

# Tight coupling of fungal community composition with soil quality in a Chinese fir plantation chronosequence

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## Abstract

Predicting changes in carbon and nutrient cycles in plantations requires a mechanistic understanding of the effects of stand age on soil quality and microbial communities. Here, we evaluated soil quality by using an integrated soil quality index (*SQI*) and traced the parallel shifts in fungal community composition using high-throughput sequencing in a chronosequence of Chinese fir (*Cunninghamia lanceolata*) plantations (stand age of 3, 16, 25, 32, >80 years). Soil properties showed pronounced changes with stand age in the topsoil. Soil organic carbon (SOC), total nitrogen (TN) and available phosphorus (AP) were 2.1, 1.9 and 2.2 times higher, respectively, in the oldest stands than in the youngest stands. *SQI* of the top 5 cm increased logarithmically with stand age. Mycorrhizal fungi initially increased in younger stands, but then they were gradually replaced by saprotrophs in older stands due to larger litterfall. Strong positive correlations between saprotrophic fungi and key soil quality indicators, such as TN, AP and  $\text{NH}_4^+$ , confirmed that abundance of decomposers is tightly linked with higher soil quality. Mycorrhizal orders *Thelephorales*, *Sebacinales* and *Russulales* increased in abundance and raised the activity of acid phosphatase to mobilize limiting phosphorus from organic matter. Consequently, mycorrhizal fungi are especially relevant in younger stands to acquire nutrients to sustain tree productivity. In developed stands, however, saprotrophic fungi are crucial in recycling nutrients from the litter. Collectively, the increase of topsoil quality during the life cycle of Chinese fir plantations is closely linked with the observed transition of fungal communities from mycorrhizae to saprotrophs.

**Keywords:** *Cunninghamia lanceolata*; Mycorrhizal fungi; Saprotrophic fungi; Soil quality index; Stand age

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## 1. Introduction

Global forest plantations have increased by over 105 million ha since 1990, and in 2015 they accounted for 291 million ha (FAO, 2016). These plantations play an important role in timber supply, offering diverse non-timber products, and sequestering CO<sub>2</sub> (van Dijk & Keenan, 2007). However, a major concern is that planting monoculture species continuously on the same site reduces soil quality and stand productivity (Guillaume, Damris, & Kuzyakov, 2015; Selvaraj, Duraisamy, Huang, Guo, & Ma, 2017; Huang et al., 2018). Silvicultural practices in plantations usually consist of harvesting mature timbers by clear-cutting, slash-and-burn of residues, and then replanting seedlings that will grow into mature stands again. These clear-cutting practices have strong negative effects on soil quality and are paralleled by shifts in soil microbial communities that alter carbon (C) and nutrient cycling as well (Lladó, López-Mondéjar, & Baldrian, 2017). Therefore, evaluating the implications of plantation practices on soil functions is of paramount importance for maintaining soil quality throughout the harvest cycle.

Healthy soils preserve environmental quality, support floral and faunal health, and sustain biological productivity (Doran & Parkin, 1994). In forestry, soil quality monitoring is fundamental to sustainable management (Burger & Kelting, 1999). Indices that integrate soil physico-chemical (texture, pH, nutrient contents, etc.) and biological properties (soil enzyme activity, microbial biomass, etc.) have been widely used to comprehensively assess the quality of soils (Bünemann et al., 2018). Most studies have developed soil quality indices (*SQI*) in the context of land-use conversion (Yu, Liu, Zhang, Li, & Zhou, 2018; Guillaume et al., 2016), vegetation restoration (Bautista-Cruz et al., 2012; Zhang et al., 2019) or mine

reclamation (Mukhopadhyay et al., 2016). These existing *SQI* have a specific purpose and are usually useful only in a particular environmental scenario. For example, Bautista-Cruz et al. (2012) identified soil organic C (SOC), pH, organic layer thickness, available phosphorus (P), and exchangeable  $Al^{3+}$  as the primary indicators of soil quality during the recovery of a deforested tropical montane cloud forest in Mexico. These indicators were selected because they represented soil functions important to the study system. However, key soil quality indicators specific to sustainable plantation management have yet to be identified.

The characteristics of the soil microbial community, including composition, taxa abundance, and diversity, have important implications for soil functions, especially in relation to biogeochemical processes (Gunina, et al., 2017; Shao et al., 2019; Wei et al., 2019, 2020). In forests, soil fungi comprise a large proportion of the microbial community, and they play central roles in C and nutrient cycling, depending on their functional group (Uroz, Buée, Deveau, Mieszkin, & Martin, 2016; Chen et al., 2019b; Guo et al., 2019; Ji et al., 2020). Therefore, any changes in fungal communities are likely to be reflected in the soil conditions. Self-sufficient saprotrophic and synergetic mycorrhizal fungi are the two main fungal functional guilds (funguilds). The former is important for decomposition and the latter for nutrient cycling. These two funguilds have overlapping fundamental niches, and they compete for space and nutrients (Bödeker, Lindahl, Olson, & Clemmensen, 2016; Chen et al., 2019a). The interactions between mycorrhizal and saprotrophic fungi have been studied well in boreal forests (Bödeker, Lindahl, Olson, & Clemmensen, 2016; Kvaschenko, Clemmensen, Hagenbo, Karlton, & Lindahl, 2017b), but less is known about subtropical or tropical forests, which differ in soil funguilds and have more widespread biodiversity. How

the composition of these two funguilds changes in relation to soil quality as forest stands develop remains unclear.

China has the world's largest area of plantations, and Chinese fir (*Cunninghamia lanceolata* [Lamb.] Hook) plantations have become especially widespread because the tree grows rapidly and produces high quality wood. Shorter crop rotations and clear-cut harvesting have become common practice due to the steady rise in timber demand since the 1980s (Chen, Zhang, Zhou, & Zheng, 1990). This increasing demand has consequently led to soil nutrient depletion and overall reduction in plantation yield (Tian et al., 2011). This outcome has only become more problematic as plantation managers become more concerned with ecosystem sustainability. Previous studies have explored how soil C stocks (Chen et al., 2013), soil properties (Selvaraj, Duraisamy, Huang, Guo, & Ma, 2017), and microbial community composition (Wang et al., 2019) change over time, but comprehensive evaluation of soil quality throughout the life cycle of a Chinese fir plantation is scarce. Productivity of tree biomass typically increases early in stand development, in parallel with early increase in nutrient uptake, but then it declines (Wu et al., 2019). Meanwhile, litterfall increases with stand age, returning C and nutrients into the topsoil as the litter decomposes. Thus, we hypothesize that (i) stand age affects soil physico-chemical and biological properties starting in the topsoil. Consequently, *SQI* will increase in topsoil much more than in the subsoil. For soil fungal communities, the competition for nutrients between mycorrhizal and saprotrophic fungi may intensify in younger stands, where soil C and nutrient contents are low. Under this scenario, species that acquire N or P efficiently may gain a competitive advantage (Bödeker et al., 2014). As stands develop, fresh litter that is

deposited constantly on the topsoil may favor saprotrophic species that produce cellulose-decomposing hydrolytic enzymes. Such enrichment of hydrolytic enzymes in older stands may ease saprotrophic competition with mycorrhizae as increasing amounts of N and P nutrients are released from decomposing organic matter. We therefore also hypothesize that (ii) the abundance of mycorrhizal fungi will increase in younger stands initially after establishment, but then they will gradually be replaced by saprotrophic fungi as stands gets older because of increased litterfall, which sustains saprotrophs.

To test these two hypotheses, we sampled soils from a chronosequence of Chinese fir plantation stands across five age groups: 3, 16, 25, 32 and >80 years. First, we quantified soil physico-chemical and biological properties and calculated *SQI* using the weighted index method to evaluate soil quality in varying ages of stands. Then, we assessed the community composition of the fungi by performing high throughput sequencing on fungal ITS amplicons and investigated linkages with the key soil quality indicators. Finally, we attempted to clarify how the fungal community composition changes in accordance with soil quality along a stand age gradient to better inform effective forest management.

