

# Phyllostachys edulis (moso bamboo) ages significantly affect soil nitrogen transformation and endophytic microbes but niche differentiations outweigh ages in shaping microbial communities of moso bamboo-soil system

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## Research

**Keywords:** moso bamboo growth, nitrogen cycling, functional gene abundances, enzyme activity, rhizospheric soil, endophytic microbes

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# Abstract

**Background:** *Phyllostachys edulis* (moso bamboo) is a key source of non-wood forest products. Clarifying the linkage among bamboo growth, soil nutrient and core microbe can expand the horizon on nutrient management practices and functional endophytic and rhizospheric microbes.

**Results:** In this study, young (0.5-yr), mature (2.5-yr and 4.5-yr) and old (6.5-yr) bamboo plants were selected in a moso bamboo field, and above-ground tissues, below-ground roots (rhizomes) and rhizospheric soils were sampled. The bamboo biomass, soil properties and endophytic microbes were determined and quantify their comprehensive relationships. Bamboo ages had negligible impacts on the bamboo height, diameter at breast height and above-ground biomass. Organic matter and nitrogen (N) contents in the rhizospheric soil of the 0.5-yr bamboo were significantly higher than those of the other three age groups. The rhizospheric soil of the 6.5-yr bamboo had the lowest N mineralization rate and urease activity. Significant differences in bacterial and fungal communities were found in the above-ground tissues but not in the rhizospheric soils associated with plants of different ages. Bacterial and fungal community structures in the above-ground tissues were significantly different from their counterparts in the rhizospheric soils.

**Conclusions:** Bamboo ages significantly affected N transformation rates, functional gene abundances and urease activities of rhizospheric soils and endophytic bacterial community structures. However, niche differentiations outweighed ages in shaping the whole microbial communities of bamboo aboveground tissues, roots and rhizospheric soils. In the future, moso bamboo management should consider balanced applications of ammonium-N and other nutrients and utilisations of *Chytridiomycota* to stimulate moso bamboo growth.

## Introduction

Bamboos are key members of the *Gramineae* plants and have approximately 90 genera and 1200 species [1, 2]. The bamboo output is a key source of non-wood forest products, and bamboo plantations and forests have been continuously increasing in tropical, subtropical and temperate regions [1, 2]. After industrial treatment, bamboos have up to 95% glucose and 73% ethanol yield [3]. Bamboo fiber has special inherent properties, such as good air permeability and excellent moisture absorbency, and has allegedly better dye-ability than cotton [4, 5]. In addition, bamboo shoot is used as healthy food due to the multiple health benefits including anticancer activity and improvement of digestion [6, 7]. Globally, 2.5 billion people are economically associated with bamboo plantations, and the value of bamboo production is about \$ 2.5 billion per year [8].

Among all bamboo species in China, the most substantial species is *Phyllostachys edulis* (moso bamboo), with more than three million hectares planted [9, 10]. After emerging, the shoot of moso bamboo grows fast, and under suitable conditions, its growth rate could reach 1 m per day [11, 12]. Therefore, different agroforest management practices, especially mineral fertilizer applications, have

been used to stimulate moso bamboo growth [10-12]. The moso bamboo generally has a rhizomatous clonal growth every second year, and this inherent biological property is markedly different from other plants belonging to the *Gramineae* [13, 14]. The rhizomatous clonal growth significantly or markedly changed the physical, chemical and biological properties of rhizospheric soils, relying on root exudates and rhizosphere size [15-16], and therefore ages of moso bamboo might also dominate the rhizospheric effects including nitrogen (N) cycling [15-18]. However, to the best of our knowledge, few studies have been conducted to reveal and quantify the linkages among bamboo biomass, age and soil nutrient cycling, especially the impacts of bamboo ages on soil N transformations.

Apart from abiotic nutrient supply, key microbes associated with plants also drive the health and biomass accumulation of host plants [19, 20]. A series of taxonomically diverse microbes (viruses, bacteria and fungi) colonizes in plants and serves as the extended secondary genome of host plants [17, 21, 22]. The relationships between plants and microbes could be classified as positive stimulation, neutral interaction and negative pathogenicity. Endophytic microbial colonizations often start with the recognition of specific root exudate which theoretically 'communicates' with symbiotic, commensalistic and pathogenic microbes [22, 23]. However, it has been proven that plants can specifically attract microorganisms for their own ecological benefits [24, 25]. For instance, endophytic microbes changed membrane lipopolysaccharides, lignins and peroxidase of host plants and improved plant resistance to *Fusarium wilt* and hazardous compound of invasive species [24-27]. Much attention has been paid to the roles of endophytic bacteria and fungi to stimulate host plant growth. Furthermore, each niche in plant-soil system, including above-ground tissue, root and rhizospheric soil, has a unique biotope for microbial community and hosts different microbial assemblies [17, 28]. The niche effects should be thereby considered, but studies disentangling impacts of niche differentiations on the key microbes in moso bamboo plantation or forestry have rarely been conducted.

The objectives of this study were to: (1) quantify the age impacts of moso bamboo on soil N transformations; (2) examine the selectivity of bamboo age and niche for key microbes; (3) identify potential endophytic and rhizospheric microbes positively affecting moso bamboo growth; and (4) link moso bamboo biomass yield with soil nutrients and key microbes. In this study, moso bamboo plants of different ages (0.5, 2.5, 4.5 and 6.5 years) were selected to serve as different treatments. The bamboo biomass, soil properties and endophytic microbes were determined to reveal and quantify the comprehensive linkages among moso bamboo biomass yield, soil nutrients and endophytic and rhizospheric microbes. Our study could help to develop strategies on nutrient management of bamboo plantations and expand the horizon in utilizing functional microbes to stimulate bamboo growth.

## Results

### Bamboo and soil properties

There were negligible differences in the heights, DBH and above-ground biomass among the moso bamboo plants of four different ages (Fig. 1). The average height, DBH and above-ground biomass of

moso bamboos ranged from 13.9 to 14.9 m, 10.4 to 10.6 cm and from 16.25 to 23.33 kg, respectively.

The organic matter and total N contents in rhizospheric soil of the 0.5-yr bamboo plant were significantly ( $P < 0.05$ ) higher than those of the other three counterparts, with the lowest organic matter and total N contents being in the rhizospheric soils of the 6.5-yr bamboo plant (Figs. 2a, b). The  $\text{NH}_4^+$ -N contents did not significantly differ among the four treatments (Fig. 2c), and the highest and lowest  $\text{NO}_3^-$ -N contents were present in the rhizospheric soils of 0.5-yr and 4.5-yr bamboo plants, respectively (Fig. 2d).

## Biological properties related to soil N transformations

The N transformation rates, functional gene abundances and urease activities of the rhizospheric soils were significantly but differently affected by moso bamboo ages (Fig. 3). The lowest N mineralization rate was detected in the rhizospheric soil of the 6.5-yr bamboo plant, which was significantly lower than those of the 2.5-yr and 4.5-yr bamboo plants (Fig. 3a). The *nprA* gene abundances in the four different rhizospheric soils ranged from  $8.16 \times 10^7$  to  $1.57 \times 10^8$  copies  $\text{g}^{-1}$  dry soil, and *nprA* gene abundances in the rhizospheric soils decreased with bamboo ages (Fig. 3b). The trend of *chiA* gene abundances among the four different rhizospheric soils was similar to that of N mineralization rates, and *chiA* gene abundance in the rhizospheric soils of the 6.5-yr bamboo plant was significantly ( $P < 0.05$ ) lower than those of the other three treatments (Fig. 3c). The highest nitrification rate was in the rhizospheric soil of 4.5-yr bamboo, while the rhizospheric soil of the 2.5-yr bamboo had the lowest nitrification rate (Fig. 3d). Soil AOA *amoA* gene abundances decreased in order of 4.5-yr > 0.5-yr > 6.5-yr > 2.5-yr (Fig. 3e). However, the AOB *amoA* gene abundance in the rhizospheric soil of 0.5-yr bamboo plant was the highest among the four different treatments (Fig. 3f). Relative to that of 0.5-yr bamboo plant, average urease activities in rhizospheric soils of 2.5-yr, 4.5-yr and 6.5-yr bamboos decreased by 81.4%, 81.1% and 88.2%, respectively ( $P < 0.05$ ; Fig. 3g).

