Seasonal Variations Affect The Ecosystem Functioning And Microbial Assembly Processes In Plantation Forests

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Abstract

Background: While afforestation mitigates climate concerns, the impact of afforestation on soil microbial compositions, ecological assembly processes, and multiple soil functions (multifunctionality) in afforested areas remains unclear. The Xiong'an New Area plantation forests (*Pinus* and *Sophora* forests) were selected to examine the effects of plantation types in four contrasting seasons on soil microbiomes.

Results: We evaluated three functional categories (nutrient stocks, organic matter decomposition, and microbial functional genes) of multifunctionality, and the average (net) multifunctionality was quantified. The results showed that net soil multifunctionality as a broad function did not change seasonally, unlike other narrow functional categories. Bacterial communities were deterministically (variable selection and homogenous selection) structured, whereas the stochastic process of dispersal limitation was mainly responsible for the assembly and turnover of fungal and protist communities. Additionally, we showed that winter triggered an abrupt transition in the bacterial community assembly from deterministic to stochastic processes in *Pinus* forests that was closely associated with a reduction in the bacterial Shannon diversity, with functional patterns of a high level of nutrient cycling (nutrient stocks and organic matter decomposition).

Conclusions: Overall, the present study contributes local-ecosystem prospects to model the behavior of soil biota seasonally and their implied effects on soil functioning and microbial assembly processes in plantation forests.

Background

One of the fundamental aims in microbial ecology is to disclose the role of ecological processes and environmental parameters in driving the assembly of microbial communities [1]. The assembly of microbial species in a local community is generally structured by four classes of ecological processes, categorized into stochasticity (homogenizing dispersal coupled with drift and dispersal limitation coupled with diversification and drift) and determinism (homogeneous selection, variable selection) [2]. Growing evidence suggests possible associations among microbial community assembly, composition, and ecosystem functioning [3, 4]. External driving determinants, such as the perturbance of land-use management using exotic vegetation, drive diversified ecosystem functions by changing assembly processes and microbiome dynamics. Subsequently, the outcomes will affect microbiome community composition by imposing feedback [3]. However, the temporal patterns of stochastic versus deterministic assembly processes and the driving environmental parameters that seasonally influence ecosystem functions in plantation forests remain unresolved.

The composition and function of ecological communities within terrestrial ecosystems can be changed greatly by anthropogenic activities, leading to notable alterations in multiple ecosystem functions (multifunctionality) [5]. Vegetation restoration through afforestation occurs in different types of land use and climatic zones, either through self-generation or by the establishment of fast-growing trees with

various physiological characteristics, which in turn can be helpful to reduce the effects of climate change [6]. The largest afforestation area is currently recorded in China [7], and the widespread planting of forest trees such as *Pinus* [8, 9] and *Sophora* [10] has recently been typical for afforestation in the northern part of China, including the Xiong'an New Area (XNA)–a leading-edge city that delivers innovative intelligence, a green ecology, and wellbeing [11]. The most cost-effective mitigation option in forestry is afforestation with sustainable management, which can provide flexibility to reduce carbon intensity and mitigate climate change [7]; thus, understanding the role of ecological processes (e.g., deterministic and stochastic) in microbial community assembly and their connections with multifunctionality in some environments, such as afforested areas, where extreme human activities are initially faced, is extremely important. To reach this goal, it is essential to focus on both plantation types and seasonal successions at the same study sites when trying to disentangle the drivers of microbial distributions, functions and their interactions in plantation forest regions.

The soil biota, mostly bacteria, fungi, and protists, is considered a key driver of primary production, nutrient cycling, and biogeochemical cycles [12, 13]. Environmental factors such as plant species [9, 14], physicochemical properties [9, 11], and seasonal variations [5, 15–17] control the abundance and/or composition of soil biota. The majority of the studies evaluated the impact of either environmental variables or ecoenzyme activities on soil biota in natural forests in temperate and boreal areas and Arctic tundra seasonally [16, 18–21]; thus, exploration of the integrated effects of different plant types, variations in seasons (multiple time points), edaphic conditions, biochemical properties and biogeochemical cycling is an essential step for ecological management practices [15], particularly for those areas that are under heavy urbanization with exotic forest plantations [9, 11].

In this study, we collected soil samples from two different plantation forest types (evergreen vs. deciduous; *Pinus tabulaeformis* Carr. and *Sophora japonica* L.) in which soil experienced considerable disturbance by human activities during land preparation during four distinct seasons. We characterized the diversity and composition of soil biota (bacteria, fungi, and protists), the gene copy numbers of soil biota, ecoenzymatic activities and stoichiometry, physiochemical properties, and the abundance of microbial functional genes involved in the transformation of C, N, P, and S as microbial functional attributes using quantitative microbial element cycling (QMEC), an approach based on high-throughput quantitative polymerase chain reaction (PCR) [22] to explore (i) the relationship between microbial diversity and composition under different plantation types seasonally with the provision of soil multifunctional services and (ii) the microbial assembly processes in plantation forests in the XNA. Our main hypothesis was that distinct assembly processes drive the structure of the different soil microbiomes. Therefore, by comparing the microbial community assembly in two different plantation types seasonally, this study presents a framework to enhance our comprehension of the mechanisms directing the microbial pattern and the benefaction of deterministic and stochastic processes to community assembly processes. The present study aimed to (i) classify the dominant operator in structuring the microbial communities in different plantation types; (ii) determine soil microbial functioning and biogeochemical cycling in different plantation types with seasonal variations; (iii) quantify the relative significance of deterministic and stochastic processes in plantation forest microbial

assembly; and (iv) examine whether bacterial, fungal, and protist taxa assemble via similar or different community assembly mechanisms in plantation forests with different plantation types seasonally.