## **2. Materials and methods**

### ***2.1. Study site and experimental design***

The chronosequence study was carried out in Huitong County (26°41'50"-26°47'08" N, 109°35'26"- 109°38'45" E), southwest of Hunan Province, China (Figure 1). This region is located in a typical humid subtropical monsoon climate and has a mean annual precipitation of 1270 mm and a mean annual temperature of 16.8 °C. The elevation of the stands ranges



from 330 to 482 m above sea level. The shale and slate-derived soil is red-yellow, with a grayish upper horizon above the reddish argillic horizon. The soil type is classified as Allit-Udic Ferrosols according to the description of an Acrisol in the World Reference Base for Soil Resources (Wu et al., 2019). Native vegetation in the mountains of this region has been replaced with Chinese fir for timber production. In the study plantation, seedlings were planted in uniformly-spaced pits. After planting, they were weeded twice a year for the first three years. No other management activities were used in this plantation until the trees were harvested. Currently, clear-cut harvesting in Chinese fir plantations generates a patchwork of various-aged stands, from recent clear-cuts to mature stands.

In 2017, a chronosequence of five stand age groups were selected: 3- (young), 16- (middle-aged), 25- (near-mature), 32- (mature) and >80-years old (over-mature) (Figure 1). All of the selected Chinese fir plantations are the second generation with the same seedling variety. Four permanent plots (20 m × 20 m) were randomly established in four separate stands per age group, resulting in twenty totally sample plots. Although climatic differences among sites can increase measured variation, careful site selection can reduce conflating factors in chronosequence studies (Walker, Wardle, Bardgett, & Clarkson, 2010). To minimize the variability among sites, these stands were within 2.0 km of each other to guarantee the same climate and soil parent materials. Moreover, all the stands had similar elevation, topography, and soil texture and were all distributed on well-drained uplands with a mean elevation of about 400 m and slopes ranging from 20° to 30°. The diameter at breast height (DBH) and total tree height (H) of all individual stems were recorded in each plot. *Rubus innominatus*, *Maesa japonica*, *Dichroa febrifuga*, *Woodwardia japonica*,

*Dicranopteris linearis* and *Miscanthus* dominated the understory. Detailed information on stand characteristics is presented in Table S1.

## **2.2. Soil sampling**

In July 2018, soil was sampled after removing the litter layer. Soil samples were collected in each plot at five points from both the top 0-5 cm (organic horizon, which was a mixture of humus and mineral soil) and the subsequent 5-15 cm (mineral horizon, which was dominated by mineral soil) (Figure 1). The samples were then combined and run through a 2-mm mesh sieve to discard visible materials, including small roots and stones. Two soil core samples (diameter 5 cm) were collected with a mater corer to determine bulk density of each horizon. Fresh soil samples were stored in a freezer during transporting to the laboratory. One set of the subsamples (500 g) was air-dried and sieved in preparation for physico-chemical analyses, and another set (200 g) was stored at 4 °C for microbial biomass and enzyme activity determination. Further, additional subsamples (10 g) were stored at -80 °C prior to extraction of DNA to support microbial community analysis.

## **2.3. Soil physico-chemical properties analyses**

Soil bulk density (BD) and water content (SWC) were measured via core cutter and gravimetric methods, respectively, by oven-drying (105 °C) the fresh soil samples to a constant weight. Soil pH was measured using a 1:2.5 mixture of soil-to-deionised water, which was shaken for 30 min and then measured with a pH meter. Total nitrogen (TN) was determined by dry combustion in an element analyzer (Vario EL III, Elementar, Germany).

Total phosphorus (TP) and total potassium (TK) were measured by wet digestion with NaOH and then determined using a flame photometer (Smith & Bain, 2008). Soil organic carbon (SOC) was determined using  $K_2Cr_2O_7$ - $H_2SO_4$  oxidation (Nelson & Sommers, 1982). Alkali-hydrolyzable nitrogen (AN) was assayed by alkaline potassium permanganate distillation (Subbiah & Asija, 1956). For extractable inorganic nitrogen, 5-8 g of wet soil was mixed with 40 mL of 0.5 M  $K_2SO_4$  and shaken for 60 min. The soil suspension was filtered and  $NH_4^+$  and  $NO_3^-$  concentrations measured using a flow injection analyzer (FIAstar 5000, FOSS, Sweden). Available phosphorus (AP) concentration was extracted using 0.05 M HCl-0.025 M  $H_2SO_4$ , and the ammonium molybdate ascorbic method was employed for the final determination (Mehlich, 1984).

Microbial biomass C (MBC), N (MBN) and P (MBP) were analyzed by the fumigation-extraction method (Vance, Brookes, & Jenkinson, 1987). The potential activity of the following three soil enzymes were measured by fluorometric 96-well microplate assay using fluorogenic methylumbelliferone-based (MUB) substrates:  $\beta$ -glucosidase (BG), which is a proxy for C release;  $\beta$ -N-acetylglucosaminidase (NAG), which is involved in depolymerizing organic N; and acid phosphatase (ACP), which is associated with P mobilization (Chen, Li, Xiao, & Wang, 2018). To prepare soil suspensions, 1.0 g of fresh soil was homogenized in 125 ml of 50 mM sodium bicarbonate (pH 5.0). Microplates were incubated at 25 °C for up to 4 h in the dark. Then, an aliquot of 10  $\mu$ l NaOH solution (1.0 M) was added to each well at the end of incubation to terminate the reaction. A microplate fluorometer was used to determine fluorescence at 365 nm excitation and 450 nm emission.

#### ***2.4. Soil DNA extraction, sequencing, and data processing***

Total DNA was extracted from 250 mg of fresh soil, using the PowerSoil DNA Isolation Kit (MoBio Laboratories, California, USA). Briefly, each soil sample was added to a bead beating tube for rapid and thorough homogenization, and cell lysis occurred by mechanical and chemical methods. Next, total genomic DNA was captured on a silica membrane in a spin column format. Finally, DNA was washed and eluted from the membrane. The quality and quantity of the extracted DNA were determined using a NanoDrop spectrophotometer. To amplify fungal ITS regions, the primer pair of ITS1F (5'-CTTGGTCATTTAGAGGAAGTAA-3') and ITS2 (2043R) (5'-GCTGCGTTCTTCATCGATGC-3') was employed (Bokulich & Mills 2013; Gardes & Bruns 1993). PCR followed the following thermal-cycling conditions: denaturing at 94 °C for 3 min, and then 35 cycles of annealing at 94 °C for 45 s, at 50 °C for 60 s, and at 72 °C for 60 s, and then a last extension phase at 72 °C for 10 min. PCR reactions were performed in triplicate with 20 µL of a mixture that contained 4 µL of 5 × FastPfu Buffer, 0.8 µL of each primer (5 µM), 2 µL of 2.5 mM dNTPs, 0.4 µL of FastPfu Polymerase and 10 ng of template DNA. Purified amplicons were grouped by equimolar and paired-end sequences (2 × 300) on an Illumina MiSeq platform (Illumina, San Diego, USA) according to the standard protocol provided by Majorbio Bio-Pharm Technology Co. Ltd. (Shanghai, China). All raw sequences were placed in the NCBI Sequence Read Archive (SRA) database under the accession nos. SRR10152476 - SRR10152515.

Raw ITS sequences were processed using the Quantitative Insights Into Microbial Ecology (QIIME) platform (Caporaso et al. 2010). A threshold of average quality scores

greater than 30 over a 50-bp window was used to filter for quality. If joined sequences had ambiguous bases and lengths less than 200-bp, they were discarded. Operational taxonomic units (OTUs) were identified using UPARSE with a 97% identity threshold after discarding the chimeras and singletons (Edgar, 2013). Finally, fungal OTUs were sorted into taxon by using BLAST against the UNITE database (Abarenkov et al., 2010). The FUNGuild tool was used for funguilds assignment (Nguyen et al., 2016).

