Differential response of soil microbial and animal communities along the chronosequence of *Cunninghamia lanceolata* at different soil depth levels in subtropical forest ecosystem

Waqar Islam, Hafiz Sohaib Ahmad Saqib, Muhammad Adnan, Zhenyu Wang, Muhammad Tayyab, Zhiqun Huang, Han Y.H. Chen

*Institute of Geography, Fujian Normal University, Fuzhou 350007, China*

**Key Laboratory for Humid Subtropical Eco-Geographical Processes of the Ministry of Education, Fujian Normal University, Fuzhou 350002, China*

*Post-doctoral Research Station of Geography, Fujian Normal University, Fuzhou, 350007, China*

*STU-UMT Joint Shellfish Research Laboratory, Shantou University, Shantou 515063, China*

*College of Life Sciences and Oceanography, Shenzhen University, Shenzhen 518060, China*

*College of Agriculture, Fujian Agriculture and Forestry University, Fuzhou 350002, China*

*Faculty of Natural Resources Management, Lakehead University, Ontario, Canada*

**Corresponding author at: Institute of Geography, Fujian Normal University, Fuzhou 350007, China.**

**Corresponding author at: Faculty of Natural Resources Management, Lakehead University, Ontario, Canada.**

[https://doi.org/10.1016/j.jare.2021.08.005](https://doi.org/10.1016/j.jare.2021.08.005)

**Abstract**

Introduction: Soil biota plays a crucial role in the terrestrial ecosystem. There is growing momentum to understand the community structure and diversity of total belowground soil biota across large ecological scales. Soil biota follow divergent trends with respect to soil physiochemical properties in different ecosystems; however, little is known about their response to stand development across multiple soil depths in Chinese fir plantations, which is the most important tree species across all over China, popular for its timber production.

**Highlights**

- We investigated the assembly of soil bacteria, fungi, archaea, protists & animals across different soil depths in five stand ages.
- Soil biotic communities exhibited a decreasing trend in alpha diversity with increasing soil depth.
- Acidobacteria, Agaricomycetes, Bathyarchaeia, Chlorophyceae and Clitellata, were most abundant classes.
- Total nitrogen, available phosphorus and pH were the most influencing factors for changes in soil biotic communities.
- As compared to soil depth, stand age was not dominantly influencing the structure of other biotic communities.

**Graphical Abstract**

**Article Info**

Article history:

Received 11 May 2021

Revised 5 August 2021

Accepted 9 August 2021

Available online 11 August 2021

**Acknowledgments**

Abbreviations: TC, Total carbon; TN, Total nitrogen; TP, Total phosphorus; AP, Available phosphorus; TK, Total potassium; AK, Available potassium; BD, Bulk density; NO₃, Nitrate; WC, Water-holding capacity; OTUs, Operational taxonomic units; ANOVA, Analysis of variance; LSD, Least Significant Difference; ANOSIM, Analysis of similarity; VIFs, Variation inflation factors; NMDS, Non-metric multidimensional scaling; RDA, Redundancy analysis.

Peer review under responsibility of Cairo University.

**Corresponding authors at:** Institute of Geography, Fujian Normal University, Fuzhou 350007, China.

**E-mail addresses:** zhiqunhuang@hotmail.com (Z. Huang), hchen1@lakeheadu.ca (H.Y.H. Chen)

https://doi.org/10.1016/j.jare.2021.08.005

2090-1232/© 2022 The Authors. Published by Elsevier B.V. on behalf of Cairo University.

This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).
Introduction

Chinese fir (Cunninghamia lanceolata (Lamb.) Hook.) is an ancient fast-growing, evergreen coniferous tree, popular for its good timber quality and high yield, particularly in subtropical China. The tree is cultivated for commercial purposes, as its timber is utilized to construct houses and furniture manufacturing. In recent years, the area under Chinese fir plantation has increased rapidly in China [1]. Apparently, this has happened due to the increasing demand for wood products because of rapid population increase and economic development in the country. Usually, the harvesting of mature Chinese fir timber stands is done at the age of 25–30 years; however, recently the harvesting time is shortened (20–25 years) to fulfill the high demands [2].

The repetitive plantation of fast-growing species at the same site can decline soil fertility [3]. Significant decline in timber yield and soil fertility due to repetitive monoculture Chinese fir plantations have already been well documented [4,5]. Soil nutrient deficiency [6], auto-toxicity of root exudates [7], reduction in biochemical activity [2] and alterations in the population of soil biotic communities are categorized as the main reasons for the decline in timber yield [8].