Results

Changes in soil physicochemical properties, extracellular ecoenzymatic activities (EEAs) and stoichiometry (EES)

The results of the edaphic conditions are summarized for different seasons specific to this investigation (Supplementary Table 1). Edaphic parameters were unsteady throughout the course of one year. Soils in spring and summer were relatively mesic and high in some elements, such as TP and TN, whereas soils were relatively high in TC, SOM, TK, SWC, and EC in autumn and winter. Soil pH was alkaline across our sampling plots; it decreased significantly between spring and winter in the CP plot, but it did not change significantly in the CS plot with seasonal variations. SWC was significantly affected by seasonal variations. Differences in the EEAs of the soils were observed based on seasonal variations and plantations (P < 0.05, Supplementary Table 1). For instance, winter was characterized by low activities of those enzymes involved in P, ALP ($F_{7, 23}$ = 3.6566, P = 0.0151), S cycling, and ASF ($F_{7, 23}$ = 6.1483, P = 0.0013). More detailed results can be found in the additional file 1. The various seasons and plantations significantly influenced the EESs in the soils. Our results showed that the BGC:LAP + NAG (C:N) activity ratio was not significantly different in the CP plot seasonally ($F_{3, 11}$ = 2.0222, P = 0.1894), whereas this ratio was significantly different between spring and other seasons in the CS plot ($F_{3,11}$ = 5.0298, P = 0.0301). The BGC:ALP (C:P) activity ratio was highest in winter, and it was significantly different between summer and spring in the CP plot ($F_{3,11}$ = 4.5608, P = 0.0383), whereas this ratio was not significantly different seasonally in the CS plot ($F_{3,11}$ = 0.8182, P = 0.5193). The LAP + NAG: ALP (N:P) activity ratio was significantly different between winter and the other seasons in both experimental plots, CP ($F_{3,11}$ = 7.6975, P = 0.0096) and CS ($F_{3,11}$ = 6.3929, P = 0.0161) (Supplementary Table 2-5). The association of edaphic factors with EEAs/EES is shown in Supplementary Figures 1-2.

Response of the composition of soil microbial communities to seasonal variations in different plots

Bacterial Communities

After quality filtering, the remaining 1,043,419 bacterial sequences were identified from 24 soil samples, and the mean value of the sequence number was 43,475 (±2,593; ±SE) for each sample. The normalized bacterial sequences were clustered into 5,157 OTUs with a 97% similarity threshold. The bacterial Venn diagram showed that the highest number of unique OTUs was found in CSU samples (60 OTUs), whereas SWI samples accounted for the lowest number of unique OTUs (23 OTUs). The core bacterial community, represented by all eight groups, included 1,746 OTUs (Supplementary Figure 3A). The abundance of the dominant Firmicutes phylum in both plant communities reached its highest level in winter, and its

abundance was significantly different between these two plant species plots (Supplementary Table 6). The overall bacterial community was dominated by sequences assigned to the class that belonged to Actinobacteria (28.48%) (Supplementary Figures 4A, B; Supplementary Table 6). The difference in bacterial abundance at the genus level increased between spring and summer in the CP (62.72%) and CS (52.71%) plots, whereas differences in abundance decreased with seasonal changes from summer to autumn and autumn to winter for both plant species (Supplementary Figure S5A). The seasons induced significant changes in the bacterial Shannon diversity ($F_{7, 23}$ = 6.8806, P = 0.0007) and richness ($F_{7, 23}$ = 3.4191, P = 0.026) of the bacterial community for both plant species plots. The results showed that bacterial diversity and richness were significantly different in spring and in winter between the two different plant species (Figs. 1A, B; Supplementary Table 7). The seasonal trends of other bacterial α-diversity indices are summarized in Supplementary Figure 6. NMDS plots revealed significant differences in the variability of the bacterial (ANOSIM, R = 0.3099, P = 0.001) community compositions among seasons and plant species. In addition, bacterial communities in the CWI samples were distinctively segregated from other seasons (Supplementary Figures 7A, B; Supplementary Table 8). There were 98 statistically significant bacterial taxa as indicators in different seasons from the phylum to the genus level, and among all seasonal samples, CWI samples exhibited the highest biomarkers (n = 28) (P⬚0.05, LDA⬚2). These biomarkers accounted for 1.89% of all taxa retrieved (Supplementary Figure 8A). The results showed the significant impact of season on different groups of soil biota. It seems that the segregation of bacterial composition mostly occurred in autumn. For instance, CAT samples formed a separate cluster, and other bacterial samples formed two segregated clusters (Supplementary Figure 9A).