### ***2.5. Soil quality index (SQI) evaluation***

Calculating *SQI* has three main steps: (1) selecting a minimum dataset (MDS) that best represents soil functions, (2) scoring the MDS indicators, and (3) consolidating the scores into single *SQI* values (Huang et al., 2018).

We analyzed 19 soil physicochemical and biological properties (Table S1) by employing principal component analysis (PCA) and Pearson's correlation analysis to identify the most suitable indicators of soil quality. According to Andrews et al. (2002), principal components (PCs) must have eigenvalues  $\geq 1.0$  that explain  $> 5\%$  of total variation to be MDS potentials. Within each selected PC, those with an absolute value within 10% of the highest loading factor were selected as the important indicators. Moreover, Pearson's correlation analysis was performed as a secondary means of eliminating factors if more than one indicator was retained (Bastida, Moreno, Hernández, & García, 2006). The lowest weighted indicator of a pair correlating at  $> 0.6$  would be removed.

After MDS indicators were selected, a nonlinear scoring function was employed to convert the soil indicators into scores ranging from 0 to 1. The equation for soil indicator

score was given in Andrews, Karlen, and Mitchell (2002):

$$S = a/[1 + (x/x_0)^b] \quad (1)$$

where  $S$  is the indicator score,  $a$  is the maximum score ( $a = 1$ ),  $x$  is the value of the soil indicator,  $x_0$  is the mean value of each indicator, and  $b$  is the value of the equation's slope. Slope values ( $b$ ) of -2.5 and 2.5 were used to illustrate a "more is better" and a "less is better" curve, respectively (Bastida, Moreno, Hernández, & García, 2006).

After scoring and weighting for all MDS indicators,  $SQI$  was calculated by the following equation (Masto, Chhonkar, Singh, & Patra, 2008):

$$SQI = \sum_{i=1}^n S_i \times W_i \quad (2)$$

where  $S_i$ ,  $W_i$  and  $n$  are the score, weighting, and number of selected indicator, respectively.

## 2.6. Statistical analysis

First, one-way analysis of variance (ANOVA), and then a Tukey's HSD test were used to test for significant differences in soil properties and fungal diversity among stand ages and between soil layers. Similarity in fungal community structure across stand ages was visualized in CANOCO 5.0 software (Microcomputer Power, Ithaca, NY, USA) by using non-metric multidimensional scaling (NMDS) ordinations based on Bray-Curtis dissimilarity matrices. To test whether fungal community composition differed significantly among stand ages and between soil layers, analysis of similarities (ANOSIM, 999 permutations) was performed using the R "vegan" package (R Development Core Team, 2015).

Pearson's correlation analysis helped assess the relationship between key soil quality indicators and fungal diversity. To test correlations with fungal community composition, the

R “ecodist” package was used to perform simple Mantel tests for each soil layer, and the Bray-Curtis index served as the dissimilarity metric. The variation in abundance of dominant fungal taxa among stand ages and their association with the key soil quality indicators was visualized using principal component analysis (PCA) with environmental metadata as additional variables.

### 3. Results

#### 3.1. Changes in soil properties with stand age

Across all stand ages, the top 0-5 cm soil layer tended to be more nutrient rich and less compact than the 5-15 cm layer (Figure 2). Soil pH was consistently acidic (from 3.79 to 4.59) in both layers, and it was less acidic in the 3-year-old stand. The most pronounced changes in C and nutrients with increasing stand age were observed in the top 5 cm. SOC increased 2.1-fold: from 36.4 g kg<sup>-1</sup> in the 3-year-old stand to 76.0 g kg<sup>-1</sup> in the >80-year-old stand. The TN content also increased gradually, from 3.49 g kg<sup>-1</sup> at 3 years to 6.53 g kg<sup>-1</sup> at >80 years. The trend for AN content was similar to those for TN. However, the TP and AP contents decreased by 21% and 33%, respectively, from 3 to 16 years, and then increased at subsequent ages.

The highest values of microbial C, N, and P contents in the 0-5 cm layer were all detected in the >80-year-old stand (Figure 3), similar to the highest SOC, TN, and TP accumulation (Figure 2). BG activity in the 0-5 cm layer increased linearly from 19.9 nmol g<sup>-1</sup> h<sup>-1</sup> in the 3-year-old stand to 84.6 nmol g<sup>-1</sup> h<sup>-1</sup> in the 25-year-old stand (Figure 3). NAG and ACP activities in the 0-5 cm layer also increased initially with stand age up to 25 years

old, and then a substantial decrease at 32 years old, but followed a second increase in stands >80 years old (Figure 3). Soil enzyme activities were lower in the 5-15 cm layer than in the topsoil, and this remained fairly constant across stand age.

### **3.2. Evaluation of *SQI***

PCA was performed separately for each soil depth. In the 0-5 cm layer, the first four components explained a total of 84.0% of the variation, and eigenvalues of PCs were all  $\geq 1.0$  (Table S2). BD, SWC, SOC, TN, AN, MBC and MBN were highly weighted indicators in PC-1 and also correlated significantly with each other. TN had the highest weighting (0.953), so it was only retained in the MDS. Although ACP and pH had similar weightage score in PC-2, ACP had a higher weightage relative to pH so it was selected for the MDS. Similarly, DON and TK were selected for PC-3 and PC-5, respectively. In sum, TN, ACP, DON, and TK were the four key soil quality indicators selected in the 0-5 cm layer. In the 5-15 cm layer, six PCs explained 84.5% of the total variance (Table S3), and AP,  $\text{NH}_4^+$ , MBC, ACP, MBP and SOC were retained in the MDS.

The weighting values based on the PCA were used to calculate the *SQI* (Table S4). In the top layer at 0-5 cm, *SQI* increased logarithmically with stand age (Figure 4). During the stand's initial development, *SQI* increased sharply from 0.32 at 3 years to 0.57 at 25 years. After stand maturation (> 25 years), *SQI* showed no further significant increase and ranged from 0.57 to 0.62. *SQI* increased less rapidly with stand age in the 5-15 cm layer.

### **3.3. Shifts in fungal diversity and community composition as stands age**



A total of 2,508,709 of fungal sequences remained from the 40 soil samples after quality filtering. This was normalized to 30,605 sequences per sample. After clustering, the fungal sequences were binned into 773 OTUs (0-5 cm layer) and 690 OTUs (5-15 cm layer) at 97% sequence identity. Fungal diversity (OTU richness or  $H'$ ) increased with stand age in the 0-5 cm layer, and was overall higher than in the 5-15 cm layer (Figure 5a and b). The dissimilarity of the fungal community composition changed substantially across stand ages (ANOSIM:  $p = 0.001$  in the 0-5 layer and  $p = 0.002$  in the 5-15 cm layer) and differed between the two soil layers (ANOSIM,  $p = 0.001$ ) (Figure 5c).

The dominant soil fungal phyla were *Ascomycota*, *Basidiomycota*, *Zygomycota* and *Rozellomycota* (Figure 6a and b). In the 0-5 cm layer, the relative abundance of *Basidiomycota* decreased with stand age, while *Ascomycota* and *Zygomycota* were more abundant in the 32- and >80-year-old stands than in the younger stands ( $p < 0.05$ , Table S5). In the 5-15 cm layer, *Ascomycota* was ~1.5 times more abundant in the > 80-year-old stand than in any other aged stands, while *Basidiomycota* were extremely rare. Functionally, the soils were dominated by saprophytic fungi across all stand ages. The mycorrhizal fungi initially increased early in stand development, but they subsequently decreased in both soil layers after the stand matured (Figure 6c and d).