## Endophytic and rhizospheric *microbial community diversities*

After filtering, average sequence length ranged from 374 to 377 bp and from 229 to 270 bp for bacterial and fungal high-quality reads in different samples, respectively (Table S2), and the measured rarefactions of bacterial and fungal sequences all reached saturation plateaus (Fig. S1). The OTU similarities and differences of the above-ground tissues, roots and rhizospheric soils of different ages were present in four-set Venn diagrams (Fig. S2). The 0.5-yr and 6.5-yr above-ground tissues had the highest bacterial and fungal OUT numbers (Figs. S2a, d). For the roots, the highest bacterial and fungal OUT numbers were both in the 6.5-yr root (1294 and 2233, Figs. S2b, e). The unique bacterial OTUs were 69, 76, 96 and 90 for the rhizospheric soils of 0.5-yr, 2.5-yr, 4.5-yr and 6.5-yr bamboo plants, respectively (Fig. S2c), and these four different rhizospheric soils shared 500 fungal OTUs (Fig. S2f).

Both bamboo plant age and niche differentiation significantly ( $P < 0.05$ ) influenced the diversity indices and richness estimators of the bacterial community (Fig. 4). Among the above-ground tissues of different ages, the 0.5-yr above-ground tissue always had the highest diversity indices and richness estimators of the bacterial community, which were significantly higher than those in the 4.5-yr tissues (Figs. 4a-d). However, for the roots, the highest diversity indices and richness estimators were in the 6.5-yr plants, and significantly ( $P < 0.05$ ) different diversity indices were observed only between the 0.5-yr and 6.5-yr bamboo plants. Rhizospheric soils had significantly ( $P < 0.05$ ) higher diversity indices and richness estimators than their above-ground tissue counterparts with the same age, although the four different rhizospheric soils shared similar values of community diversity. In the roots, bacterial richness estimators increased with the bamboo root ages (6.5-yr > 4.5-yr > 2.5-yr > 0.5-yr). Bamboo plant ages only significantly ( $P < 0.05$ ) affected fungal community diversity in the above-ground tissues, with negligible impacts being observed in the roots and rhizospheric soils (Figs. 4e, f). Meanwhile, there were no significant differences in fungal insimpson diversity among the above-ground tissues, roots and rhizospheric soils in the same age group. In contrast, apart from the above-ground tissue, fungal richness estimators in the roots were also significantly influenced by the bamboo ages, with the lowest richness estimators being in the 0.5-yr roots (Figs. 4g, h).

## Endophytic and rhizospheric *microbial structures*

All the bacterial OTUs belonged to the predominant phyla (> 0.1% relative abundance): *Actinobacteria*, *Acidobacteria*, *Armatimonadetes*, *Bacteroidetes*, *Chlamydiae*, *Chloroflexi*, *Dependentiae*, *Elusimicrobia*, *Entotheonellaeota*, *Firmicutes*, *Gemmatimonadetes*, *Latescibacteria*, *Nitrospirae*, *Planctomycetes*, *Proteobacteria* and *Verrucomicrobia* (Fig. S3a). At the genus level, the *Enterobacter* has a higher relative abundance (Fig. S3b), and in terms of the biological function, the relative abundance of bacteria related with carbohydrate transport and metabolism decreased in the order of above-ground tissue > root > soil (Fig. S3c).

Bamboo plant ages significantly ( $P < 0.05$ ) affected the relative abundances of *Actinobacteria* and *Firmicutes* in the above-ground tissues, *Bacteroidetes*, *Chloroflexi*, *Elusimicrobia*, *Nitrospirae* and *Planctomycetes* in the roots, and *Gemmatimonadetes*, *Latescibacteria* and *Verrucomicrobia* in the rhizospheric soils (Fig. S4). The relative abundances of *Acidobacteria*, *Chloroflexi*, *Dependentiae*, and *Nitrospirae* in rhizospheric soils were the highest among the three niches and significantly ( $P < 0.05$ ) higher than those in the above-ground tissues. Notably, there were no *Latescibacteria* detected in any above-ground tissues (Fig. S4j), and phyla *Elusimicrobia*, *Nitrospirae*, *Planctomycetes* and *Verrucomicrobia* were not detected in the 2.5-yr or 4.5-yr above-ground tissues, either.

The predominant phyla of fungal community were: *Ascomycota*, *Basidiomycota*, *Chytridiomycota*, *Glomeromycota*, *Mortierellomycota*, *Mucoromycota* and *Rozellomycota* (Fig. S5a). The genus *Mortierella* were detected in all samples, and 70% of the total *Mortierella* were from the soil samples (Fig. S5b). The relative abundances of endophyte fungi in the 48 samples ranged from 2.90% to 28.8% (Fig. S5c). In the

above-ground tissues, roots and rhizospheric soil, the largest phylum was *Ascomycota* among all the phyla, (Fig. S6a). The relative abundances of *Basidiomycota*, *Chytridiomycota*, *Glomeromycota* and *Mortierellomycota* in the above-ground tissues and the relative abundances of *Ascomycota* and *Basidiomycota* in the roots were significantly ( $P < 0.05$ ) affected by bamboo ages (Figs. S6b-e).

## Comparisons of endophytic and rhizospheric microbial structures

The PCoA1 and PCoA2 of *two-dimensional* plots explained 27.0%-60.0% of the total variances in endophytic and rhizospheric microbial structures, and as elicited in Fig. 5, divergences in endophytic and rhizospheric microbial community structures among the same niches with different ages occurred, although to various extents. There were significant ( $P = 0.01$  and  $P = 0.001$ ) differences in bacterial and fungal communities among the above-ground tissues of different ages (Figs. 5a, d). Significant differences in the bacterial communities occurred between the 6.5-yr and 0.5-yr or 2.5-yr above-ground tissues (Fig. 5a). For the fungal communities in the above-ground tissues, significant differences were found between the 6.5-yr and the other three age groups (Fig. 5d). In the roots, bacteria rather than fungi had significantly ( $P = 0.024$ ) different community structures among different age groups (Figs. 5b, e). Significant differences in bacterial communities occurred between the 6.5-yr and 0.5-yr or 2.5-yr roots, which was consistent with the results of the bacteria in above-ground tissues. Compared with the microbial community in the above-ground tissues and roots, microbes (both bacteria and fungi) in the rhizospheric soils markedly overlapped ( $P > 0.05$ ) among different age groups (Figs. 5c, f).