Fungal Communities

After quality filtering, the remaining 1,618,594 fungal sequences were obtained from 24 soil samples, and an average of 67,441 (±3,636; ±SE) sequences were shown per sample. The normalized reads were grouped into 3,902 OTUs with a 97% similarity threshold. The fungal Venn diagram revealed that the core fungal community consisted of 266 OTUs among all samples (Supplementary Figure 3B). The abundance of the dominant Ascomycota reached its highest level in winter for both plant species, and its abundance did not significantly differ between spring and winter in the CS plot (Supplementary Figure 4C; Supplementary Table 6). The highest abundance of fungal classes belonged to Sordariomycetes (42.20%) (Supplementary Figure 4D). Furthermore, the relative abundance of fungal genera did not significantly increase with changing seasons from summer to autumn and from autumn to winter for both plant species plots (Supplementary Figure 5B). The fungal Shannon diversity ($F_{7, 23}$ = 4.4671, P = 0.0063) and richness ($F_{7, 23}$ = 2.7379, P = 0.0463) in the plant species plots differed significantly with seasonal variations. We observed that fungal diversity and richness decreased with changing season to cold (winter), particularly in the CP plot (Fig. 1). The seasonal trends of other fungal α-diversity indices are summarized in Supplementary Figure 6. NMDS plots revealed significant differences in the variability in fungal (ANOSIM, R = 0.4451, P = 0.001) community compositions among seasons and plant species. In addition, fungal communities of the CWI samples were distinctively segregated from other seasons. For

the fungal community, CAT samples showed separate variation and distinctiveness (Supplementary Figures. 7C, D; Supplementary Table 9). Our LDA results (total = 119 biomarkers) showed that the highest number of biomarkers for fungal taxa was observed in SSP samples (n = 24) ($P < 0.05$, LDA > 2) (Supplementary Figure 8B). These biomarkers accounted for 3.04% of all taxa retrieved. The hierarchical clustering results showed that the segregation of the fungal compositions mostly occurred in autumn. For instance, the structure of the fungal communities of the CAT samples and CS samples formed two segregated clusters, and the remaining fungal samples formed one cluster (Supplementary Figure 9B).

Protist Communities

After quality filtering and removal of the Metazoa, Streptophyta, and Fungi [11], 546,303 protist sequences remained among all soil samples, and an average of 22,763 (±1,415; ±SE) sequences were calculated for each sample. The normalized sequences were clustered into 2,162 protist OTUs with a 97% similarity threshold. The core protist community consisted of 370 OTUs among all samples (Supplementary Figure 3C). The protist communities were dominated by the phyla Cercozoa (26.87%), Chlorophyta (14.51%), and Apicomplexa (12.77%), followed by unclassified Eukaryota (10.98%) and Lobosa (8.75%) (Supplementary Figure 4E). The abundances of the most dominant protist phyla and classes, Cercozoa and Filosa-Sarcomonadea, respectively, were the highest in both plant species plots in spring (Supplementary Figure 4F; Supplementary Table 6). Another example of the impact of season on protist abundance was Chlorophyta, which had a high frequency in winter (Supplementary Table 6). The abundances of protist genera increased from spring to summer for both plant species. In contrast, their abundances showed different trends within seasonal changes from summer to autumn and autumn to winter for both plant types (Supplementary Figure 5C). The protist Shannon diversity ($F_{7,23}$ = 1.9374, P = 0.0486) and richness ($F_{7,23}$ = 2.7219, P = 0.0461) were significantly different among seasons. The results showed that the protist richness was high in winter but was not significantly different from that in other seasons, whereas the protist Shannon diversity did not follow this trend (Figs. 1E, F). The seasonal trends of other α-diversity indices are summarized in Supplementary Figure 6C. The homogeneity in the protist community composition was corroborated by the concentric ordination space covered in the NMDS (ANOSIM, R = 0.1667, P = 0.032) analysis in comparison with bacterial and fungal compositions (Supplementary Figures 7E, F; Supplementary Table 10). Our LDA results showed that the highest number of biomarkers for protist taxa was observed in CSP samples (n = 12) ($P < 0.05$, LDA > 2) (Supplementary Figure 8C). These biomarkers (in total = 42) accounted for 1.94% of all taxa retrieved. The hierarchical clustering results showed that the protist compositions of the *Pinus* and *Sophora* forests formed segregate clusters in spring and winter, respectively, and other seasons grouped in the same cluster (Supplementary Figure 9C).

Relationships Among Diversity, Abundance, And Eeas

Although the microbial Shannon diversity was linked with several edaphic factors, such as soil pH, fewer soil factors showed a significant association with microbial richness (Chao1) (Fig. 2). Four (PD, Chao1, Sobs, and ACE) and one protist alpha diversity (Simpson) index were negatively and positively correlated with PPO and POD activities, respectively (Supplementary Figure 10).

Quantification Of Microbial Abundance Using Real-time Quantitative Pcr

Quantitative PCR showed that the fungal gene copy number was lower than that of other soil biota and followed the trend bacteria > protists > fungi (Supplementary Figure 11). The 16S rRNA gene copy number of bacteria ranged on average from 8.65×10^8 to 34.84×10^8 copies/g dry soil, the fungal gene copy numbers ranged on average from 0.68×10^8 to 6.84×10^8 copies/g dry soil, and the protist gene copy numbers ranged on average from 1.17×10^8 to 7.22×10^8 copies/g dry soil.

Drivers Of Soil Microbiome Community Variations

The PERMANOVA showed that the impact of season on bacterial and fungal compositions was significant, explaining 26.83% ($P = 0.029$) and 43.30% ($P = 0.002$) of the bacterial and fungal variations, respectively (Figs. 3A, B; Supplementary Tables 11-13). In contrast, the influence of season on protist compositions was not significant, explaining 17.91% ($P = 0.163$) of the variation in protists (Fig. 3C, Supplementary Table 13). The second most important factors for predicting the β-diversity of bacterial, fungal and protist taxa were AGC, TP, and POD, which explained 17.02% ($P = 0.011$), 35.57% ($P = 0.003$), and 12.18% ($P = 0.029$), respectively, of the variance (Fig. 3C). Bacterial and fungal RDA analyses of soil enzymes did not separate hydrolytic and oxidative activities onto separate axes (Figs. 4A, C). However, interestingly, RDA of soil enzymes condensed hydrolytic and oxidative activities onto separate axes (Fig. 4E). In addition, fungal RDA of edaphic parameters was much concentric compared with bacterial and protist RDA analyses of soil properties (Figs. 4B, D, and F).