### **3.4. Relationship between key soil quality indicators and fungal community**

Analysis through Pearson's correlation demonstrated that fungal diversity had a strong positively correlation with ACP ( $p = 0.04$ ),  $\text{NH}_4^+$  ( $p < 0.001$ ) and DON ( $p = 0.013$ ) in the top 5 cm soil. In the 5-15 cm layer, fungal diversity was found to correlate negatively with AP ( $p$

= 0.015) and to positively correlate with  $\text{NH}_4^+$  ( $p = 0.035$ ) and ACP ( $p = 0.045$ ) (Table S6).

In the 0-5 cm layer, the Mantel test results indicated that the fungal community matrices correlated positively with all the key soil quality indicators, including TN ( $p = 0.001$ ), ACP ( $p = 0.004$ ) and DON ( $p = 0.03$ ). Also, fungal community composition correlated significantly with AP ( $p < 0.001$ ),  $\text{NH}_4^+$  ( $p < 0.01$ ) and ACP ( $p = 0.048$ ), which were the key soil quality indicators in the 5-15 cm layer.

Principal component analysis revealed similarity in the key soil quality indicators among fungal orders (Figure 7). In the 0-5 cm layer, the saprotrophic fungal orders *Auriculariales*, *Mortierellales*, *Agaricales* and *Pleosporales* were associated with older stands with high TN content (Figure 7a). Furthermore, the P-acquiring enzyme ACP was closely associated with the abundance of orders *Sebacinales*, *Hymenochaetales*, *Thelephorales*, and *Russulales*, most of which are mycorrhizal fungi. In the 5-15 cm layer, the key soil quality indicators of AP and  $\text{NH}_4^+$  correlated positively with saprotrophic orders *Archaeorhizomycetales* and *Eurotiales*, respectively (Figure 7b).

## 4. Discussion

### 4.1. Changes in soil quality with increasing stand age

Stand age strongly affected soil properties, starting in the topsoil (Figure 2). The 3-year-old stand had the lowest SOC and TN content, mainly because the removal of the burnt materials after the previous clear-cut harvest and the high intensity rainfall had contributed to erosion (Guillaume, Damris, & Kuzyakov, 2015; Selvaraj, Duraisamy, Huang, Guo, & Ma, 2017). The pattern of SOC enrichment in the 0-5 cm layer with stand age is directly related to

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photosynthesis, which enables C input via litter and rhizodeposition (Kuzyakov, 2001; Pausch & Kuzyakov, 2018). The increase in TN and AN with stand age are mainly due to N<sub>2</sub>-fixation and atmospheric deposition (Lovett et al., 2018). However, the source of all P in the soil is from weathered rocks and those retained and recycled by vegetation and microbial biomass (Osman, 2013; Gao, Li, Zhao, & Kuzyakov, 2019). In young stands, rapidly growing trees take up soil P, transferring it into aboveground biomass, thus leading to soil P depletion (Selvaraj, Duraisamy, Huang, Guo, & Ma, 2017). In Chinese fir plantations, it has been reported that P uptake increases in stands from age 8 to 24, at levels of 2.2 kg P ha<sup>-1</sup> yr<sup>-1</sup> to 2.8 kg P ha<sup>-1</sup> yr<sup>-1</sup> (Ma et al., 2007). As the stands develop, the litter input increases, thus returning P to the soil (1.8 kg ha<sup>-1</sup> yr<sup>-1</sup> in 32-year-old stand), and leading to an increase of P in the topsoil (Wu et al., 2019). Collectively, the shift from nutrient acquisition to recycling as stands aged resulted in enriched C, N, and P in the 0-5 cm soil layer of these Chinese fir plantations. Nutrient levels in the 5-15 cm layer remained stable or only a slight increase with stand age, possibly due to the equilibrium between uplift of nutrients from deeper layers and percolates from the top 0-5 cm layer. Plant cover resources in topsoil increase with stand age, and this leads to a corresponding rise in microbial biomass (Li et al., 2018). The increase in soil enzyme activity from 3 to 25 years was attributed to enhanced mineralization that helps overcome nutrient stress (Lungmuana et al., 2017). The subsequent decrease in soil enzyme activity in older stands (32 and > 80 years) may be due to microbial nutrient demands that are satisfied in old plantations, where microbial communities do not require the production of extracellular enzymes to mine nutrients.

The *SQI* based on our data increased in tandem with the selected key soil indicators

(Figure 4). TN contributed the most to *SQI* in the 0-5 cm layer (Table S4). ACP, an important indicator of P mineralization, was also a vital indicator, but it was secondary to TN. The AP and  $\text{NH}_4^+$  emerged as the two most important indicators for *SQI* in the 5-15 cm layer. Consequently, Chinese fir trees require high N and P for optimal growth (Zou et al., 2014; Huang et al., 2018). Zhang et al. (2019) also identified TN and AP as important and sensitive soil indicators in subtropical forest ecosystems. In Chinese fir plantations, clear-cut harvesting practices caused dramatic nutrient loss, leading to decreased soil fertility with the lowest *SQI* values in the newly regenerated stands (Selvaraj, Duraisamy, Huang, Guo, & Ma, 2017). Although the post-harvest recovery of soil quality occurs gradually as stands aged, management measures should be adopted to retain soil nutrients to allow successive rotation of Chinese fir.

#### ***4.2. Shifts in soil fungal community with stand age***

Several factors contribute to changes of the soil fungal community: soil nutrients and water availability (Clemmensen et al., 2015), soil microclimate conditions (Castaño et al., 2018), litter quantity and quality (Chen et al., 2019a), and root density (Peay, Kennedy, & Bruns, 2010). After the clear-cut phase when seedlings are just establishing, diverse microbial populations are expected to rapidly colonize the soil (Shao et al., 2019). As these stands developed, the shift in litter input and composition, as well as environmental conditions, leads to an increase in both nutrient availability and fungal diversity (Figure 5a and b). The tendency in older stands for declining fungal diversity may be explained by niche competition among funguilds (Kyaschenko, Clemmensen, Hagenbo, Karlun, & Lindahl,

2017b). The variance in community composition (Figure 5c) demonstrated shifts in fungal community functions as stands aged. The vertical gradients in fungal diversity and composition are common due to the main input of organic materials starting from the topsoil (Uroz, Buée, Deveau, Mieszkin, & Martin, 2016; Chen et al., 2019a; Ovsepyan, Kurganova, de Gerenyu, & Kuzyakov, 2019).

*Ascomycota* was substantially greater in the oldest stand relative to the youngest stand, but the opposite was true for *Basidiomycota* (Figure 6 and Table S5). This result is inconsistent with Wang et al. (2019), who found that *Ascomycota* dominated Chinese fir stands of all ages and that its relative abundance first declined and then increased from ages 6 to 49 years. The shift from high abundances of *Basidiomycota* to *Ascomycota* was associated with soil changes related to the increase in resource availability, which also mirrored a transition in fungal functional groups as stand age increased. Among the soil funguilds, mycorrhizal fungi (most affiliated with *Basidiomycota*) assist most in a plant's nutrient uptake, especially in low-nutrient conditions (Read & Perez-Moreno, 2003). In contrast, the free-living saprotrophic fungi (mostly belonging to *Ascomycota*) contribute to litter and organic matter degradation (Baldrian et al., 2011). In young stands, the high nutrient requirements for tree growth and mycelia proliferation drives the root-associated symbiotic fungi to free organic N and P from organic matter. With litter constantly being deposited on topsoil in older stands, both nutrient availability and the abundance of saprotrophic fungi increase. Proliferating saprotrophic fungi may colonize niches of mycorrhizal fungi, in response to the increased resource availability. The finding that soil fertility relates positively to fungal saprotrophic abundance is also supported by Kyaschenko,

Clemmensen, Karlton, and Lindahl (2017a) and Castaño et al. (2018). These inter-guilds relationships are especially relevant due to their potential effects on crucial ecosystem processes, such as C sequestration and N and P cycling (Clemmensen et al., 2015; Averill & Hawkes, 2016; Kyaschenko, Clemmensen, Karlton, & Lindahl, 2017a; Chen et al., 2019a).

