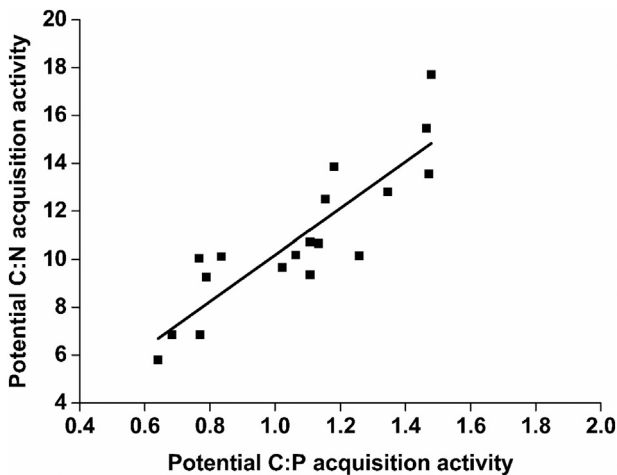


Fig. 5. Potential C: N acquisition activity, obtained by ratio of β-glucosidase: alkaline phosphatase activities, in relation to potential C: P acquisition activity, calculated by β-glucosidase: urease activities. The regression C: N = 9.72 (C: P) + 0.46 has an R<sup>2</sup> value of 0.75, n = 18.

**Table 2**  
Pearson correlations between soil physicochemical properties and biological variables across the survey sites on Chongming Island.

Microbial communities	Soil physicochemical properties							
	pH	Moisture	SOC	Total N	C/N ratio	NH <sub>4</sub> <sup>+</sup> -N	NO <sub>3</sub> <sup>-</sup> -N	Net N mineralization
Total PLFAs	-0.02	0.17	<b>0.87**</b>	<b>0.77**</b>	-0.32	0.34	<b>0.70**</b>	<b>0.64**</b>
Bacterial PLFAs	0.05	0.26	<b>0.79**</b>	<b>0.64**</b>	-0.16	0.41	<b>0.74**</b>	<b>0.62**</b>
G + bacterial PLFAs	-0.03	0.25	<b>0.79**</b>	<b>0.62**</b>	-0.13	0.47	<b>0.83**</b>	<b>0.67**</b>
G- bacterial PLFAs	0.12	0.26	<b>0.76**</b>	<b>0.63**</b>	-0.19	0.34	<b>0.61**</b>	<b>0.54*</b>
Saprotrophic Fungal PLFAs	-0.12	0.06	<b>0.93**</b>	<b>0.87**</b>	-0.45	0.15	<b>0.57*</b>	<b>0.55*</b>
AM Fungal PLFAs	-0.08	0.14	<b>0.78**</b>	<b>0.74**</b>	-0.39	0.24	<b>0.68**</b>	<b>0.56*</b>
F/B ratio	-0.33	-0.37	<b>0.48*</b>	<b>0.64**</b>	<b>-0.65**</b>	-0.44	-0.23	-0.07
G +/G- ratio	-0.30	-0.00	-0.05	-0.15	0.21	0.25	0.41	0.25
G + bacteria%	-0.06	0.24	-0.22	-0.40	<b>0.51*</b>	0.34	0.39	0.16
G- bacteria%	<b>0.53*</b>	0.34	-0.25	-0.35	0.42	0.03	-0.23	-0.27
Saprotrophic Fungi%	-0.33	-0.32	<b>0.51*</b>	<b>0.61**</b>	<b>-0.55*</b>	-0.46	-0.22	-0.12
Bacteria%	0.30	0.44	-0.36	<b>-0.59*</b>	<b>0.73**</b>	0.32	0.20	-0.04
β-Glycosidase activity	-0.30	-0.34	0.14	0.11	-0.09	<b>0.69**</b>	<b>0.65**</b>	<b>0.50*</b>
Urease activity	-0.32	-0.15	0.27	0.27	-0.20	0.46	<b>0.67**</b>	<b>0.66**</b>
Phosphatase activity	-0.28	-0.04	0.31	0.24	-0.08	<b>0.47*</b>	<b>0.76**</b>	<b>0.64**</b>
Catalase activity	-0.40	-0.25	0.24	0.23	-0.18	0.42	<b>0.60**</b>	<b>0.53*</b>