Effect of seasonal variations and plantations on microbial trophic groups

The results showed that protist consumer taxa were the dominant group across our experimental plots (42.24%, on average). The second most dominant protist trophic group was phototrophs (eukaryotic algae, 31.68%), with the highest abundance in SWI samples and the lowest abundance in CSU samples. The protist communities were composed of taxa putatively assigned as 11.64% parasites, with the highest abundance in SSU samples and the lowest abundance in CSP samples. The photophagotroph group was found across sampling plots with a lower abundance compared with other trophic groups (0.35%, on average); the lowest abundance of this group was observed in CAT samples (0.15%), and the highest abundance was observed in SAT samples (0.53%). Across sampling plots, 14.07% of the overall protist communities were composed of the unclassified group (Supplementary Figure 12). The results of

PICRUSt2, fungal guilds and modes as well as the bacterial TAX4FUN analysis are presented in Supplementary Figures. S13-16.

Co-occurrence Patterns In Microbial Networks

To determine how plantation types and seasonality affected microbial network complexity, topological network parameters such as the 'average degree (AD)', 'average clustering coefficient (ACC)', and 'average path length (APL)' were considered. The higher the AD and ACC, the more complicated the networks. The more moderate the APL, the closer the association among the members. Applying this rationale and considering plantation type, our results indicated that the soil−biome relationship was more complex and intense in the *Pinus* forest; considering seasonality, the microbial co-occurrence network was more complex in spring, whereas this network was more intense in winter (Fig. 5, Supplementary Table 14). Interestingly, the highest number of microbial links (bacteria to protists) was recorded in spring (Supplementary Table 14). Based on the key nodes, our results suggested that spring, which was characterized by those nodes, was affiliated with bacterial and protist networks and abiotic factors such as soil enzymes that belonged to the N and P cycles (Supplementary Table 15). More details of microbial interactions are presented in Supplementary Table 16. All network parameters were significantly associated with the three different classes of air temperature. Remarkably, oxidative enzymes such as PPO and N-related enzymes such as LAP were also associated with most network parameters (Supplementary Figure 17).

Abundance And Composition Of Microbial Functional Genes

Absolute functional gene abundance differed significantly ($P < 0.05$) among soil samples (Supplementary Figures. S18-S20). More specifically, the results of QMEC based on the HT-qPCR approach showed that the abundance of those genes involved in C degradation (ANOVA, $P = 0.0139$), N cycling (ANOVA, $P = 0.0026$), and S cycling (ANOVA, $P = 0.009$) was significantly affected by plantation type seasonally (Supplementary Figure 21). The PCA revealed that the CNPS cycling gene compositions in different plantation types with seasonal variations formed distinct but concentric clusters in which the CNPS cycling genes of the *Pinus* forest in autumn and winter separated from other clusters (Supplementary Figure 22). Additionally, a significant impact of different plantations with seasonal variations on the overall profile of CNPS cycling genes was observed (Adonis, $P = 0.004$, Anosim, $P = 0.006$, and MRPP, $P = 0.008$). The abundance of keystone C-hydrolysis genes significantly differed seasonally among plantation types, and the abundance of those genes involved in hemicellulose degradation was higher than that of other C-hydrolysis genes (Supplementary Figure 23). Our results also showed that those genes involved in C degradation and N cycling significantly differed between the two plantations (Supplementary Figure 24).

Contribution of CNPS cycling genes to microbial diversities and compositions

Spearman analysis showed that there was no association between microbial richness and diversity and the microbial CNPS cycling genes, whereas fungal and protist β-diversity was significantly associated with microbial C degradation and C fixation. In contrast, bacterial β-diversity was significantly associated with microbial P cycling genes (Supplementary Figure 25). Interestingly, the protist RDA results of the microbial CNPS cycling genes indicated significantly condensed C degradation and C fixation on separate axes ($P < 0.05$) (Supplementary Figure 26).

Variation in ecosystem functioning is impacted by biotic and abiotic parameters

Among the four functional categories (Supplementary Table 17), narrow functional categories (nutrient stocks, organic matter decomposition, and microbial functional genes) significantly differed between plantation types with seasonal variations (ANOVA, $P < 0.05$), whereas 'net soil multifunctionality' did not differ significantly ($P = 0.4306$) (Supplementary Figure 27). Different levels of statistical associations were observed between multifunctionality indices and microbial compositions (Supplementary Figure 28) and microbial diversities (Supplementary Figure 29). The nutrient stocks and organic matter decomposition showed the highest number of significant associations with environmental variables (Supplementary Figure 30). Among the four functional groups, only the 'nutrient stocks' function was associated with the minimum, average, and high air temperatures (Supplementary Figure 31). Among the four functional categories, 'nutrient stocks' was significantly linked with most network parameters (Supplementary Figure 32). Net soil multifunctionality explained 17.66% and 10.55% of the variation in the fungal and protist communities, respectively (Supplementary Figure 33).