#### **4.3. Relationship between key soil quality indicators and fungal community**

Soil properties and environmental factors are major drivers of microbial communities in forests (Uroz, Buée, Deveau, Mieszkin, & Martin, 2016; Lladó, López-Mondéjar, & Baldrian, 2018). On the other hand, microorganisms can also reflect soil quality and play a vital role in ecological processes (Baldrian, 2016; Lladó, López-Mondéjar, & Baldrian, 2017; Cui et al., 2020). Fungal species are the central mediators that drive forest ecosystem development (Lindahl & Clemmensen, 2016). Correlation analysis suggested that soil fungal composition shifts were associated with changes in key soil quality indicators (Figure 8 and Table S5). Our study further links fungal taxa to trophic strategies and nutrient cycles (Figure 7 and Table S7). These relationships highlight fungal roles in liberating soil C, N, and P for subsequent trees uptake. The abundance of certain mycorrhizal taxa, such as the orders *Sebacina*, *Thelephora* and *Russula*, correlated positively with ACP activity. This association suggests that these functional species possess the capacity to mobilize P from soil organic matter (Lindahl & Tunlid, 2015). We propose that early establishment of mycorrhizal fungi that have high access to complex nutrient pools benefits tree growth and favors stand development. The positive correlation between most saprotrophic taxa (e.g. *Auriculariales*, *Mortierellales*, *Agaricales*, and *Pleosporales*) and soil TN highlights that

these decomposers facilitate increased nutrient availability (van der Wal, Geydan, Kuyper, & de Boer, 2013). Collectively, the observations regarding mycorrhizal and saprotrophic fungi suggest that shift in fungal community composition is closely link with the changes of soil quality.

#### ***4.4. Implications for plantation management***

Establishing management strategies that maintain soil quality throughout a harvest cycle of Chinese fir plantations will be important for continued sustainable forestry. Here, soil quality was more desirable in older stands than in the youngest 3-year-old stand, which had recently experienced a clear-cut slash and burn. The stem and wood removal that occurs during this destructive harvest and controlled burn lowers C and N content significantly in the soil surface due to high temperatures and erosion (Guo et al., 2010). This C and N loss could be reduced by intercropping with suitable plants (e.g. perennial grasses) that can reduce harvest-associated nutrient loss and maintain ecological balance among funguilds (Kyaschenko, Clemmensen, Karlton, & Lindahl, 2017a; Li et al., 2018). Cultivation of annual plants during the forest fallow period was found to improve soil fertility by enhancing microbial activity and increasing nutrient availability (Lungmuana et al., 2017). As Chinese fir seedlings establish, they experience rapid growth from 10-20 years of age, which depletes soil nutrients. The current study reiterates that the level of soil nutrients, especially P, decreases until stands are 16 years old. Therefore, it is reasonable to employ the most effective and feasible fertilization practices to improve soil fertility and to relieve competition between funguilds during the rapid growth stages (Subedi, Jokela, Vogel, & Martin, 2019). Our

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results indicated that soil C and nutrients increase with stand age to a steady level for at least 30 years after harvest. Thus, to maintain soil quality, we recommend that Chinese fir stands should not be clear-cut harvested until they are over 30 years old in order to best enable soil quality recover (Figure 8).

## 5. Conclusions

Stand age has strong effects on topsoil C, N, and P contents, as well as on the microbial communities and their function in Chinese fir plantations. The previous clear-harvest practice strongly reduced soil quality, with the lowest *SQI* occurring in the youngest stands. As stands developed, *SQI* increased gradually, with an obvious logarithmic increase occurring in the top 0-5 cm layer only. *Ascomycota* and *Basidiomycota* dominated soil fungal communities during stand development, with a clear transition of funguilds as the stands aged. Mycorrhizal fungi increased in abundance in younger stands initially, but then they were gradually replaced by saprotrophic fungi in older stands, due to increased litter input and resource availability for saprotrophs. Correlation analysis indicated that funguilds shifts were closely associated with the changes in soil quality. The key soil quality indicators (i.e. total N, available P and  $\text{NH}_4^+$ ) were positively correlated with the abundance of saprotrophic fungi, indicating that increased soil quality was tightly linked with the enrichment of decomposers. The abundance of mycorrhizal fungi in rapidly growing stands was positively correlated with acid phosphatase, a key indicator of organic P mineralization. This relationship suggests that mycorrhizal fungi sustain tree productivity in low-quality soil. Such close association between the key soil quality indicators and fungal composition



suggest that soil quality shifts during stand development in conjunction with fungal succession.

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### **Conflicts of Interest**

The authors have no conflicts of interest to declare.

### **Data Availability Statement**

The fungal DNA sequences of the 40 soil samples have been deposited in the SRA of the NCBI database under the Accession nos. SRR10152476 - SRR10152515. Other data that support the findings of this study are available from the corresponding author upon reasonable request.

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## Figure captions

**Figure 1** Map of sampling sites showing the location of the five Chinese fir stands along an age gradient. The square boxes (yellow on the right map) show the sampling plots, and the circular points (red) show the individual sampling points.

**Figure 2** Soil physico-chemical properties in Chinese fir stands along an age gradient. Error bars correspond to standard deviation ( $n = 4$ ). Different lowercase letters indicate significant differences among stand ages at the same depth, and different uppercase letters indicate significant differences between soil depths (one-way ANOVA,  $p < 0.05$ ).

**Figure 3** Soil microbial biomass and enzyme activities in Chinese fir stands along an age gradient. Error bars correspond to standard deviation ( $n = 4$ ). Different lowercase letters indicate significant differences among stand ages at the same depth, and different uppercase letters indicate significant differences between soil depths (one-way ANOVA,  $p < 0.05$ ).

**Figure 4** Non-linear (logarithmic) regression between stand age and soil quality index (*SQI*) in the 0-5 cm and the 5-15 cm layers of Chinese fir plantations. The calculation of *SQI* is presented in Section 2.5.

**Figure 5** Changes in soil fungal diversity and community composition in the Chinese fir chronosequence. (a) Number of fungal OTUs, (b) Shannon-Weiner Index, and (c) Bray-Curtis similarity of the fungal community structure as indicated by non-metric

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multidimensional scaling (NMDS) analysis based on dataset of OTUs (species level). ANOSIM (999 permutations) was used to evaluate the significant differences in soil fungal community composition among stand ages and between the two soil depths. Error bars correspond to standard deviation (n = 4). Different lowercase letters indicate significant differences among stand ages at the same depth, and different uppercase letters indicate significant differences between soil depths (one-way ANOVA,  $p < 0.05$ ).