Soil biotic communities constitute prokaryotes and eukaryotes that may be further divided into soil microbial (Bacteria, Fungi, Archaea, Protists), animal and plant communities [9]. A fundamental role of soil biotic communities include nutrient and hydrological cycling, litter decomposition and supporting terrestrial primary production [10,11]. Literature highlights that stand age in monoculture forests [12,13] and soil depth/vertical profiles greatly impact soil biotic communities [14]. Soil organisms are strongly influenced by changes in the soil ecosystem and alteration in available nutrients [15]. Moreover, soil biotic communities’ response is accepted as an ecological indicator for soil quality [16]. Previous reports indicate that successive plantations of Chinese fir on the same site led to a significant decrease in nitrogen fixation (%), ammonification, fiber decomposition, respiration (%), soil pH, organic matter, humus carbon, C/N-ratio, total and available nitrogen, potassium and phosphorus [2,5], which likely altered the composition, structure, and diversity of soil biotic communities [8].

Nevertheless, soil arbuscular mycorrhizal fungal composition is recently attributed to the chronosequence of Chinese fir using high-throughput sequencing on an illumina miseq platform [17]. Similarly, another recent study evaluated the soil microbial (Fungi & Bacteria) community diversity along with three different rotations of 26 years old Chinese fir plantations [8]. However, despite strong interactions among soil organisms [18], the response of all the soil biotic communities (Bacteria, Fungi, Archaea, Protists and Animals) to different stand ages and soil vertical profiles across the chronosequence of Chinese fir has not been previously studied.

High-throughput sequencing technology has shown great advantages in understanding the soil biotic diversity and community composition because of its unprecedented sequencing depth [19]. Therefore, in this study, we employed the high throughput (illumina miseq2500) sequencing to assess the composition of soil microbial (Bacteria, Fungi, Archaea, Protists) and animal communities across a chronosequence of Chinese fir plantations at five different stand ages (5, 8, 20, 27, 40 years) with three different vertical soil depth levels (0–10, 10–20, 20–40 cm). We assumed that Chinese fir plantations with different stand ages might result in shifts among soil physiochemical properties, microbial and animal communities at various vertical soil depth profiles. Specifically, we aimed to understand that (a) How soil microbial (Bacteria, Fungi, Archaea, Protists) and animal diversity and community composition changes between different Chinese fir plantation stages/ages at various vertical soil profiles? (b) How the changes in soil microbial and animal communities’ structure are correlated with environmental factors across various vertical soil profiles and different stand ages of Chinese fir?

Material and methods

Experimental sites, sampling and soil analysis

Our experiment site was located at Baisha forest farm (25°15’ N, 116°61’ E, altitude 1181 ft) near Longyan city (Southwestern China, Fujian Province), which is a well-managed forest site between Baopingling and Nanzhao mountains (Wuyi mountain range). The climate is monsoon-influenced humid subtropical (Köppen climate classification), having mild short winter, long hot and humid summer. Average annual precipitation is 1738 mm while average daily precipitation is 162 mm (frost-free period-280 days) with a mean annual temperature of 20.4°C (highest in July i.e., 29.2°C; lowest in January i.e., 6.8°C). The mean evapotranspiration is 1598 mm, and the annual mean relative humidity is 75%. According to IUSS Working Group-WRB-2014, the soils in the Longyan area belongs to Oxisol (thickness > 62 cm), developed from granite [20]. Historically, our experimental forest area was impacted by typhoons and consecutive intensive harvesting. For Chinese fir monoculture plantations, the site was prepared by removing logging residues and additional vegetation, followed by establishing the contoured...
strips and digging of plant holes. The plantation of seedlings (3600 stems/ha) was done leading towards the application of synthetic fertilizers (N: 54, P2O5: 54, K2O: 54 kg/ha per seedling). Weeds and other competing vegetation removal processes were performed manually on a regular basis for the first three years of seedling establishment and growth. Furthermore, the standard thing process was followed at the ages of 8, 18, and 25 years.

To understand the comparative composition and diversity of total belowground soil microbiota with relation to soil physiochemical properties in Chinese fir plantations, we employed a chronosequence approach to collect the soil samples among each stand. Chronosequence approach has been previously criticized by assuming that the sample stands may follow a similar development history along a temporal sequence [21]. However, careful site selection and replications enable us to investigate the ecological changes / processes on yearly, decadal, continental and time scales basis [22]. Soil samples among five age chronosequences were done in June 2019. We selected five different ages (5, 8, 21, 27, and 40 years) to represent different classes i.e., early stand initiation, canopy closure, stem exclusion, canopy transition, and gap dynamics, respectively [23]. We selected visually homogenous stands (>1 km distance among stands) on the basis of their structure, composition and spatial distribution across large areas for minimizing the impact of spatial autocorrelation. For each sampled stand, plots of 20 m × 30 m were assigned randomly, which were at least 100 m away from the forest edge that abutted to agricultural lands, roads, and forests of different age classes. After removal of detritus and litter in each plot, we used 3.8 cm auger to collect soil samples (Bulk soil) from ten random locations to prepare a composite sample by mixing. A total of 45 composite samples from three soil depths (0–10, 10–20, 20–40 cm) were collected. After the removal of loose gravel, stones, and plant debris, the samples were stored in plastic bags with ambient field moisture content at 4°C, labelled and transported to the laboratory for downstream experimentation.