Assembly Processes Of Microbial Communities

We observed that the βNTI distributions differed significantly across plantation type, with seasonal variations for the bacterial ($F_{7, 575} = 3.5549$, $P = 0.0009$), fungal ($F_{7, 575} = 6.9355$, $P = < 0.0001$), and protist communities ($F_{7, 575} = 3.6975$, $P = 0.0006$) (Figs. 6A, B, and C). Null model analysis showed that the relative contributions of deterministic ($|βNTI| \geq 2$) and stochastic ($|βNTI| < 2$) processes in various microbial compartments differed greatly. The deterministic processes of variable selection and homogenous selection were primarily responsible for the assembly and turnover of the soil bacterial communities (average: 62.49%) (Fig. 6D), whereas the stochastic process of dispersal limitation was mainly responsible for the assembly and turnover of the fungal and protist communities (average: fungi: 42.36%, protists: 76.39%) (Figs. 6E, F). Interestingly, among microbial compartments, the relative contribution of undominated processes to the fungal community (average 42.36%) was greater than that to the bacterial and protist communities (bacteria: 25.34%, protists: 14.93%, on average). The results showed that the bacterial deterministic process of homogenous selection shifted toward the dominance of stochastic processes (dispersal limitation) in the *Pinus* forest in winter (Fig. 6D).

Influence of biotic and abiotic parameters on microbial diversity, composition, assembly processes and multifunctionality

We built theoretical frameworks using PLS-PM to disentangle direct and indirect relationships by focusing on the community diversities and compositions of soil biota with climatic and environmental variables, multifunctionality and microbial community assembly. In both models based on α-diversity (Fig. 7A) and β-diversity (Fig. 7B) indices, annual air temperature substantially and negatively structured the microbial meta-community co-occurrence network parameters and soil enzyme activities. In the model based on β-diversity indices, annual air temperature negatively influenced multifunctionality. In both models, soil enzyme activities and soil properties were greatly and positively associated with multifunctionality. In the model based on α-diversity, soil enzyme activities significantly influenced protist diversity. In both models, a significant link between soil enzymes and soil properties was observed.

Discussion

Microbial diversities under seasonal variations and in different plantations

The seasonal dynamics of the observed richness and Shannon diversity of each soil microbiome showed different patterns (Fig. 2, Supplementary Table 7). These findings suggest that seasonal variations had an additional influence on soil microorganisms concerning different plant species, which is consistent with other studies [17, 19, 20, 23–28]. As an example, unlike other studies in which potential biotic and abiotic environmental variables such as soil moisture [29, 30], plant types [31], pH [12, 13, 29, 32], litter chemistry [33], and fertilization/season-induced changes [34] contributed to shaping the composition of the protist structures, our PERMANOVA profiling revealed that the key factor driving the protist community's structure was peroxidase (POD) (Fig. 3). PERMANOVA showed that the protist community, compared with the bacterial and fungal communities, was not influenced by either seasonal variation or plantation type, suggesting that protist taxa have a broad tolerance to the wide fluctuation of seasonal variabilities. Thus, we suggest that the protist composition is strongly but indirectly influenced by soil bacterial and fungal metabolic activities, particularly C-related functions. If this pattern holds true across a range of ecosystem types, then it implies that protists appear to be impacted to a unique degree compared with other soil biotas by alterations in soil oxidative activities and nitrogen fluctuations. Moreover, the GAM results indicated the importance of N cycles and oxidative enzymes to predict the diversity and composition of the soil protist taxa (Supplementary Figure 10). This finding is partly consistent with previous finding which highlighted that protists were the most susceptible soil biota to the application of nitrogen fertilizers [34]. PERMANOVA also showed that seasonal variations were the best predictor of bacterial and fungal β-diversity community variations. In accordance with the present results, previous studies have demonstrated that the soil bacterial and fungal communities showed more seasonal than spatial variation in alpine tundra soils [18, 20, 35]. Interestingly, the number of soil variables that significantly explained fungal β-diversity and variation was higher than that of soil parameters, which proportionally explained the bacterial β-diversity variation. A possible explanation might be that fungal lineages are fostered to better access more soil volume due to their hyphal growth and thereby obtain access to more substrate and nutrients than other biotas [36]. Thus, the potential

alteration in edaphic parameters might have a substantial influence on the fungal structure rather than bacterial taxa. Additionally, our results showed that unlike the β-diversity of the fungal and protist taxa, soil moisture strongly and significantly explained bacterial β-diversity variation, which is consistent with a previous study that highlighted the importance of the soil water content for soil bacterial communities [25]. However, PERMANOVA showed that none of the microbial β-diversity variations were significantly explained by the impact of different plantations. This finding is consistent with previous report that the effect of soil type on shaping the bacterial rhizosphere was stronger than that of plant species [37].