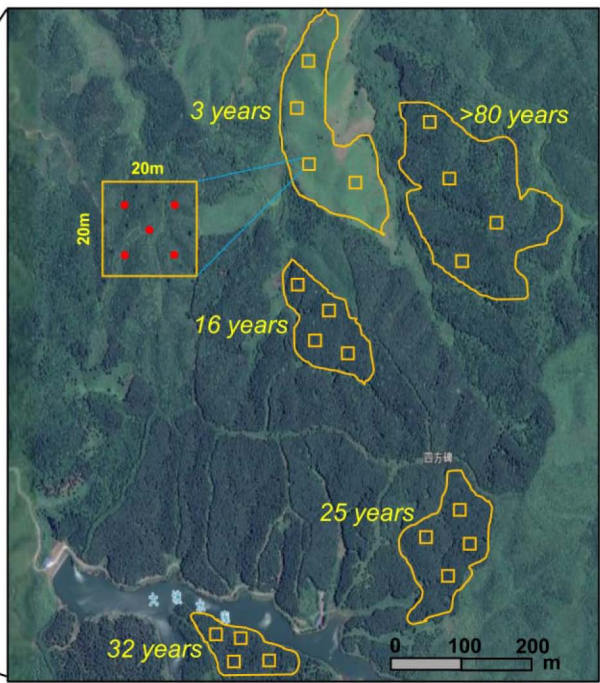
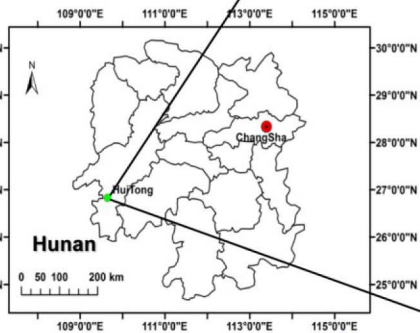
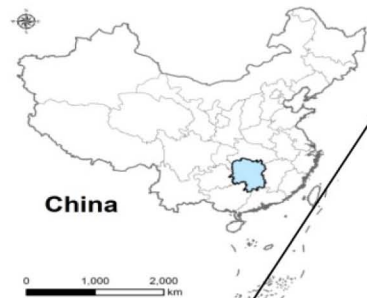
**Figure 6** Relative abundance of dominant fungal phyla (top) and funguilds (bottom, compositions of funguilds were inferred by FUNGuild) in the 0-5 cm (a, c) and 5-15 cm layers (b, d) of Chinese fir chronosequence. All data are presented as the mean value (n = 4).

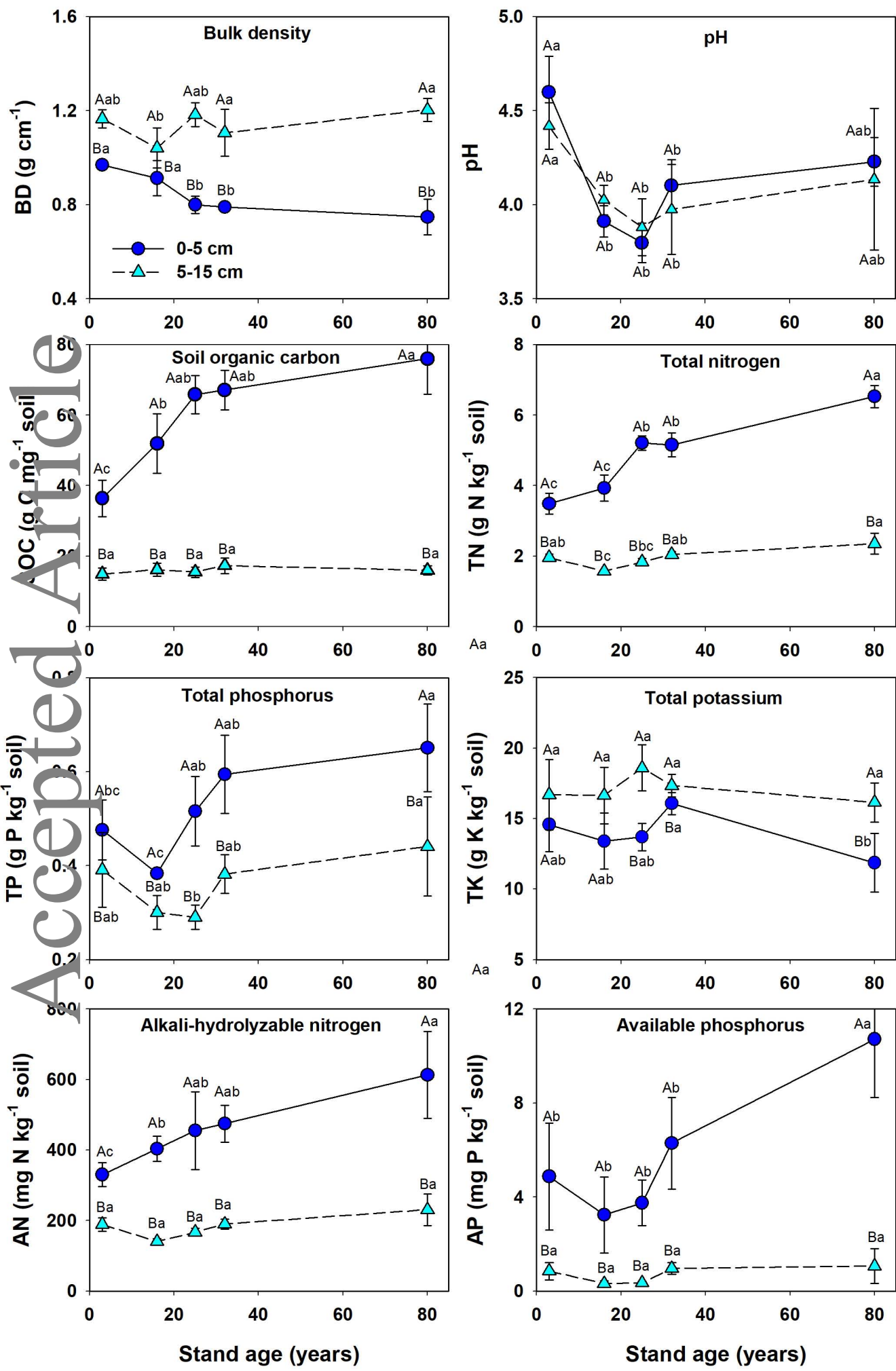
**Figure 7** Links between key soil quality indicators and fungal orders in the Chinese fir chronosequence, as visualized by principal component analysis (PCA) of soil environmental factors. Only orders accounting for more than 1% of total sequences in two or more samples are shown. In the 0-5 cm layer, PCA 1 (34% of variability) is loaded mainly by mycorrhizal fungi, and *Auriculariales* (saprophytic fungi) is mainly responsible for PCA 2 (18.8%). In the 5-15 cm layer, *Archaeorhizomycetales* and *Thelephorales* are mainly responsible for PCA 1 (41.8%) and PCA 2 (13.8%), respectively.