All the samples were sieved through ≤ 2 mm mesh and were divided into two portions, with one immediately stored −80°C for molecular analysis, while the other was air-dried for measurement of soil physiochemical properties as quantified previously [24] including pH (measured in 0.01 M CaCl2), total carbon (TC- g/kg), total nitrogen (TN- g/kg), C/N-ratio, total phosphorus (TP- mg/kg), available phosphorus (AP- mg/kg), total potassium (TK- g/kg), available potassium (AK- mg/kg), bulk density (BD- g/cm³), nitrate (NO3– mg/kg) and water-holding capacity (WC- g/kg).

**DNA extraction, primer selection, PCR and high-throughput sequencing**

Extraction of genomic DNA was done through mechanical lysis followed by homogenization (triplicate) using 0.20 g soil from each sample by using the DNeasy PowerSoil Pro Kit (QIAGEN, USA). As our soil was acidic, the samples were pre-treated with 750 μL of 1 M CaCO3, to improve the PCR performances [25]. Extracted DNA was stored at −20°C till further preparation of amplicon library.

Amplicon libraries were generated using primers for rRNA marker genes, specifically for the V3 + V4 region of the 16S rDNA gene targeting bacteria and archaea [515F/806R] [26], ITS1 targeting fungi (ITS5/5.8S_Fungi) [27], and the V4 region of the 18S rDNA gene (TAReuk454FWD1/TAReukREV3) [28] targeting a wide range of eukaryotes (Protists, Animals and Plants), but not all eukaryotic organisms. The amplification of samples was performed in triplicate keeping no-template controls, followed by pooling of triplicate PCR amplicons and detection via electrophoresis in 2% (w/v) agarose gel [29]. High quality (bright bands) PCR products were mixed in equal ratio leading to purification using the GeneJET Gel Extraction Kit (Thermo Fisher Scientific). Final library preparation and Illumina HiSeq2500 (300 bp paired-end reads) sequencing was conducted at Bio-marker (Pvt) limited, Beijing. The obtained sequences were filtered by following a standard protocol [26]. USEARCH tool (UCHIME algorithm) was used to remove chimeric sequences, if any [30]. UPARSE pipeline was used for grouping the sequences and assigning the operational taxonomic units (OTUs) according to their taxonomy at a 3% dissimilarity level [30]. Non-classified OTUs at all taxonomic levels and the OTUs having less than two sequences were removed followed by classification of sequences according to Greengenes 13.8 [31], UNITE 7.2 [32], SILVA 128 [33] for 16S, ITS1, 18S, respectively. OTUs belonging to eukaryotes in the 16S OTU table, non-fungal OTUs in ITS OTU table, as well as fungal, non-soil animals and plant OTUs from the 18S OTU table were removed.

**Statistical analysis**

The linear response of soil physiochemical properties at various soil depths and stand ages were assessed using linear regression model followed by the analysis of variance (ANOVA) and Fisher’s Least Significant Difference (LSD) Post-hoc tests [34]. The relative abundance of top 15 microbial and animal communities’ classes among all the samples was calculated using phyloseq [35] and microbiomeSeq [36] packages in R, followed by testing the linear response of each taxon at various age and depth. The Chao1 and Shannon-Wiener diversity indices were calculated in each sample, and their linear responses were measured at various soil depths and stand ages using linear regression models followed by ANOVA and the LSD Post-hoc tests. Beta-diversity was estimated via non-metric multidimensional scaling (NMDS) using the Bray–Curtis distance matrix between the samples. Moreover, perMANOVA (permutational multivariate analysis of variance) was used to model the influence of stand ages and soil depths on the Bray–Curtis dissimilarity matrix by applying the Adonis and analysis of similarity (ANOSIM) tests in vegan after 999 permutations [37]. Pearson’s correlation test was used to show the relationships of soil microbial and animal classes with soil physiochemical properties (AK, AP, BD, TC, C/N-Ratio, NO3−, PH, TK, TN, TP and WC). Moreover, the associated p-values were also calculated and adjusted with the Benjamin and Hochberg procedure [38] to show the significance of each correlation. Heatmaps were drawn to show the correlations by following the R-codes provided by Torondel et al. [39].

A multivariate approach using redundancy analysis (RDA) was used to show each taxa’s linear response to various environmental variables. Before performing RDA, community metrics were Hellinger transformed. This transformation enables us to use the ordination methods on datasets containing many zeros. We used variation inflation factors (VIFs) to test the goodness of fit to each RDA model. Since environmental variables with VIF > 10 had collinearity with other environmental variables [40], they do not significantly explain the model’s variance, and these were removed from our final model. Moreover, an ANOVA-like permutation test was used to test RDA models’ significance and environmental variables [41]. All calculations were conducted, and graphs were drawn in R (v.3.6.3), using “phyloseq” [35], “microbiomeSeq” [35], “ggplot2” [42], “vegan” [37], “dplyr”, “forcats”, “multcompView” and “extrafont” packages [43].