The relative abundance of bacterial taxa was disproportionately higher than that of other soil biotas

Our results showed that the high proportion of assigned reads and quantitative PCR belonged to bacterial taxa in our experimental plots in the plantation forest over the course of one year. This observation might be described by some plausible reasons. First, the annual accumulation of aboveground litter in the young plantation forest floor in the XNA (forest stand age ~ 4 years) is much lower than that in hyperdiverse and layered forests (mature temperate forests). Second, other studies highlighted that alkaline pH was not optimal for fungal growth [38]. Unlike fungal diversity indices without an association with soil pH, bacterial Shannon diversity was significantly associated with soil pH (Fig. 3), suggesting that the bacterial diversity was more significantly affected by pH than the fungal diversity, which might be due to comparatively narrow optimal pH ranges for bacterial growth but wide pH ranges for fungal growth [39]. Third, the fungal growth network is more sensitive to anthropogenic activities [11, 40] and forest management [41] than the bacterial community by creating plantation forests. Our results suggest that the development of the new forest may have resulted in a loss of niches for other soil biotas. Moreover, we infer that the microbial community structure can be altered by the potential effect of alkalinity by favoring high-pH adapted or alkaliphilic microorganisms. We concluded that several reasons, such as the pH level, the paucity of plant litterfall, anthropogenic activities and land management practices, created an opportunity for bacterial taxa to take over other soil biota, such as fungal and protist lineages, in young plantation, seasonal, open-canopy forests in the XNA. Further studies that take these variables into account will need to be undertaken.

Our results showed that the spring and summer microbial communities were significantly bacterial-dominated genera, whereas the autumn and winter communities were fungal-dominated genera, but these fungal differences were not significant (Fig. S6). Previous studies that evaluated the seasonal fluctuations of the microbial community in diverse ecosystems [18, 20, 42] observed consistent results on whether fungi that dominated under-snow biomass and bacterial taxa were more active in summer. The increase in bacterial abundance from spring to summer was associated with an increased abundance of protist genera, some of which may be bacterial feeders (Supplementary Figure 5). Several reasons can explain the domination of bacterial taxa in summer. First, fungi commonly target recalcitrant substrates such as plant litter (higher C to N ratio), whereas bacteria generally target labile substrates such as root exudates. In summer, warmer temperatures can increase root exudates compared with winter, and fresh plant litter input produces a relatively more moderate C to N ratio [40]. Therefore, the domination of

bacterial taxa was expected in summer. Another reason that can explain the trend mentioned above might be related to temperature and soil moisture as the two main factors that drive soil biota abundance [43, 44]. We realized that the soil moisture was relatively high in winter, particularly in the *Pinus* forest (Table S5). Additionally, the GAM analysis showed that both fungal and bacterial Shannon diversity indices decreased with increasing soil moisture. The decrease in the bacterial Shannon diversity was much greater than that of the fungal Shannon diversity (Supplementary Figure 10), suggesting a threshold for soil moisture and that fungi will exhibit less of a response to changes in moisture compared with bacteria [40]. However, it has been highlighted that the bacterial community was more sensitive to lower soil moisture than the fungal community [41]. A consistent pattern for the effect of soil moisture on soil biota abundance remains to be elucidated. Another possible explanation for the increased abundance of fungi in cold seasons could be partially related to their natural resistance to freeze-thaw perturbations [45, 46] and may not be related to substrate preference. More discussion about these reasons can be found in the additional file 1.

Effect Of Seasonality On Soil Enzymes

Consistent with previous literatures [47–49], the PLS-PM results predicted that the activity of soil enzymes was highly temperature-dependent (Fig. 7). In analyzing the projected future climate, the XNA would become warmer and wetter [50], in which the rising temperature had a more significant influence on respiration than on assimilation, suggesting the suppression of vegetation growth at any increase in temperature in the future in this area [50]. Consequently, we inferred that less plant growth would be followed by more miniature plant rhizodeposition and subsequently less microbial enzyme activities. Thus, this outcome likely can explain the negative association of the temperature and enzyme activities in our study. It has been highlighted that some environmental constraints, such as acidic pH and high phenolic compounds, can greatly decrease enzyme activities [49], consequently leading to weak or no temperature sensitivity of soil enzymes. Thus, it appears that the alkaline pH of the XNA, in part, drove the soil enzymes to be substantially affected by the temperature. There are similarities between our results and those of other studies [21, 51], highlighting the association of temperature with many EEAs. However, it has been suggested that soil peroxidase activity can decline with warming and that other enzymes may be less sensitive to warming, indicating that EEAs are generally more sensitive to nutrient addition than atmospheric and climate change [52]. The results of the ecoenzyme activities indicated that seasonal variations and plantation types significantly influenced the EEA (Supplementary Table 1), indicating different isoenzyme pools and consequently, the domination of different microbial taxa in each season, plantation, gene pool, and expression pattern. Other researchers documented comparable seasonal alterations in the EEA of soils [53–56]. As an example, the production of BGA increased in winter in the *Pinus* forest, whereas its activity was high in autumn in the *Sophora* forest. A possible explanation is that different plantation types have individual ecological and physiological features [14], resulting in different effects on the edaphic conditions and microenvironment [57], conclusively impacting the secretion of microbial nutrient-acquiring exoenzymes and plants [58]. High bacterial and fungal gene copy numbers generally showed higher enzyme activity in the *Pinus* forest than in the *Sophora* forest, indicating that key