## Niche selection of key microbes

On the whole (48 samples), there were no significant differences in the bacterial communities detected in the above-ground tissues, roots or rhizospheric soils of different age bamboos (Fig. 6). However, bacterial communities were significantly different between the above-ground tissues and their corresponding rhizospheric soils, but identical in the above-ground tissues and roots (Fig. 6a). The fungal community had the same trend with its bacterial counterpart, with the microbes in the above-ground tissues and rhizospheric soils being significantly different in the PCoA1 value (Fig. 6b). The same results were also evident in the unweighted UniFrac clustering analyses (Figs. 6c, d). Regardless of bamboo ages, fungal communities in the above-ground tissues were firstly separated, and the fungi in the roots and rhizospheric soils tended to be grouped together (Fig. 6d). The microbes (both bacteria and fungi) from the above-ground tissues had longer distance from their counterparts in the rhizospheric soils, relative to the microbes in the roots. Based on the above, sample niches outweighed bamboo ages in shaping key microbial structures in the moso bamboo plant-soil system. Random forest analysis revealed that the phylum *Nitrospirae* displayed the most variable importance in the bacterial composition in the above-ground tissues, roots and rhizospheric soils (Fig. 6e). For the fungal community, the phylum *Glomeromycota* had the largest decrease accuracy (Fig. 6f).

# Comprehensive linkages among moso bamboo growth, soil nutrient and microbes

The  $R^2$  values in stepwise regression models of N mineralization and nitrification rates to the regulating factors were 0.264 and 0.483, respectively (Table 1). The N mineralization rate was best described and positively correlated with *chiA* gene abundances. The potential nitrification rate was positively correlated with both AOB *amoA* gene abundance and bamboo age. The moso bamboo biomass had positive relationships with the total N and  $\text{NH}_4^+$ -N contents of rhizospheric soils (Table S3). Among all the predominant bacteria and fungi, only the relative abundances of phylum *Chytridiomycota* in the above-ground tissues, roots and soils were all positively correlated with the moso bamboo biomass (Table S3).

Table 1

Quantitative relationships among N transformation rates, functional gene abundances and soil and bamboo properties

Stepwise regression models	$R^2$	<i>P</i> value
Net mineralization rate = $1.10 \times 10^{-9} \times \textit{chiA}$ gene abundance - 0.25	0.264	0.042
Potential nitrification rate = $1.81 \times 10^{-8} \times \textit{AOB amoA}$ gene abundance + $0.874 \times$ bamboo age - 4.91	0.483	0.033

## Discussion

### Impacts of moso bamboo ages on nutrient contents and N transformations in rhizospheric soils

The rhizospheric soils of young bamboo plants had higher organic matter and total N contents, compared with those of the old bamboo plants (Fig. 2). Root exudates and dead fine roots were the major sources of organic matter input into the rhizospheric soils. We found that the rhizospheric soil of 0.5-yr bamboo had the highest organic matter content (Fig. 2a), suggesting that higher amounts of exudates from the young roots contributed to soil organic matter accumulation. During the bamboo invasion, organic C chemical properties could also be altered [29]. Young bamboo growth decreased alkyl C (hydrolyzable polysaccharides) but increased O-alkyl C (recalcitrant substances) contents [29]. Furthermore, Li et al. [30] confirmed that in broadleaf forests, the bamboo invasion could stimulate  $\text{CO}_2$  fixation potential in soils by enhancing soil *cbbL* gene abundance and RubisCO enzyme activity,. The moso bamboo is bred from its rhizome, and its under-ground rhizome and roots are connected. Apart from nutrient uptakes from the soils, young bamboos might also obtain nutrients from old and mature bamboo rhizomes, resulting in lower organic matters in rhizospheric soils of old and mature bamboos. Chou and Yang [31] have shown that even the extracts of bamboo leaves could be detected in the

rhizospheric soils. The young moso bamboo has a fast growth rate, which might also contribute to increases in organic matter in the rhizospheric soil of the 0.5-yr bamboo plant.

Both *Nitrospirae* ratio and random forest analysis showed the impacts of moso bamboo age on N transformation, and thus more attention should be paid to the rhizospheric microbes related to N cycling (Figs. S4 and 6). The entire N cycling includes plant N uptake, N-fixation, N mineralization, nitrification and denitrification. Soil N mineralization is the key source of  $\text{NH}_4^+\text{-N}$  that can be easily oxidated to  $\text{NO}_3^-\text{-N}$  [32]. Relative to the  $\text{NO}_3^-\text{-N}$ , plant growth prefers to utilize the  $\text{NH}_4^+\text{-N}$  [33]. In this study, the 6.5-yr bamboo plant was the oldest, and its nutrient intake rate might be slow, which resulted in the lowest N mineralization rate in the rhizospheric soil of 6.5-yr bamboo plant (Fig. 3). Soil nitrification is mainly mediated by the nitrifying microorganisms, and therefore soil nitrification rate generally has positive correlations with (AOA and AOB) *amoA* gene abundances [34, 35]. The AOA *amoA* gene was more abundant than its AOB counterpart in the same rhizospheric soils (Fig. 3), but we found that the potential nitrification rate was best described by the AOB *amoA* gene abundance (Table 1). To date, there is no consensus regarding the relationship between acidic soil nitrification and AOA and AOB *amoA* gene abundances. Jiang et al. [34] found that nitrification in acid soil was stimulated with increases in both AOB and AOA *amoA* gene abundances. Some studies suggested that AOA *amoA* gene controlled soil nitrification in acid soils [35]. However, Huang et al. [36] suggested that ammonia oxidation in acidic forest soil was driven by the bacteria rather than archaea. Young bamboo plants also had higher total soil N content than mature and old bamboos (Fig. 2b), which might be also explained by the reasons for increasing organic matter as mentioned above. As a key parameter describing soil fertility, urease activity could also affect the soil N supply and cycling [37]. Our results found that the highest total N and urease activity were both detected in the rhizospheric soil of 0.5-yr bamboo plant (Fig. 3g), implying that young bamboo roots favoured soil organic N accumulations.

## Niche selection of key microbes

Soil hosts a plethora of microbes and has been widely accepted as the “base camp” and ideal habitats for various bacteria, and it is also the origin of plant-associated microbes [38, 39]. Generally, microbes in plants origin from two pathways: horizontal and vertical transmissions [40, 41]. After seeds germinate, plant roots exudate different chemicals into the soils to attract soil rhizospheric microbes to enter into the roots. Some microbes prefer to colonize and flourish in the rhizospheric soils or roots, and other microbes gradually translocate from the roots and inhabit in the above-ground tissues, flower and fruit, and finally colonize embryo and endosperm of seed [42]. The vertical transmission from seed has also been reported in plants: endophytic microbes spread into different plant components with the seedling developments and re-enter the next generation seeds [17, 42]. However, relative to the horizontal transmission, the vertical transmission efficiency is lower. During plant development and growth, many microbes were excluded or killed by the host plant [42]. There was no seeding management in our current study, and all test moso bamboo plants were produced by vegetative propagation, and thereby, the horizontal transmission was the only pathway of endophytic microbial colonization in the moso bamboos.

Furthermore, anthosphere, carposphere, caulosphere and phyllosphere could also contribute to the endophytic microbes [41, 42]. However, these moso bamboo plants have not blossomed and born any fruit yet, and therefore only caulosphere and phyllosphere were alternative sources of endophytic microbes in the test bamboo plants.