**Results**

**Soil physiochemical properties**

With the change in soil depth profiles and stand age, the soil physiochemical properties showed diverse trends (Supplementary
Fig. S1). For example, TC and TN significantly decreased with the increase in soil depth (0–40 cm) \((p < 0.001)\) (Supplementary Fig S1-A,B). A similar decreasing trend was observed for TP \((p = 0.11)\), AP \((p < 0.001)\), soil pH \((p = 0.34)\) and NO\(_3\) \((p = 0.05)\) (Supplementary Fig S1-D,E,F). Other soil physiochemical properties, such as C/N-Ratio, TK, AK, and BD exhibited variable patterns with increased soil depth. However, with respect to increasing age, TC, TN and AP showed an increase from 8 years to the age 27 significantly \((p = 0.01)\), followed by a slightly decreasing trend up to age 40 years (Supplementary Fig S1-A). On the contrary, soil pH \((p = 0.001)\) and NO\(_3\) \((p = 0.001)\) exhibited a significant decrease up to 27 years followed by an increase in 40 years forest stand (Supplementary Fig S1-F). All other properties showed variable trends with respect to an increase in stand age.

**Distribution of soil microbial and animal communities**

Among the entire samples, Illumina Hiseq2500 sequencing resulted in a total of 1,889,175 (Bacteria-16 s:V3 + V4), 3,557,061 (Fungi-ITS1), 1,737,854 (Archaea-16 s:V3 + V4), 196,257 (Protists-18 s:V4) and 909,546 (Animals-18 s:V4) clean paired reads, grouped into 1120 bacterial, 599 fungal, 99 archaea, 317 protist and 284 animal OTUs at 97% sequence similarity cutoff rate. Significant decreasing and increasing trends for the relative abundance of microbial and animal communities (top 15 classes) were observed with respect to changes in stand age and soil depth profiles (Figs. 2,3; Supplementary Fig S2,3; Supplementary Table 1). Briefly, among bacteria, Acidobacteria were the most abundant class i.e., 36%, followed by Alphaproteobacteria (16%) and Verrucomicrobiae (7%) (Fig. 1). Relative abundance of Acidobacteria increased with the increase in depth however an opposite trend was observed for the Alphaproteobacteria as their abundance reduced with the increase in depth symmetrically. Interestingly, Verrucomicrobiae showed increased abundance at 10–20 cm soil depth for all the stand ages. With an increase in stand age, the relative abundance of Alphaproteobacteria increased up to 27 years significantly \((p = 0.05)\), while Verrucomicrobiae showed a significant decrease \((p = 0.05)\) (Supplementary Fig S3a; Supplementary Table 1). Agaricomycetes were the most abundant (27%) class among fungi, followed by Sordariomycetes (14%) and Mortierellomycetes (12%) (Fig. 1). A contrasting trend was observed for all the fungal classes with the response to increasing age and soil depth. However, Sordariomycetes abundance was recorded relatively high at 0–10 cm soil depth and for 21 and 40 years stand age \((p = 0.001)\), while Mortierellomycetes were found significantly abundant at the early stage of plantation \((p < 0.001)\) i.e., 5 years age (Supplementary Fig S3b; Supplementary Table 1). On a similar note, dominant Archaea classes were Batharchaeae (60%), Nitrososphaeria (16%) and Thermoplasmata (13%) (Fig. 1). Batharchaeae exhibited an increasing trend with respect to an increase in soil depth, while contrastingly, both Nitrososphaeria and Thermoplasmata reduced with the increasing soil depth (Supplementary Fig S3c). Interestingly, Nitrososphaeria significantly reduced with the increase in stand age up to 27 years \((p = 0.05)\), while Thermoplasmata increased with an increase in stand age from 8 to 20 years (Supplementary Fig S3c; Supplementary Table 1). Most of the sequences for protists were unclassified (56%) and unassigned (19%) at the class level (Fig. 1). Among the classified protists classes, Chlorophyceae, Conoidasida and Peronosporomycetes were dominant, exhibiting 5.1%, 4.8% and 3.2% relative abundance respectively (Fig. 1). Conoidasida significantly reduced with the increase in soil depth \((p < 0.001)\) while Peronosporomycetes showed an opposing trend. Chlorophyceae showed inconsistent trends with a change in soil depth profiles \((p < 0.001)\) (Supplementary Fig S3d; Supplementary Table 1). Interestingly, a significant high relative abundance of Peronosporomycetes was observed in 40 years age stands \((p = 0.001)\). Among animal communities, Clitellata (34%) were the most dominant class, while Chromadorea (21%) and Arachnida (16%) were also among the dominant classes (Fig. 1). For most of the stand ages of Chinese fir plantation, Clitellata abundance reduced with the increase in soil depth \((p = 0.001)\), while Arachnida \((p = 0.05)\) and Chromadorea \((p < 0.001)\) followed an opposite trend (Supplementary Fig S3e; Supplementary Table 1).