microbial-mediating biogeochemical processes vary seasonally in different plantations (Supplementary Figure 11). Additionally, these higher gene copy numbers likely explain the high potential enzyme activities in winter, but our oxidative enzyme activities were higher in summer. A similar finding showed that oxidative activities were more variable than hydrolytic activities and increased with soil pH [59]. In addition, as mentioned previously, microbes typically invest more in enzymes, especially lignin-degrading enzymes, in summer [60]. In contrast, potential enzyme activities were potentially expected to be high in winter. The most recent study, highlighted that the same EEA dataset could be interpreted in contrasting ways [61]. Higher enzyme activities can be interpreted as more nutrient availability [59] or reduced nutrient availability [62]. In accordance with the resource allocation strategy, soil biota may secrete more soil ecoenzymes under low nutrient conditions [7, 63–69]. In our study, we inferred that the higher activity of some enzymes in cold seasons might be related to the lack of plant litter and plant photosynthesis products. However, other studies showed that higher enzyme activities in hyperdiverse forests were limited to autumn, which provided fresh and readily available substrates for more secretion of soil ecoenzymes [56, 70, 71], suggesting the synchronous intensification of belowground biomass and plant litter driving a considerable increase in the metabolism of microorganisms to produce more soil ecoenzymes. Our study showed the positive relationships of BGC: ALP, LAP + NAG: ALP with TC, LAP + NAG with TP and SOM (Supplementary Figure 1), as well as significant associations between BGC: LAP + NAG and BGC: ALP with TC/TN ratio (Supplementary Figure 1); these associations might not be the conclusion to match enzymatic acquisition ratios with nutrient stoichiometry [64] because nutrients such as organic matter elements can be stored in various forms, and only a tiny portion can be used by microbes [72].

The results of the investigated C:N:P stoichiometry ratios indicated a deviation from the global [59, 66] and regional scale of 1:1:1 for China's forests based on a nationwide dataset [64, 69], suggesting that ecoenzyme activity stoichiometry in the plantation forests of the XNA was mostly based on nutrient resource availability and demand for microbial nutrients and was not homeostatic. This result is consistent with that from several studies conducted in China [57, 73, 74], indicating that this ratio can potentially be different based on the type of ecosystem and soil. Our results also showed that the *Sophora* forest faced more substantial P limitation (with an average lower TP) than the *Pinus* forest with comparatively lower C:P and N:P ecoenzyme activity ratios, particularly in warmer seasons (Supplementary Tables 2-5). This is similar to the previous finding, which highlighted that P deficiency is a common problem in forest ecosystems and often intensifies in the summer season [75]. However, the abundance of P cycling genes in both forest types did not show a significant difference (Supplementary Figure 21). A detailed discussion is presented in the additional file 1.

Microbial trophic regulation by seasonal variations

Unlike the most recent study, in which biotic factors were identified as the major biogeographical predictor for protist consumers and mean annual temperature was the best predictor of the diversity and composition of phototrophs on a large scale in the Southern Hemisphere [76], our findings showed that seasonality and plantation type were important factors shaping protist trophic interactions at the local

scale. The highest level of protist consumers was observed in spring and summer in the *Pinus* forest. The possible explanation may be related to the temporal variation in the supply of carbon and other nutrients in warm seasons from Chinese pine roots to soil, as well as other compounds such as amino acids, organic acids, and sugars lost via this route, which provide high-quality nutrient sources for microbes, stimulating their growth and thereby increasing prey availability for consumers such as protists, which in turn can enhance nutrient uptake by plants [42, 77, 78]. The high abundance of consumers indicates the importance of protists' role in controlling the frequency of other soil biotas [13]. In support of this, PLS-PM analysis predicted that the protist α-diversity indices strongly influenced the microbial cooccurrence network parameters (Fig. 7A), indicating the robust impact of predator community compositions on microbial networks. This strong influence of predator diversity on the microbial co-occurrence network indicates that the predation pressure of protists significantly influenced the microbial co-occurrence network, and in turn, bacteria and fungi, as the key food source of protists, may shape the diversity of protist taxa, suggesting the bottom-up regulation of soil biota [79]. The high presence of protist parasites was observed in summer in the *Sophora* forest, which might be related to the preference of this group for a more arid atmosphere and the existence of favorable hosts. Interestingly, the relative abundance of 'phototroph-dominated' protist taxa was high in winter in the *Sophora* forest, which might be related to the fact that plant photosynthesis and consequently plant exudates are less abundant in the cold season, and this group of protists try to compensate for the paucity of root exudates to fix carbon and soil nutrients [80]. The results of predictive tools such as Tax4Fun, FUNGuild, and PICRUSt2 showed that the abundance of gene families differed seasonally for bacteria, fungi, and protists in each plantation plot, suggesting that a given microbial lifestyle might play unique roles within different plantations at different seasonal stages.

Soil microbial co-occurrence network complexity under seasonal successions and in different plantations

The segregated soil biota co-occurrence network patterns and their topological characteristics in each plantation plot showed obvious seasonality (Fig. 5, Supplementary Table 14). Our results showed that the spring microbial network was complex in comparison with that of other seasons. The upward shift in network complexity from one season to another may have happened because of, or was induced by, several biotic or abiotic factors that generated an adaptive response in soil microbiomes seasonally. More specifically, many factors might contribute to this intricacy, such as controlling the temporary partitioning of nutrients between soil biota and plants due to the rapid alterations in microclimate from winter to spring, which consequently lead to transitions in some groups of soil microbiomes, abiotic stresses (wet-dry and freeze-thaw cycles), and the consumption of labile C composites, which drives the turnover of microbial community attendants to release labile N for plant uptake [42]. The increased network complexity in spring can indirectly be attributed to an increase in soil thermal variability and increased resource availability in spring that foster microbial diversity and network complexity. It has been highlighted that network complexity is closely related to stable ecosystem functioning [81]. Furthermore, spring consisted of nodes with high BC values (Supplementary Table 15), indicating the importance of the control potential that an individual node exerts over the interactions of other nodes