The highest bacterial diversity indices and richness estimators were observed in the rhizospheric soils (Figs. 4a-d). The above-ground tissue, root or rhizospheric soil each provides a unique ecological niche for key microbes in the moso bamboo-plant system despite these niches were of the same age. Many factors could limit bacterial colonization and flourishing inside the moso bamboo plants: (1) the significant differences in oxygen, osmotic pressure and nutrient supply between bamboo plants and soils firstly impose selective pressure for suitable bacteria; (2) bacterial colonization is a kind of invasion to host plants, and the immune system of the host plants could exclude or inhibit some bacteria [40]; and (3) even if successful colonization, limited intercellular space and environmental heterogeneity may be unsuitable for the reproduction of some bacteria [43]. Fungi and moso bamboo both belong to the eukarya domain, but fungi still need to overcome niche barriers to enter into the above-ground tissues and form plant-endophytic fungi association. Interestingly, the highest fungal alpha diversities were not always in the rhizospheric soils. This result was also supported by Chhipa and Kaushik [44] who suggested that soils and above-ground stems of *Aquilaria malaccensis* shared similar fungi. Gond et al. [45] also showed that relative to soils, a higher Shannon diversity of fungal community was in the stems of *Nyctanthes arboristis*. The reason for the differences in bacterial and fungal diversities might be that the host biogeography was the key factor dominating endophytic fungi, while plant tissues primarily shaped endophytic bacteria [46, 47]. Simultaneously, phylogenetic profiling of key fungi further confirmed that plant compartments had minor impacts on fungal assemblages including community diversity and structure, which might be evolutionary conservation of endophytic fungi [17, 46, 47].

## **Linking moso bamboo growth with soil nutrient management and endophytic microbes**

**No differences in plant biomass were observed among age groups in our study. Moso bamboo growth is a “slow-fast-slow” process, and the maximum growth rate is generally in the second month after shoot emergence, which contributes to more than 50% of biomass accumulation [11]. The growth of moso bamboo involves soil nutrient uptake by root systems, translocation from roots and then accumulation in the above-ground tissues, and therefore, moso bamboo biomass might be stimulated by the**

increasing availability of soil nutrients, especially N and phosphorus (P) fertilization. Our previous study has shown that P fertilization alone has minor effects on moso bamboo growth in acidic soil [10], but the previous and current studies confirmed that bamboo biomass might be enhanced by increases in  $\text{NH}_4^+$ -N content in soil (Table S3). Piouceau et al. [12] have also found that photosynthetic activities of bamboos increase after combined applications of N and other nutrients. Endophytic microbial colonization in plants could also increase plant biomass and commercial production and thus have useful applications in agroforestry [19, 27, 39]. Endophytic microbes in plants confer various bioactive compounds (protease, lipase, laccase, and antiviral or insecticidal compounds) and protection against harsh conditions of contamination, drought and pathogen [48-50]. The *Chytridiomycota* relative abundances in above-ground tissues, roots and rhizospheric soils were all positively correlated with moso bamboo biomass (Table S3). The *Chytridiomycota*, widely distributed in terrestrial system, could solubilize  $\text{CaHPO}_4$  to enhance plant P bioavailability [51]. Some genera of the *Chytridiomycota* could generate  $\alpha$ -tubulin, effectively decompose straws and litters, mineralize cellulosic substrates to enhance available nutrient content, and the *Chytridiomycota* and arbuscular mycorrhizal fungi had similar molecular systematics and molecular identification, which might be utilized as growth-promoting microbes of moso bamboos [52-54]. Therefore, in the future, moso bamboo management should consider balance applications of  $\text{NH}_4^+$ -N and other nutrients, especially at the

# fast growth stage, and utilisations of endophytic *Chytridiomycota* to stimulate moso bamboo growth.

## Conclusion

There were significantly higher organic matter and total N content in the rhizospheric soils of young bamboo, relative to the mature and old moso bamboos, although the height and above-ground biomass remained relatively similar among the four different age groups. Bamboo ages significantly influenced N transformation rates, functional gene abundances and urease activities of the rhizospheric soils, and the rhizospheric soil of old bamboo plant had the lowest N mineralization rate and urease activity. Bacterial diversity indices and richness estimators in the rhizospheric soils were significantly higher than those of the above-ground tissues. Bamboo ages also significantly affected endophytic bacterial community structures in the above-ground tissues and roots, but niche differentiations outweighed ages in shaping the whole microbial community of the moso bamboo-soil system. In the future, moso bamboo management should consider balanced applications of  $\text{NH}_4^+\text{-N}$  and other nutrients and utilisations of *Chytridiomycota* to stimulate moso bamboo growth.

## Materials And Methods

### Moso bamboo plantation site and sampling

This experiment was conducted in the Bamboo Education Base of Jiangxi Agricultural University (28°76'N, 115°83'E), which has been enclosed since 1998 to prevent anthropological disturbance. The moso bamboo was the main vegetation, with a minor proportion of understory weeds. Every second year, new moso bamboo plants emerged in spring (March). In August 2019, young (0.5-yr), mature (2.5-yr and 4.5-yr) and old (6.5-yr) moso bamboo plants were selected to serve as different treatments, with four replications for each treatment. The ages of bamboo plants were identified based on the stalk colours and fallen leaf traces on twigs. The moso bamboo plants (4 different ages × 4 replicated bamboo plants for each age) were randomly selected, but the distance between adjacent moso bamboo plants was > 8.0 m.

The bamboo plants were harvested in August 2019, and the above-ground plants were cut off, rinsed with clean water and wiped with absorbent paper, prior to determining heights and biomass. Simultaneously, diameters at breast height (DBH) of the bamboo plants were measured at 20 cm above the ground. Below-ground roots (rhizomes) and rhizospheric soils (0-20 cm) were also sampled. The above-ground tissues (approximately 20 cm from the ground) and below-ground roots (approximately 20 cm from the ground) were used to analyze endophytic microbes. Plant samples were surface sterilized with the following procedures: (1) washing with double-distilled  $\text{H}_2\text{O}$  (dd $\text{H}_2\text{O}$ ) for 30 s; (2) sequentially soaking in 70% (v/v) ethanol solution for 2 min, 2.5% (v/v) sodium hypochlorite solution for 5 min and 70% ethanol solution for 30 min; and (3) rinsing with dd $\text{H}_2\text{O}$  for three times. The surface-sterilized plant samples were

used for endophytic and rhizospheric microbial analyses and storage. Every rhizospheric soil sample was divided into four parts: the first part was air-dried and ground to determine soil nutrient contents; the second fresh part was used for quantifying soil mineral N content, N transformation rate and enzyme activity; the third fresh part was used for molecular analyses; and the last part was kept at -20 °C for storage.

## **Soil nutrient content *analyses***

*Soil organic matter and total N were determined according to the methods used in previous studies [55, 56]. In brief, after digesting with sulfuric acid and potassium dichromate, total carbon (C) and N contents were quantified by the titrating method and Discrete Auto Analyzer (SmartChem, USA), respectively, and total C was then converted into organic matter contents. Fresh and sieved soil samples were shaken with 2 M KCl (1:5 soil/water ratio) and filtered. The mineral N contents were analyzed with the Discrete Auto Analyzer, and the same filtered KCl solution was used as a blank.*

## **Soil N transformation rates and urease activity analyses**

*Net N mineralization and potential nitrification rates of rhizospheric soils were quantified with the methods described by Zhang et al. [56]. The rhizospheric soils (10.0 g dry soil equivalent) were incubated in 100 mL flasks at 28 °C for 7 days. Net N mineralization rate was calculated with the changes in mineral N contents before and after incubations and expressed with mg N kg<sup>-1</sup> dry soil d<sup>-1</sup>. For determination of potential nitrification rate, solution of (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> was added into test soil (10.0 g dry soil equivalent) at 100 mg N kg<sup>-1</sup> dry soil as the substrate of nitrification, and then soil microcosm was incubated at 28 °C for 7 days. The potential nitrification rate was quantified with the changes in nitrite and nitrate (NO<sub>2</sub><sup>-</sup>+NO<sub>3</sub><sup>-</sup>-N) contents before and after incubations and expressed with mg N kg<sup>-1</sup> dry soil d<sup>-1</sup>.*