Alpha diversity of microbial (Bacteria, Fungi, Archaea, Protists) and animal communities was estimated by Chao1 and Shannon indices. Significant trends among alpha diversity of all the biotic communities were observed with changes in stand age and soil depth profiles (Fig. 4; Supplementary Fig S4). For example, on average, bacterial alpha diversity decreased significantly up to the age of 27, followed by an increase at 40 years stand in both Chao1 \((p = 0.001)\) and Shannon indices \((p < 0.001)\). However, Shannon indices indicated a significant decrease in bacterial alpha diversity with the increase in soil depth \((p < 0.001)\) (Fig. 4-A). A similar trend was recorded in fungal diversity by Chao1 indices \((p < 0.001)\) (Fig. 4-B), while archaea exhibited a contrasting significant opposite trend (Shannon indices; \(p < 0.001)\) (Fig. 4-C). For protist (Fig. 4-D) and animal (Fig. 4-E) communities, a significant decreasing trend was observed with an increase in soil depth by Chao 1 and Shannon indices. Other than the bacterial communities, contrasting significant trends for the alpha diversity of all the other communities were recorded with an increase in stand ages (Chao 1 and Shannon indices) (Fig. 4; Supplementary Fig S4).

Non-metric multidimensional scaling (NMDS) analysis revealed that soil samples from different soil depths and stand ages formed distinct and overlapping clusters in ordination space (Fig. 5) with significant difference (ANOSIM & Adonis test; \(p = 0.001)\) (Supplementary Table 2). We observed significant differences in microbial and animal communities with respect to soil depth and stand age. These differences were larger for soil archaea (Age-\(R^2 = 0.245\), Depth-\(R^2 = 0.571)\), bacterial (Age-\(R^2 = 0.208\), Depth-\(R^2 = 0.538\)) and fungal communities (Age-\(R^2 = 0.441\), Depth-\(R^2 = 0.187)\), followed by protists (Age-\(R^2 = 0.229\), Depth-\(R^2 = 0.258)\) and animals (Age-\(R^2 = 0.166\), Depth-\(R^2 = 0.149)\), which indicates that the soil fungal, bacterial and archaea communities were more influenced by stand age and soil depth (Fig. 5A-E; Supplementary Table 2). Other than the soil fungi and animal communities, all other microbial communities were more sensitive to soil depth as compared to stand age (Adonis Test) (Supplementary Table 1).

**Potential drivers of soil microbial and animal community diversity**

To understand the main drivers for the diversity of microbial and animal communities, we tried to find a correlation between soil physiochemical properties and soil biotic communities through Redundancy analysis (RDA) (Fig. 6; Supplementary Table 3) and creating a heatmap through Pearson correlation (Supplementary Fig S5). The abundance of microbial and animal communities’ classes was variable and differently coordinated with soil physiochemical properties. For example, TC and TN were the most significant influencing factors \((p < 0.001)\) for all the communities with variable \(p\)-values followed by pH, TP, AP, NO\(_3\) and AK. We found collinearity among TC, TN and C/N-Ratio on the basis of VIF. Therefore, we selected TN in the final model (Supplementary Table 3). For bacteria, TN was predicted to have a significant influence on Alphaproteobacteria \((p < 0.001)\). We also observed a significant positive correlation among Gammaproteobacteria and Planctomycetacia with pH \((p = 0.001)\), TP \((p = 0.01)\) and NO\(_3\) \((p = 0.05)\) (Fig. 6-A). For fungi, TN \((p = 0.001)\), pH \((p = 0.001)\), NO\(_3\) \((p = 0.001)\) and AP \((p < 0.001)\) exhibited a significant positive correlation with Sordariomycetes and Mortierellomycetes (Fig. 6-B). Thermoplasmata had a significant positive influence on TN.
While Nitrososphaeria was predicted to have been associated with changes in AP ($p < 0.001$) and pH ($p < 0.001$) (Fig. 6-C). For protists, RDA predicted that Spirocheta had a positive correlation with TN ($p < 0.001$) (Fig. 6-D), while for animals, TN was influenced by the majority of unassigned animal classes ($p < 0.001$) (Fig. 6-E).

**Discussion**

Soil biotic communities are vital ecological indicators for forest ecosystems. Different forest management and rotation practices continuously change soil biotic communities’ structure, composition, diversity and correlations with soil nutrients, impacting plant growth and metabolism. In this study, we utilized high-throughput sequencing to unveil the diversity of soil biotic communities amongst the chronosequence of Chinese fir across different vertical soil profiles. The results revealed both expected and novel trends for abundance, diversity and dynamic relationships between soil organisms and environmental drivers.