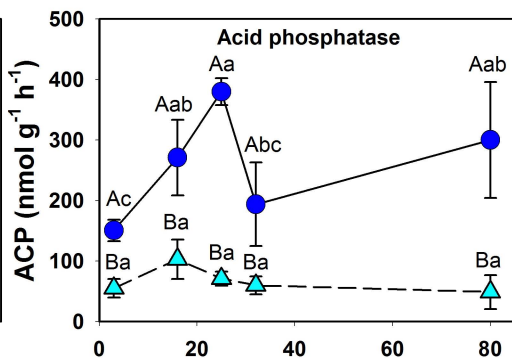
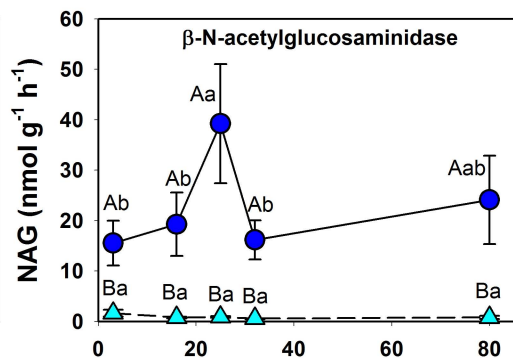
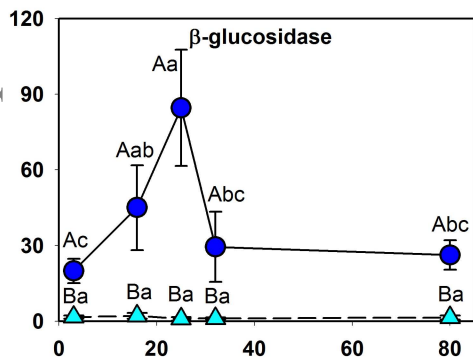
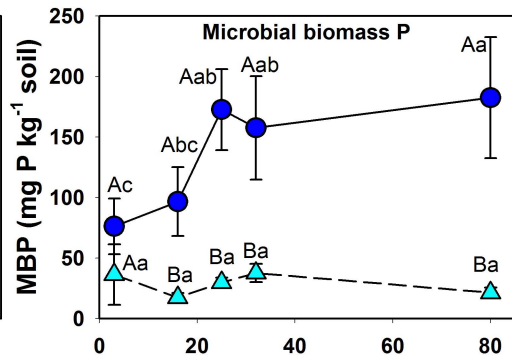
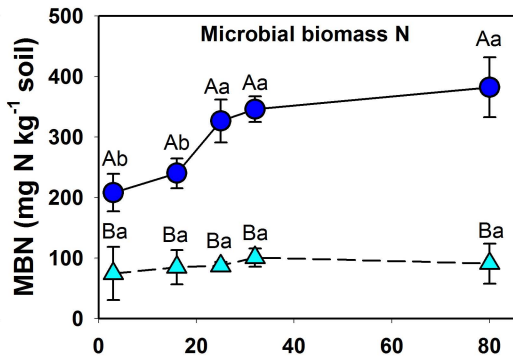
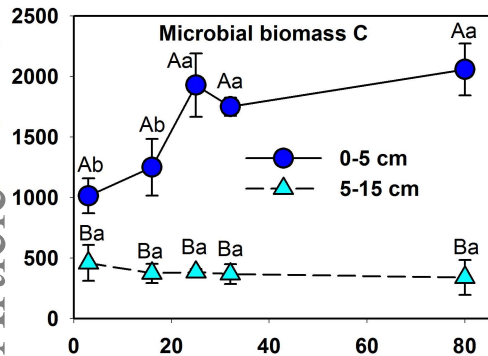
**Figure 8** Schematic representation of soil quality index (*SQI*) changes (red lines) and linkages between the key soil quality indicators and fungal community along Chinese fir stand development. The numbers before the key soil quality indicators represents the

weighting values in the *SQI* calculation (e.g. maximal for TN). Blue lines connect soil quality indicators and fungal diversity and community composition reflects the significance ( $*p < 0.05$ ,  $**p < 0.01$ ). The recommended management strategies for maintaining soil quality throughout the harvest cycle of Chinese fir plantations are presented in blue text at the top above the trees.





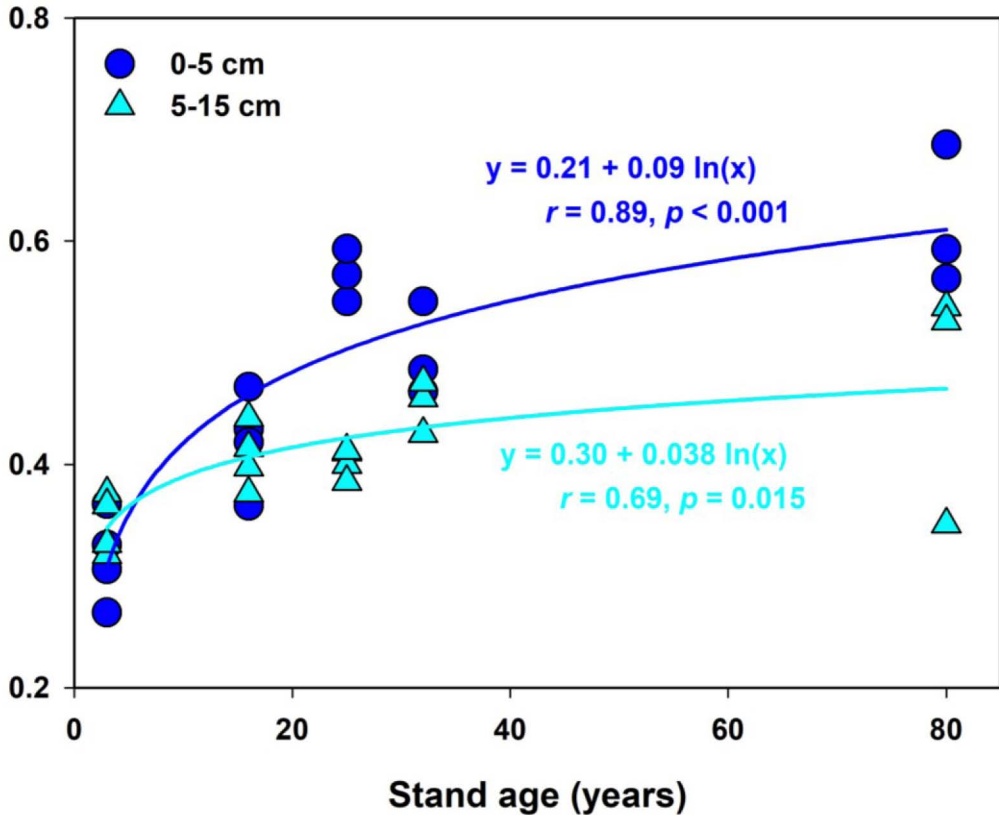


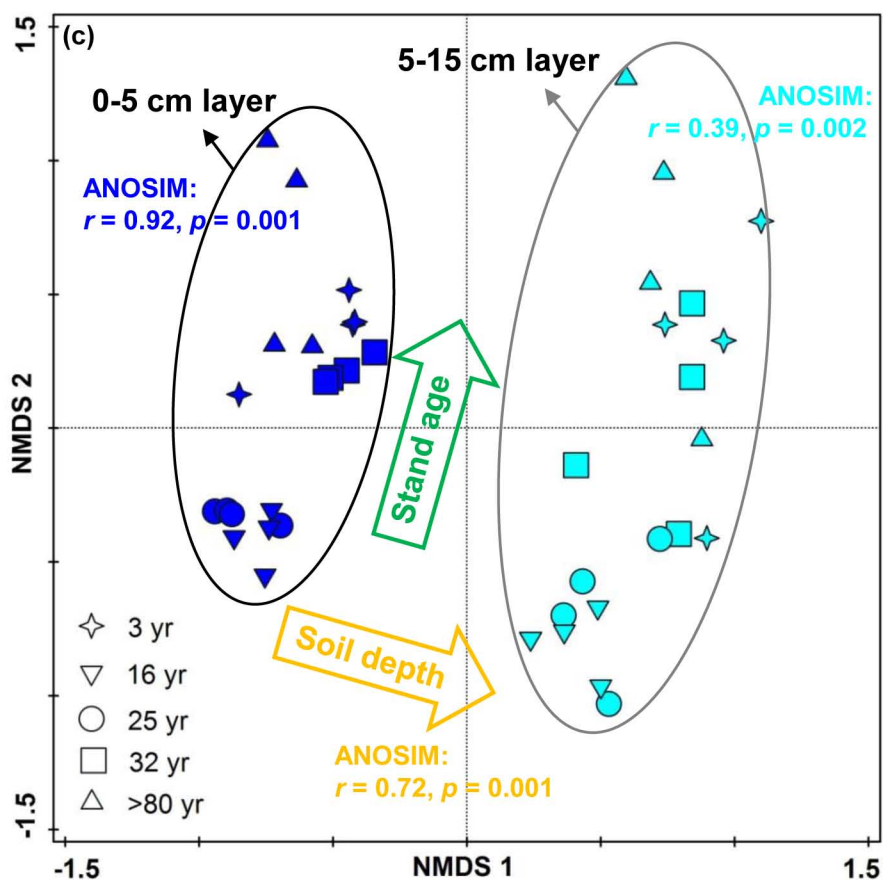
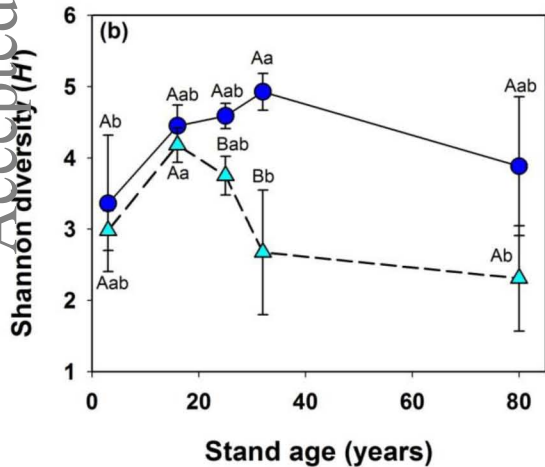
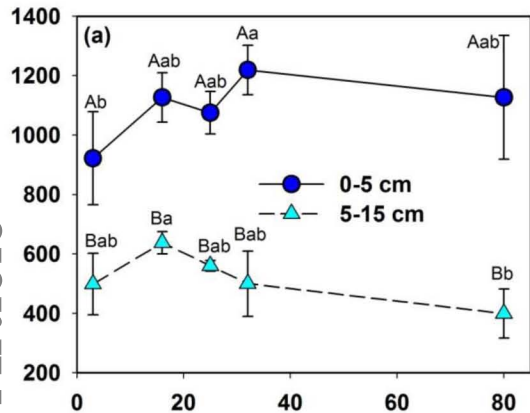