*Soil urease activity was analyzed with the method described by Guan [57]. After treated with toluene for 15 min, fresh and sieved soil samples (5.0 g dry weight equivalent) were mixed with the substrate (urea) and citrate buffer (pH = 6.7) and incubated at 37 °C for 24 h. Soil ammonium-N (NH<sub>4</sub><sup>+</sup>-N) concentrations before and after the incubation were extracted with 2 M KCl and then determined with Discrete Auto Analyzer. Soil urease activity was expressed as mg NH<sub>4</sub><sup>+</sup>-N kg<sup>-1</sup> dry soil d<sup>-1</sup>.*

## **Real-time quantitative PCR (qPCR) analysis**

*Genomic DNA of rhizospheric soils was extracted with Fast DNA SPIN Kit for Soil (MP, USA) and dissolved in 100.0 μL ddH<sub>2</sub>O. The DNA suspensions were evaluated, sub-packed and stored at -20 °C prior to molecular analyses. The qPCR was employed to determine abundances of functional *nprA* and *chiA**

*genes related to N mineralization and ammonia-oxidizing archaea and bacteria (AOA and AOB) amoA genes related to nitrification. The determination methods, including the reaction system, calibration curves and PCR product confirmation, were identical to those reported in our previous study [56]. Primer sequences and amplification efficiencies of qPCR were listed in Table S1. Quantifications of diluted DNA suspension suggested that there was no detectable amplification inhibition for the qPCR in this study.*

## ***PCR amplification of endophytic and rhizospheric microbes***

*The surface-sterilized plant samples were ground and extracted for DNA with the method described by Wang et al. [58]. The V5-V7 region of bacterial 16S rRNA gene was amplified with the nested PCR: in the first-round PCR, the forward and reverse primers were 779F and 1392R, and the amplified product fragment was approximately 593 bp; the forward and reverse primers for the second-round PCR were 779F and 1193R, and the final product fragment was approximately 394 bp. The internal transcribed spacer (ITS) region of fungi was amplified, and the forward and reverse primers were ITS1F and ITS2R. All the primers were added with special barcodes, and after amplification, the products were estimated by 2.0% agarose gel electrophoresis and purified with a gel extraction kit (Qiagen, Germany).*

## ***Illumina Miseq sequencing, quality control and data processing***

*The Illumina Miseq platform (Majorbio, China) was employed to analyse purified PCR products. The fastp software was used to filter raw reads to obtain high-quality reads: (1) raw reads were truncated at the end side, and quality score < 20 within 10 bp sliding window and truncated reads < 50 bp were removed; (2) raw reads with nitrogenous bases were eliminated; (3) reads were merged to one sequence with at least 10 overlapped bases; and (4) the unassembled reads were discarded [59-61]. A series of high-quality reads was classified into operational taxonomic units (OTUs) with > 97% sequence similarity. Based on the OTUs, rarefaction curves, diversity indices (Shannon and invsimpson) and richness estimators (Ace and Chao1) of microbial communities were analyzed. Meanwhile, bacterial and fungal OTUs were identified with the SILVA and UNITE databases and classified into different bacterial and fungal taxonomies, respectively, and presented at phylum and genus levels. Bacterial and fungal functional profilings were predicted with the PICRUSt and FUNGuild analyses, respectively.*

## ***Calculation and statistical analysis***

*The impacts of bamboo plant ages, sample niches and their interactions on microbial properties were evaluated by the two-way analysis of variance (ANOVA), and significant differences ( $P < 0.05$ ) among the same samples with different ages or among different niches were tested by the one-way ANOVA, followed by a Duncan multiple range test. Bamboo properties, soil nutrient contents and functional gene abundances served as candidate variables in stepwise regression analyses of N transformation rates.*

*The ANOVA and stepwise regression analyses were conducted with the SPSS 24.0 software (IBM SPSS Inc., USA). Principal coordinate analysis (PCoA) and unweighted UniFrac clustering were employed to reveal the divergences in endophytic or rhizospheric microbial communities. The PCoA was based on the Bray-Curtis distances, and statistical analyses of similarity with 999 random permutations were also utilized to compare the mean of ranked dissimilarities among different communities to the mean of ranked dissimilarities within the same community. The rarefaction curves, Venn figures, circos diagrams revealing microbial genus levels, PCoA plots and unweighted UniFrac clustering were completed with the online platform of Majorbio Cloud Platform ([www.majorbio.com](http://www.majorbio.com)).*

## ***Declarations***

### ***Ethics approval and consent to participate***

*Not applicable*

### ***Consent for publication***

*Not applicable*

### ***Availability of data and material***

*The sequencing raw data of bacterial and fungal communities have been submitted to the NCBI, and bacterial and fungal BioProject accession numbers were SRP256129 and SRP256169, respectively.*

### ***Competing interests***

*The authors declare that they have no conflict of interests.*

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### ***Authors' contributions***

*MYZ and WYZ designed the study. MYZ, YZ and WYZ sampled the soils and plants, determined soil nutrient contents and N transformation rates, extracted DNA and performed bioinformatics and statistical analysis. MYZ wrote the manuscript, and WJW, SHB and ZHX revised the manuscript. All authors read and approved the final manuscript.*