Soil physiochemical properties exhibited notable contrasting trends across different stand ages and soil depth profiles. We observed that key soil physiochemical factors, such as TC, TN, TP, AP, pH and NO₃ decreased with the increase in soil depth (0–40 cm) (Supplementary Fig S1). It is verified that TC [44], TN and TP [45] decrease consistently with increasing soil depth (0–60 cm) across Chinese fir chronosequence. In acidic soils, AP and pH are considered closely related factors showing similar trends, as it has already been reported that soil pH constantly decreased with stand age across Chinese fir plantations, resulting in AP decrease because the low pH in acid soils drives phosphate to bind...

**Fig. 1.** Sankey diagram of relative abundance (%) of top 15 classes for soil microbial (Bacteria, Fungi, Archaea, Protists) and animal communities. The figure was created using the online website http://sankeymatic.com/build/. The vector code (SVG) for this figure is available in supplementary materials.
to Fe and Al precipitates [46,47]. The decrease in NO₃ is correlated with irrigation because continuous and heavy rainfall results in the leaching of NO₃ [48]. We observed variable patterns for TK, AK, BD, consistent with the findings of Selvalakshmi et al. [49] and Chen et al. [44], respectively. For most of the soil nutrients (TN, TP, TK, AP, Ak) (Supplementary Fig S1), we observed variable increase/decrease trends with respect to stand age, confirming that the stand age played a minor role in changing the status of most of the soil nutrients [44]. However, some soil nutrients (TC, pH, NO₃) exhibited changes with stand age. For example, up to the age 27, TC showed an increase followed by a slightly decreasing trend at 40 years stands. On the contrary, soil pH and NO₃ exhibited a sig-
significant opposite trend. These results are in line with the findings of Chen et al. [44] and Zhang et al. [50].

Illumina Hiseq2500 sequencing revealed variable significant increase/decrease trends among the top 15 classes of soil biotic communities with respect to changes in stand age and soil depth profiles. For bacterial classes, Acidobacteriia, alphaproteobacteria and verrucomicrobiae were the most abundant bacterial classes among all the stand ages and soil profiles. These findings are consistent with the previous reports [8,15]. Moreover, it is established that the high dominance of Acidobacteriia (36%) (Fig. 1) is due to

Fig. 3. Combined relative abundance (%) of top 15 soil microbial (Bacteria, Fungi, Archaea, Protists) and animal communities for all samples after pooling the replications. Here, D1, D2, D3 are about different soil depths i.e., D1 (0–10 cm), D2 (10–20 cm) and D3 (20–40 cm).
Fig. 4. Box plots for alpha diversity (Chao 1 and Shannon indices) of soil microbial (Bacteria, Fungi, Archaea, Protists) and animal communities. Here, D1, D2, D3 are about different soil depths i.e., D1 (0–10 cm), D2 (10–20 cm) and D3 (20–40 cm). Error bars in the boxplots with different lowercase letters show significant differences between different depths, while different uppercase letters describe significant differences among stand ages (LSD test).
the soil acidity (4 < pH < 5)) [51], which was in good agreement with our findings. We found that the relative abundance of Acidobacteria increased with the increase in soil depth however, an opposite trend was observed for the alphaproteobacteria (Supplementary Fig S3a). A similar shift in bacterial community composition was observed along with deep soil profiles in monospecific and mixed stands of *Eucalyptus grandis* and *Acacia mangium* [52]. Different Chinese fir stand ages posed significant narrow variations in the relative abundance of major bacterial classes [8]. These observations are connected with the narrow range of pH change (approximately 0.3 unit) detected among different stand ages in our findings (Supplementary Fig S1-F), which is supported by earlier research that demonstrated pH as the best predictor for changes in bacterial community structure and composition by surveying 88 soils across America [51]. As reported by Liu et al. [8] and Buée et al. [53], we recorded high abundance of Agaricomycetes (27%), phylum Basidiomycota (Fig. 1). These fungi are considered lignin decomposers, and therefore, with the increase in forest age, the litters are increased, leading to the rapid growth and abundance of Basidiomycota (Agaricomycetes) [54]. Stand age and soil depth profiles did not exhibit any particular trend among the major classes of fungi; however, we observed a
relatively high abundance of Sordariomycetes in 0–10 cm deep soil samples (Supplementary Fig S3b) that was already a confirmed finding [54]. A comparatively high dominance was observed for Mortierellomycetes in 5 years age stands of Chinese fir (Supplementary Fig S3b), which is perhaps due to the capability of this particular fungi to decompose the limbs of plants and animals remains of the previous plantation after the rotation [55], and to support root development during initial years of tree growth through phosphorus transformation from an insoluble to a soluble form that can then be directly utilized by plants [56]. Our findings revealed Bathyarchaeia (60%) as the most abundant archaeal class, belonging to the phylum Crenarchaeota (Fig. 1). According to our
brief knowledge, there are no previous reports regarding the abundance of archaea, protists and animals with respect to various soil profiles in Chinese fir chronosequence. However, these archaea are found abundantly in soils that have been subjected to strong ecological and management treatments (clear-cutting and burning) [57], which is very common in Chinese fir plantations [2]. Few reports disclosed that archaeal dominance is related to ammonium oxidation at Alaska and Qinghai-Tibetan forest sites [58,59]. Increasing soil depth showed an increase in Bathymarchaeota, as supported by an early report [58], while Thaumarchaeota (Thaumarchaeota) and Thermoplasmata (Euryarchaeota) exhibited reduction with the increasing soil depth (Supplementary Fig S3c). In rhizospheres, Thaumarchaeota exist as ammonia-oxidizers, playing vital roles in biogeochemical cycles (nitrogen & carbon cycle) [60], while Euryarchaeota seems to be directly proportional to the existence of mycorrhizal fungi [61].