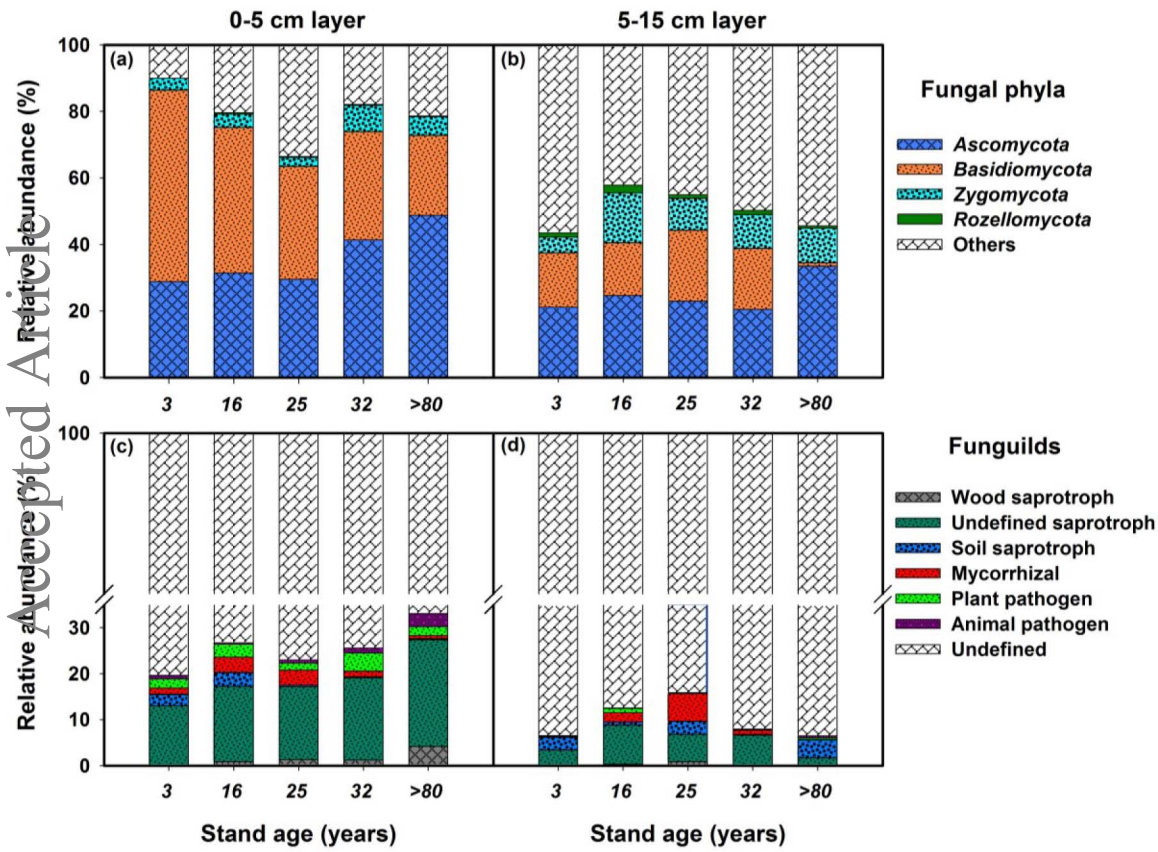
Stand age (years)

Stand age (years)

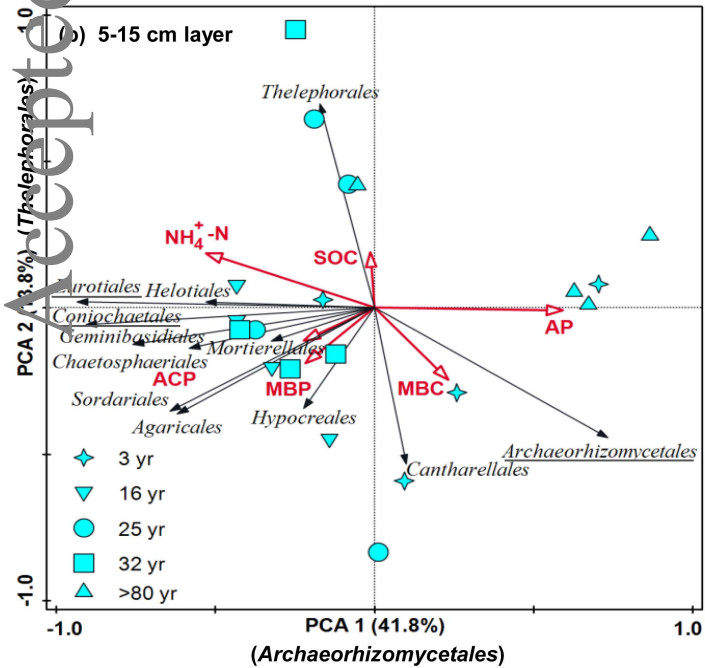
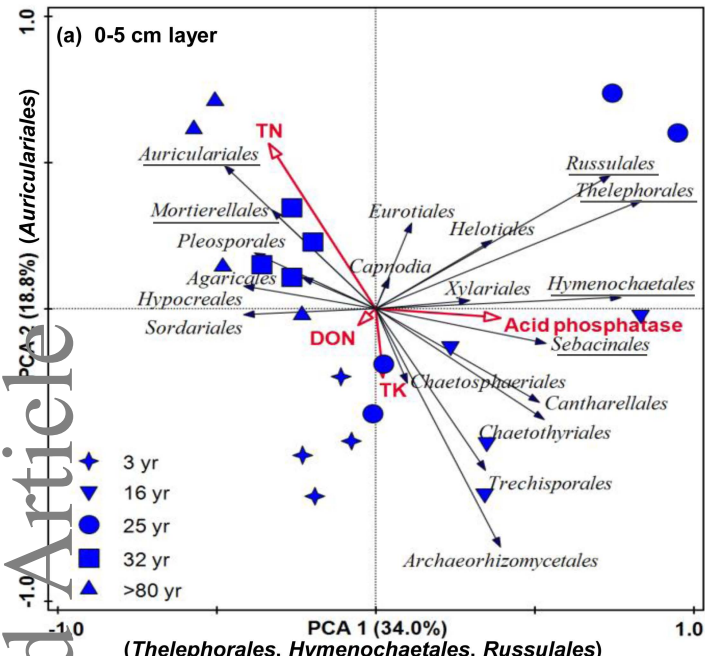
Stand age (years)











# Chinese fir plantation

Management suggestion:

Intercropping with perennial grasses in fallow period

Fertilization of P (and some N)

Prolongation of harvest periods to ~30 years

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