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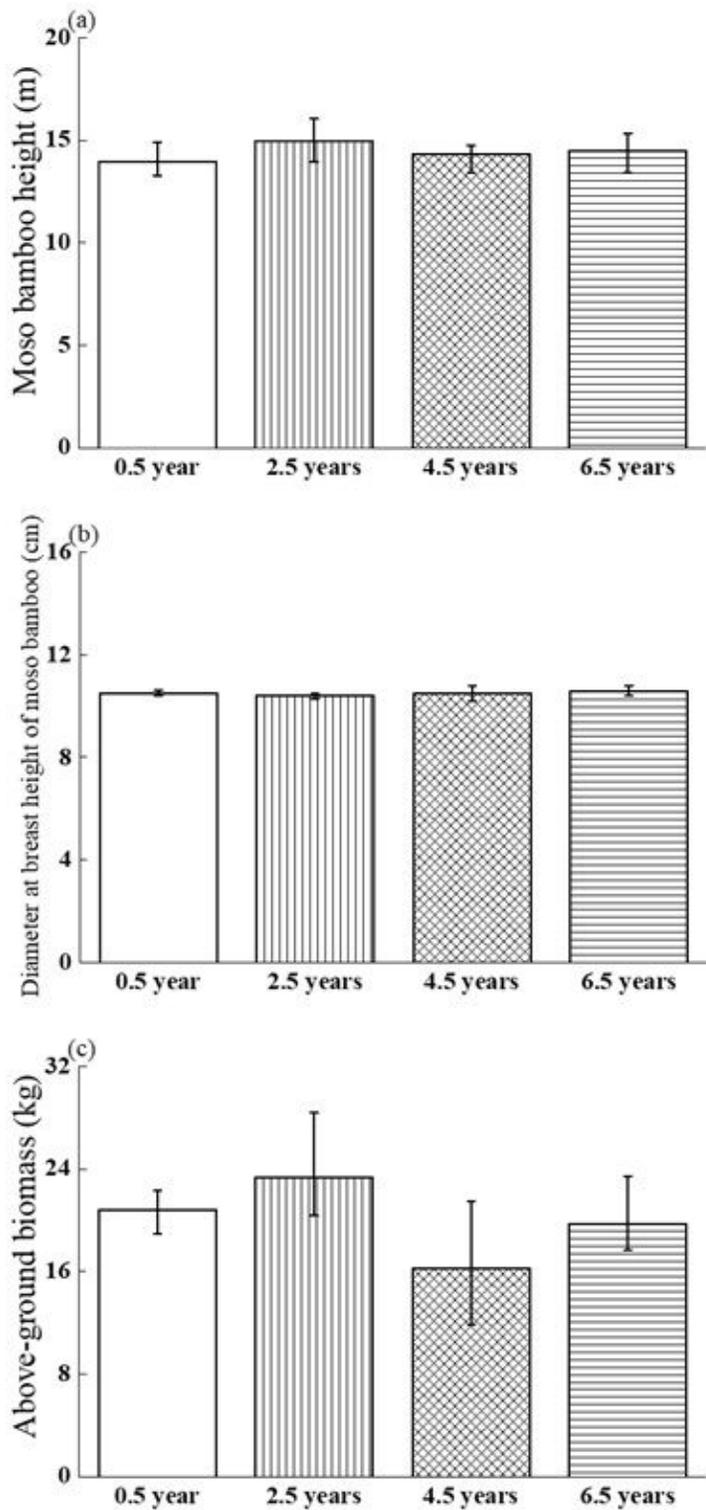
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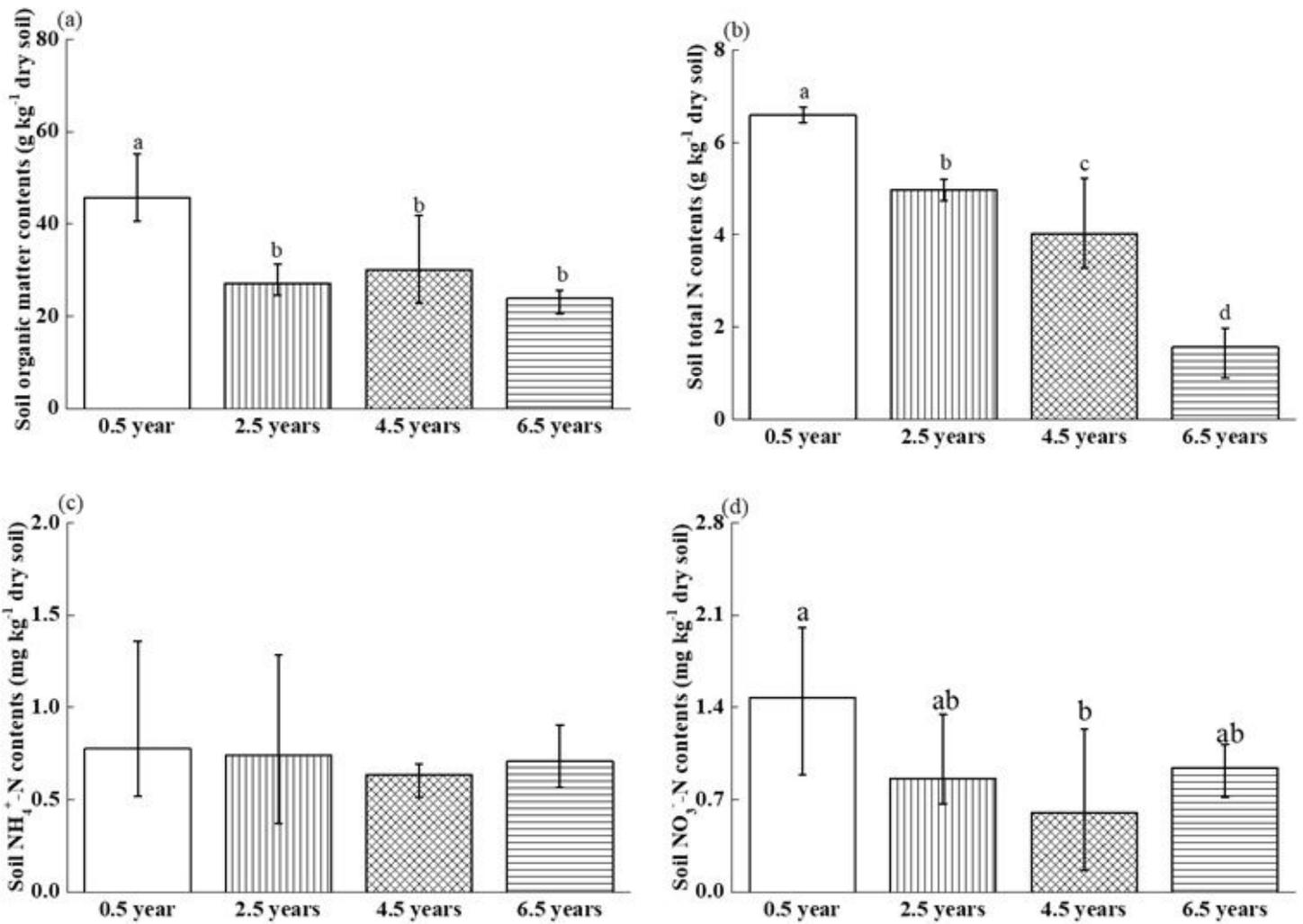
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## **Figures**