Protists are overlooked and are not well studied, but we believe that all the soil biotic communities play some of the other role in balancing the soil ecosystem. Our results exhibited unclassified (56%) and unassigned (19%) protist classes (Fig. 1), clearly indicating that these have been poorly understood and more research is required to classify them at various taxonomic levels. Among other classified protists, Chlorophyceae (phylum Chlorophyta) were found most abundant (5.1%) (Fig. 1). Some previous reports related to biological soil crusts [62] and forest forming tree species suggest the higher abundance of Chlorophyta in soil [63]. In our findings, Conoidasida (phylum Apicomplexa) reduced while Peronosporomycetes (Clade Rhizaria) increased with an increase in soil depth (Supplementary Fig S3d). Not much information is available about the Conoidasida or Peronosporomycetes regarding their trend with the changes in stand age and vertical soil depth profiles in literature. However, some researchers observed the spacio-temporal structure of Rhizaria for temperate agriculture and grasslands ecosystem [64,65]. Among animal communities, we recorded the higher relative abundance of Clitellata (34%) belonging to phylum Annelida (Fig. 1). Not many reports exist in the literature about the abundance and diversity of soil animal communities in the forest ecosystem. But it is reported that the Annelida population is relatively higher in woody savannahs as compared to pastures, grasslands and fields [66]. We observed that for most of the stand ages, Clitellata abundance reduced while Arachnida and Chormodorea increased with increasing soil depth (Supplementary Fig S3e). In our understanding, mostly the members of Arachnida are spiders that don’t like the places with greater human activity and management practices that may lead them to stay at increasing soil depth (40 cm) or even more [67]. Similarly, Chormodorea are Nematodes whose population is correlated with the soil moisture content in the forest ecosystem [68]. The dynamic shift and trends among nematodes may be linked to the trend of WC in our study. Members of Clitellata (phylum Annelida) are earthworms that burrow in the soil or live near the surface, generally in moist leaf litter [69]. These are very important contributors to soil fertility as they loosen the soil by burrowing and help ion mixing the organic and mineral matter through acceleration in the decomposition process [70].

Our results explained that the stand age impacted the alpha diversity of bacteria showing a significant decrease and increasing trend. However, a significant decreasing trend in alpha diversity was observed with an increase in soil depth for all the biotic communities except archaea that showed a contrasting opposite trend (Fig. 4; Supplementary Fig S4). For example, on average, bacterial alpha diversity decreased up to the age of 27 significantly, followed by an increase at 40 years stand, which is directly proportional to the changes in soil pH. Significant correlations have been frequently found between the pH and bacterial alpha-diversity in different soils [71,72] and lake sediments [73], indicating that pH is a predictor for soil microbial diversity. This is recently confirmed by a global meta-analysis, stating that soil pH is the top most indicator for changes in microbial diversity [74]. The decreasing trend in fungal [14], protists [75] and animal [11] communities diversity, while the opposite contrasting trend in archaea communities was observed across reforested, agricultural and temperate ecosystems [11]. Protist communities are considered the most diverse and dominating in neotropical forests [76] and are influenced by changes in soil nitrogen [75]. However, in the tropical forest ecosystem, our findings are novel with respect to different soil depths and stand ages that may also be correlated with nitrogen availability in soil, but we believe that more specific research is required to understand this dynamic aspect. Generally, regarding richness, Protist and animals follow a similar trend like bacteria and fungi while archaea exhibit an opposite trend [11], which is similar to our findings as well (Chao1 index). As mentioned earlier, Animal diversity is also related to TC and least disturbance and tillage activities [77].

Among all the soil biotic communities, we found that the beta diversity of fungal communities was more influenced by stand age (Adonis test) (Fig. 5), previously confirmed by Lu et al. [17]. Our results explained that TN was the most significant influencing factor for all the communities, followed by TP, pH and NO3. TN correlation with bacterial, fungal [8,78], archaeal [79] and animal communities [80]; pH, TP and NO3 correlation with Proteobacteria [14,51] has been previously documented.