G +, gram-positive; G -, gram-negative; AM, arbuscular mycorrhizal; F/B ratio, the ratio of saprotrophic fungi to bacteria; G +/G - ratio, the ratio of gram-positive bacteria to gram-negative bacteria; % represents the relative abundance of a group. Significant values are shown in bold.

\*  $P < 0.05$ .

\*\*  $P < 0.01$ .

this study, experiments based on more sites and more species are needed to evaluate the performances of broadleaved and coniferous trees in improving soil biological characteristics.

#### 4.2. Relationships between soil elemental contents and microbial community structure

Soils from the camphor forest had higher F/B ratio and higher proportion of fungi than paddy field (Fig. 3). This finding agreed with Deng et al. (2016) who proposed that afforestation could increase F/B ratio by introducing litter with higher C/N ratios. However, to our surprise, the relative abundance of fungi and F/B ratio in dawn redwood soils were significantly lower compared to camphor ones, although F/B ratios are usually thought to be higher in conifer than in broadleaf forests (Ushio et al., 2008; Chang et al., 2016). Compared to broadleaved forests, coniferous soils may provide more recalcitrant C and greater concentrations of tannins, both favoring fungi (Scalbert, 1991; Ushio et al., 2010); the higher ratio of gram-positive bacteria also indicated depletion of easily-decomposable substrate in dawn redwood soils in this study. However, it is worth noting that low pH level favoring fungal growth (Winsborough and Basiliko, 2010) was found for none of the six land use types in our study (Table 1).

There was a negative relationship between F/B ratio and soil C/N ratio (Table 2). This was unexpected given the well-established link between the two ratios (Fierer et al., 2009; Waring et al., 2013). The range of C/N values in the present study is quite narrow (12–15), whereas an earlier study in which this correlation was observed covered a wide range from 4 to 38 (Fierer et al., 2009). Rather, we found both SOC and total N contents were positively correlated with F/B ratio and the relative abundance of fungi. This finding partly agreed with the results of Bailey et al. (2002) and they associated increased fungal activities with increased soil C. Although some studies (Six et al., 2006; Busse et al., 2009) have proposed that fungal-dominated soils have greater soil C accumulation because (1) fungi incorporate more C into biomass than bacteria, (2) they have more recalcitrant cell walls than bacteria, and (3) they facilitate C stabilization and protection by enhancing soil aggregation, it is still unclear whether fungal-dominated soils favor more stored C or more stored C in soils favor fungi (Bailey et al., 2002). Overall, our findings indicate that microbial community structure and, in particular, F/B ratio may reflect the integrated effects of a suite of soil characteristics, including pH, moisture, SOM quantity

and quality, litter quantity and quality and other factors (Smith et al., 2014).

#### 4.3. Relationships between soil inorganic N and microbial communities

We found soil inorganic N levels were positively related to PLFA absolute abundances, but not to the relative abundances (Table 2). This is consistent with the finding of Muruganandam et al. (2010) who concluded that greater N transformation rates in no-till than tilled soil were due primarily to increased microbial biomass (i.e., microbial population size) rather than altered microbial community composition. We also found close links among N availability and all the four enzyme activities. Other studies have also shown increased activity of cellulase (including β-glucosidase) in response to N fertilization (Sinsabaugh et al., 2005). The strong association among soil inorganic N and the activity of C-cycling enzyme (β-glucosidase) suggests a shift toward increased C acquisition in sites with greater available N (Bowles et al., 2014). Urease activity was also reported to be correlated with soil inorganic N supply (Li et al., 2014) or net N mineralization (Gomez-Rey et al., 2013). Marklein and Houlton (2012) stated that N fertilization enhanced phosphatase activity in a meta-analysis. Ratliff and Fisk (2016) found that phosphatase activity was related to N availability across hardwood forests.