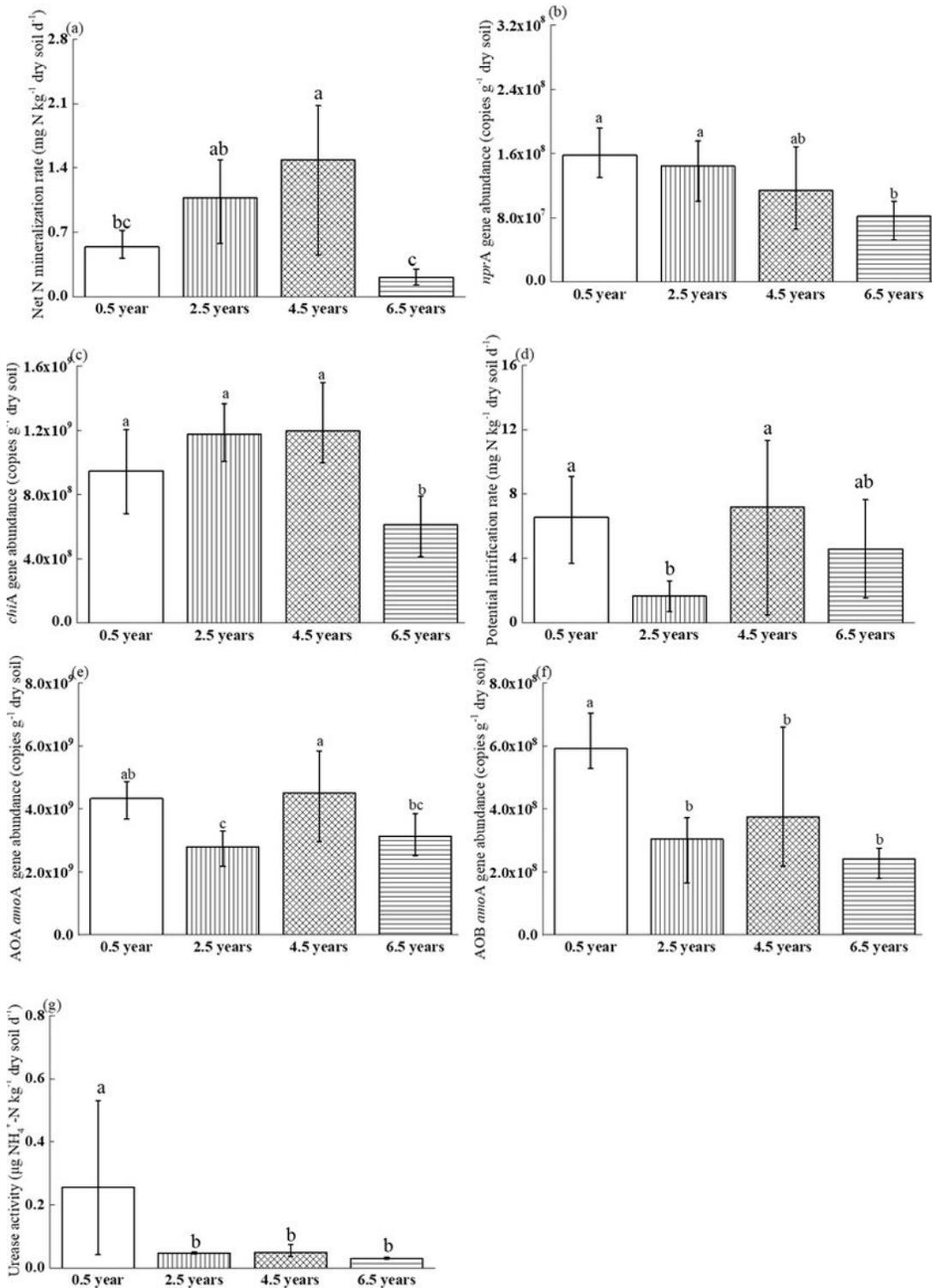
**Figure 1**

*Impacts of bamboo ages on moso bamboo properties. (a) heights, (b) diameter at breast height and (c) above-ground biomass of moso bamboo. Vertical bars indicate the maximum and minimum values*



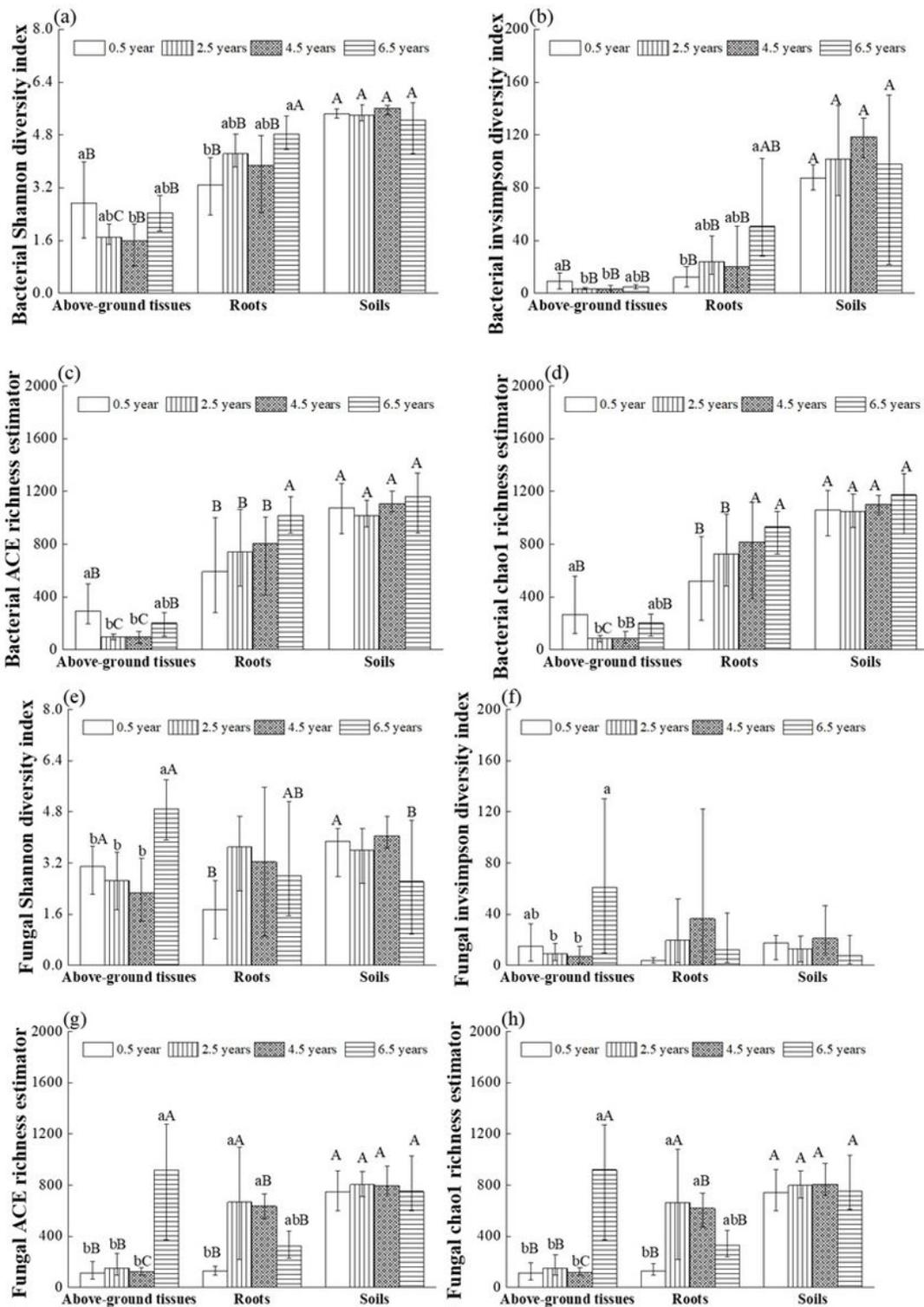
**Figure 2**

**Impacts of bamboo ages on soil properties. (a) organic matter, (b) total N, (c) NH<sub>4</sub><sup>+</sup>-N and (d) NO<sub>3</sub><sup>-</sup>-N contents in rhizospheric soils. Vertical bars indicate the maximum and minimum values. Lowercase letters show significant differences among the different rhizospheric soils**



**Figure 3**

*Impacts of bamboo ages on the biological properties related to rhizospheric soil N cycling. (a) net N mineralization rate, (b) nprA gene abundance, (c) chiA gene abundance, (d) potential nitrification rate, (e) AOA amoA abundance, (f) AOB amoA gene abundance and (g) urease activity. Vertical bars indicate the maximum and minimum values. Lowercase letters show significant differences among the different rhizospheric soils*



**Figure 4**

*Impacts of bamboo ages on the microbial community diversities. (a) Shannon and (b) invsimpson diversity indices, and (c) ACE and (d) chao1 richness estimators of bacterial communities, and (e) Shannon and (f) invsimpson diversity indices, and (g) ACE and (h) chao1 richness estimators of fungal communities. Vertical bars indicate the maximum and minimum values. Lowercase letters show*

significant differences among the same samples of different ages, and capital letters show significant differences among different niches of the same age