Conclusions

Using various soil vertical profiles to study the assemblage of soil biotic communities across the chronosequence of Chinese fir plantations, we find perhaps the most comprehensive evidence showing the complexity of belowground ecology. Our results showed that with the increase in soil depth, soil biotic communities exhibited a decreasing trend in alpha diversity of bacteria, fungi, protists and animals; however, archaea showed an opposite contrasting trend. The most abundant soil bacterial, fungal, archaeal, protist and animal classes were Acidobacteria, Agaricomycetes, Bathymarchaeia, Chlorophyceae and Clitellata, respectively. Correlation of vertical distribution of biotic communities and variations in soil physiochemical properties explained that TN, AP and soil pH were the most influencing factors for changes in soil biotic communities. Our results highlighted that microbial and animal richness is strongly influenced by soil properties in a near-uniform manner thus explaining the importance of the dynamics between biotic and abiotic processes that drive the organization of belowground biological diversity. Soil can be regarded as “ecological black boxes” as it provides differential insights among belowground micro and macro-biota. Therefore, we believe that this study represents a step toward an insightful understanding of all the soil biotic communities in continuous plantations of Chinese fir. We believe that our study will facilitate in better understanding not only the major soil communities (Bacteria and Fungi) but also the other important communities (Archaea, Protists, Animals) diversity and structure in response to different stand ages and vertical soil profiles. It will serve as a theoretical base for developing more sustainable management practices in the forest ecosystem.

Declarations
Ethics approval and consent to participate. Not applicable.
Consent for publication
Not applicable.
Availability of data and material
The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements
We are thankful for the support of our fellow Dr. Zaipeng Yu (Fujian Normal University) and Mr. Wang Sirong (Forest Farm Manager, Baisha Forest Farms, Longyuan) for their valuable efforts in establishing the research plots and helping in soil sampling. Funding for this experiment was provided by the National Science Foundation of China [31917548] and for Distinguished Young Scholars (31625007).

Compliance with Ethics Requirements
This article does not contain any studies with human or animal subjects.

Appendix A. Supplementary data
Supplementary data to this article can be found online at https://doi.org/10.1016/j.jare.2021.08.005.

References
[1] Chen G, Yang Y, Yang Z, Xie J, Guo J, Gao R, et al. Accelerated soil carbon turnover under tree plantations limits soil carbon storage. Scientific Reports 2016;6. doi:10.1038/srep19693.
[2] Farooq TH, Yan W, Rashid MHU, Tabuha M, Giliani MM, Zou XH, et al. Chinese fir (Cunninghamia lanceolata) a green gold of China with continues decline in its productivity over the successive rotations: A review. Appl Ecol Environ Res 2019;17:11055–67. doi: https://doi.org/10.15666/aeer/1705_1105511067.
[3] Kooch Y, Rostayee F, Hosseini SM. Effects of tree species on topsoil properties and productivity over the successive rotations: A review. Appl Ecol Environ Res 2018;51:91–101. doi:10.2478/cerce-2018-0020.
[4] Sagova-Mareckova M, Cermak L, Novotna J, Plhackova K, Forstova J, Kopecky J. Central Punjab, Pakistan – A Case Study. Cercetari Agronomice in Moldova 2016;6. doi:10.1038/srep19699.
[5] Selvaraj S, Duraisamy V, Huang Z, Guo F, Ma X. Influence of long-term successive rotations and stand age of Chinese fir (Cunninghamia lanceolata) on soil physiochemical properties. ZH and HYCH supervised and provided funding for the project.

Declaration of Competing Interest
The authors declare that they have no competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

The datasets generated and/or analyzed during the current study are available at www.datadryad.org. via https://doi.org/10.1016/j.jare.2021.08.005. via https://doi.org/10.1016/j.jare.2021.08.005.

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements
We are thankful for the support of our fellow Dr. Zaipeng Yu (Fujian Normal University) and Mr. Wang Sirong (Forest Farm Manager, Baisha Forest Farms, Longyuan) for their valuable efforts in establishing the research plots and helping in soil sampling. Funding for this experiment was provided by the National Science Foundation of China [31917548] and for Distinguished Young Scholars (31625007).

Compliance with Ethics Requirements
This article does not contain any studies with human or animal subjects.

Appendix A. Supplementary data
Supplementary data to this article can be found online at https://doi.org/10.1016/j.jare.2021.08.005.
[79] Rughöft Saskia, Herrmann Martina, Lazar Cassandre S, Cesarz Simone, Levick Shaun R, Trumbore Susan E, et al. Community composition and abundance of bacterial, archaeal and nitrifying populations in savanna soils on contrasting bedrock material in Kruger National Park, South Africa. Front Microbiol 2016;7. doi: https://doi.org/10.3389/fmicb.2016.01638.

[80] Sun X, Zhang X, Zhang S, Dai G, Han S, Liang W. Soil nematode responses to increases in nitrogen deposition and precipitation in a temperate forest. PLoS ONE 2013;8. doi:10.1371/journal.pone.0082468.