Across land use types, the ratio of C: N: P acquisition activity was 10: 1: 10 (Fig. 5), suggesting that the capacity of microbial communities to alter relationships among enzymatic C, N and P acquisition activities in response to land use change is limited. This finding, together with the similar correlations between the three acquisition activities and N availability, confirms the finding of Sinsabaugh et al. (2009) that microbial communities of diverse habitats share a common pattern of functional organization.

## 5. Conclusion

Our study on Chongming alluvial island in subtropical China suggests that compared to agricultural soils, despite the initial decreases in soil elemental concentration and microbial biomass, long-term afforestation could significantly enhance SOM contents, accumulate microbial biomass and improve potential enzyme activities, while deforestation showed negative or no effects. The lower fungal-to-bacterial ratio in coniferous soils than broadleaved soils indicates that apart from litter quality, it may also be governed by many other



factors. Shifts in soil microbial community structure and function across different land covers strongly interacted with soil nutrient levels as well as N cycling. The fixed C: N: P acquisition ratio across land use types shows that the plasticity of these relationships is constrained. Overall, in order to improve soil quality, long-term afforestation is needed and tree species should be carefully selected. Our research findings have important implications for afforestation which is one of the most significant ecological changes facing China and the world.

## Declarations

The author's declare that they have no conflicts of interest with the submitted material and declare that the sponsor did not play any role

## Appendix A

**Table A.1**

ANOVA results of the effects of land cover: (A) on soil physicochemical properties; (B) on soil microbial community composition; and (C) on soil microbial enzyme activity.

Subject	Effect	DF	F value	$P_r > F$
<b>A. Soil physicochemical properties</b>				
pH	Land use type	17	1.48	0.268
Moisture	Land use type	17	3.99	0.023
SOC	Land use type	17	126.22	<0.001
Total N	Land use type	17	114.97	<0.001
C/N ratio	Land use type	17	22.50	<0.001
NH <sub>4</sub> <sup>+</sup> -N	Land use type	17	29.89	<0.001
NO <sub>3</sub> <sup>-</sup> -N	Land use type	17	15.86	<0.001
Net N mineralization rate	Land use type	17	13.57	<0.001
<b>B. Soil microbial community composition</b>				
Total PLFAs	Land use type	17	74.55	<0.001
Bacterial PLFAs	Land use type	17	50.82	<0.001
G + bacterial PLFAs	Land use type	17	40.02	<0.001
G - bacterial PLFAs	Land use type	17	53.49	<0.001
Saprotrophic fungal PLFAs	Land use type	17	39.77	<0.001
AM fungal PLFAs	Land use type	17	11.91	<0.001
F/B ratio	Land use type	17	7.08	0.003
G +/G- ratio	Land use type	17	17.57	<0.001
G + bacteria%	Land use type	17	11.55	<0.001
G - bacteria%	Land use type	17	8.77	0.001
Saprotrophic Fungi%	Land use type	17	6.07	0.005
Bacteria%	Land use type	17	6.97	0.003
<b>C. Soil microbial enzyme activity</b>				
β-Glycosidase activity	Land use type	17	268.37	< 0.001
Urease activity	Land use type	17	647.10	< 0.001
Phosphatase activity	Land use type	17	67.53	< 0.001
Catalase activity	Land use type	17	303.83	< 0.001

DF, degree of freedom; G +, gram-positive; G -, gram-negative; AM, arbuscular mycorrhizal; F/B ratio, the ratio of saprotrophic fungi to bacteria; G +/G - ratio, the ratio of gram-positive bacteria to gram-negative bacteria; % represents the relative abundance of a group.

## Appendix B. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2017.12.180>.

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