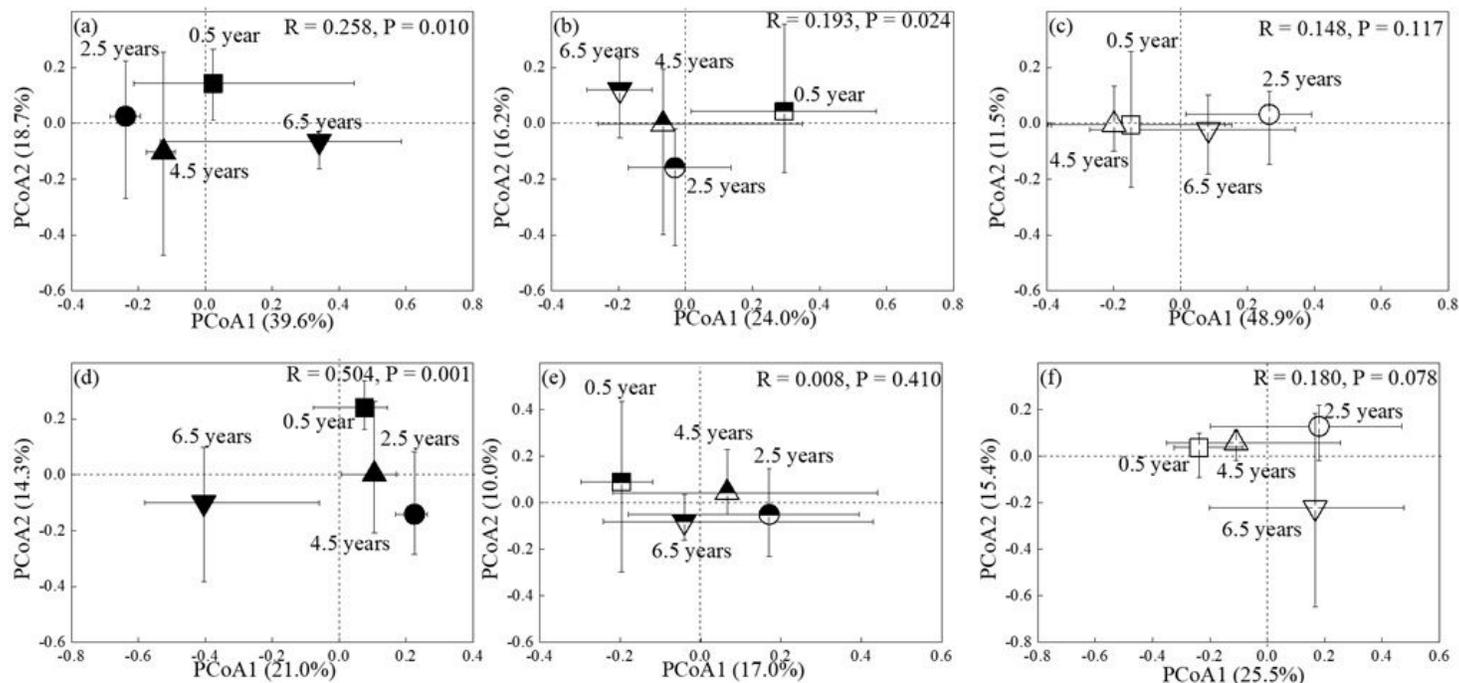


Figure 5

Impacts of bamboo ages on microbial community structures at different niches. Bacterial community structures in (a) above-ground tissues, (b) roots and (c) rhizospheric soils and fungal community structures in (d) above-ground tissues, (e) roots and (f) rhizospheric soils

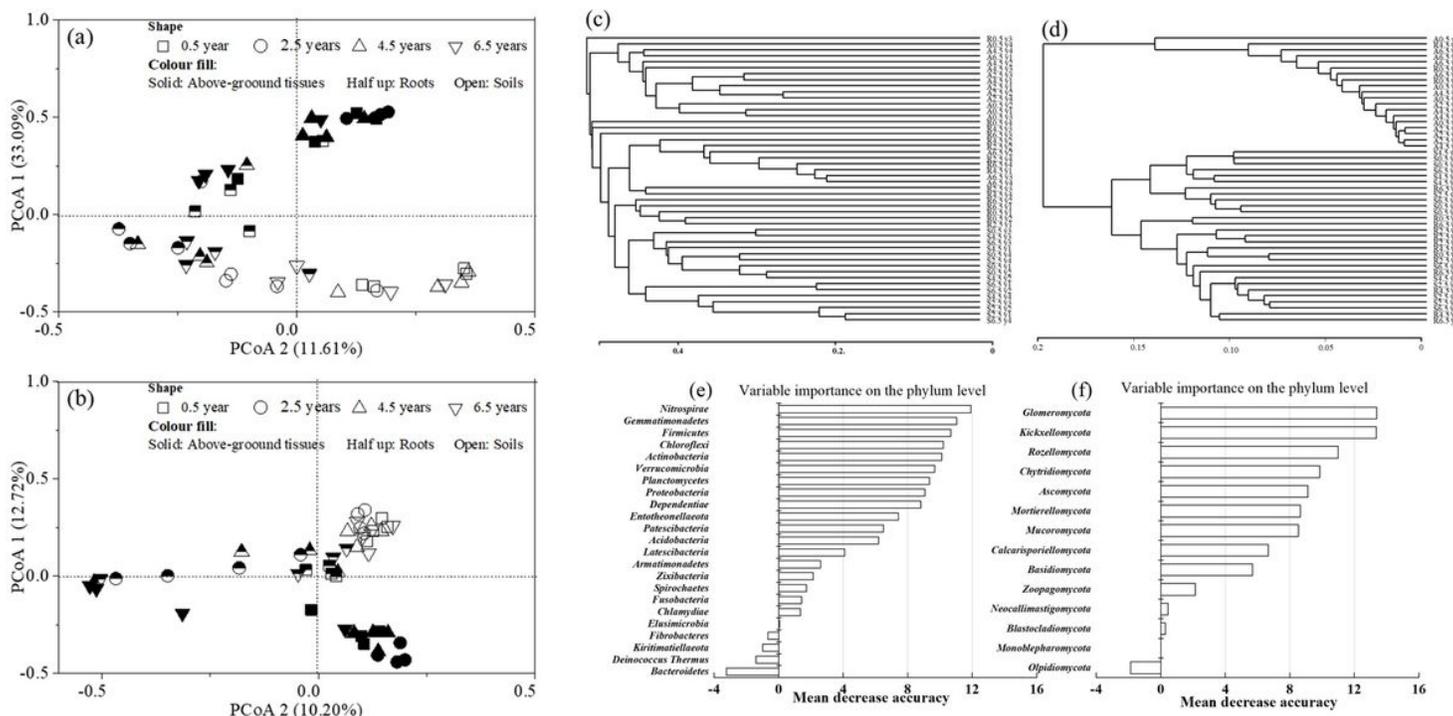


Figure 6

*The principal coordinate, unweighted UniFrac clustering and random forest analyses of microbial communities. Principal coordinate analyses of (a) bacterial and (b) fungal communities, unweighted UniFrac clustering of the (c) bacterial and (d) fungal communities, and random forest analyses of the (e) bacterial and (f) fungal communities. A0.5y, 0.5-year above-ground tissues; A2.5y, 2.5-year above-ground tissues; A4.5y, 4.5-year above-ground tissues; A6.5y, 6.5-year above-ground tissues; R0.5y, 0.5-year roots; R2.5y, 2.5-year roots; R4.5y, 4.5-year roots; R6.5y, 6.5 roots; S0.5y, rhizospheric soils of 0.5-year bamboos; S2.5y, rhizospheric soils of 2.5-year bamboos; S4.5y, rhizospheric soils of 4.5-year bamboos; and S6.5y, rhizospheric soils of 6.5-year bamboos*

## ***Supplementary Files***

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