[82]. These complex interactions in spring might consequently enhance system durability and resistance to adverse environmental conditions such as wet-dry and freeze-thaw cycles with changing seasons [83]. Additionally, the soil microbiome networks could be used to visualize the scenarios in which the highest percentage of links among soil biota was observed between bacteria and protists (spring), indicating that bacteria and protist taxa were tightly linked within the microbiomes (Supplementary Table 14). It has also been shown that protist communities create a dynamic hub in soil biota [34, 84]. As bacteria and fungi are principal prey for phagotrophic and/or consumer protists as the dominant soil protist functional group in soils [12, 13, 77, 85, 86], biotic interactions within the soil microbiome can influence protist diversity. The taxon-specific manner of protist taxa was observed for their potential microbial prey by detecting different and/or specific links between bacteria/fungi and protist lineages. These results are consistent with previous studies showing that protist taxa selectively graze on fungal or bacterial lineages [34, 77, 87]. Interestingly, however, we observed changeable connections of some protist groups with different microbiome taxa in different seasons and the different plantations, suggesting that the substitution of preferable feeding impacts the predator composition structure [86]. The network results showed that the young plantation forests induced more positive interactions (mutualism or commensalism) than negative interactions (competitive) in each plantation type and even in different seasons, suggesting that cooperative interactions might play a key role in shaping microbial interactions and structures in different plantations seasonally. This might correspond to stable ecosystem functioning [11]. However, competitive interactions do not necessarily match unstable and/or poor ecosystem functioning [88]. Whether these positive and negative interactions mutually impact the microbial network assembly seasonally needs to be further assessed. The association of environmental variables with co-occurrence network parameters (Fig. 5; Supplementary Figure 17) showed that the assembly of the ecological network was shaped by several vital parameters, among which temperature appeared to be the strongest. This striking observation is consistent with the pervious study, which highlighted that microbial co-occurrence networks are mainly modulated by temperature, followed by precipitation, soil nitrogen, latitude, and plant diversity [89].

Diversity-function-assembly relationship with seasonality

Our results suggest that different afforested plantations changed ecosystem functioning seasonally, consistent with previous finding [60]. We found a positive relationship between microbial diversity and composition and multifunctionality in plantation forests seasonally, corroborating the positive biodiversity-ecosystem function relationships. These results are in line with recent studies on the northeastern and central Chinese Tibetan Plateau at the local scale [90] and the global scale [91]. First, these outputs suggest that microbial diversity and composition have a leading role in maintaining ecosystem functioning [36]. Second, this association indicates high functional redundancy in soil biotas [92]. Previous study highlighted that functional redundancy is a part of microbial communities [93], and the functional redundancy of the microbial communities may explain why microbial taxonomic and functional gene diversity were correlated with different environmental variables in our study. Therefore, any changes in microbial diversity resulting from both soil biotic and abiotic factors might influence the soil's multifunctionality, suggesting that the estimation of the causal association between microbial

diversity and ecosystem functioning would be complex [94]. Furthermore, our results suggested that broad functions, such as net soil multifunctionality, may be more functionally redundant and thus better buffered against microbial shifts that are caused by seasonality in different plantation types or under other biotic and abiotic disturbances.

The null model supports the notion that distinct assembly processes drive the structure of different soil microbiomes, supporting our main hypothesis. Deterministic processes, particularly variable selection, tended to be more critical in shaping the assembly of the soil bacterial communities. In contrast, stochastic processes dominated the soil fungal and protist community assemblies, with dispersal limitation playing a more critical role in both plantation types. A similar finding was recently reported for the assembly of the soil bacterial community [4, 95]. In accordance with the present results, previous study demonstrated that stochastic processes are more important than deterministic processes for microbial community assembly at small scales [96]. Moreover, our results showed that seasonality played a decisive role in mediating the balance between stochastic and deterministic processes and showed a significant association with the diversity of soil microbiomes seasonally. The soil bacterial community assembly was governed by both deterministic and stochastic processes, with deterministic processes exerting a more substantial influence than stochastic processes. However, the relative importance of deterministic processes versus stochastic processes in bacterial community assembly varied between the different plantations, with seasonal variations in the *Pinus* forest. More specifically, seasonal transition in particular plantations markedly diminished the relative importance of homogenous selection and increased dispersal limitation of the bacterial community assembly in winter in the *Pinus* forest, corresponding to significantly lower bacterial Shannon diversity (Fig. 2), higher bacterial gene copy numbers (Supplementary Figure 11), the association of the bacterial Shannon diversity with soil pH (Fig. 3) and the bacterial βNTI value (Supplementary Figure 34), suggesting seasonality significantly increased the importance of stochastic processes, specifically in the bacterial community with stronger deterministic assembly. This result is likely related to the seasonal transition leading to the selection of a particular group of soil microbiomes and influencing several soil edaphic conditions. Thus, selected microbes may have eminent potential to increase functions connected to nutrient stocks and organic matter decomposition and to decrease functional genes involved in CNPS cycling in the *Pinus* forest in winter (Supplementary Figure 27), which was associated with a shift in the deterministic process to the stochastic process for the bacterial community with the transition of season from autumn to winter for this plot (Fig. 6). In accordance with the present results, previous studies have demonstrated that determinism-dominated assembly processes generally selected limited taxa, which led to limited stress and perturbance tolerance [97]. Therefore, we propose that microbial communities such as bacteria, which were characterized by the highest gene copy numbers and the lowest Shannon diversity in winter, are potentially more susceptible to the assembly transition from deterministic to stochastic with seasonal variations. In contrast, microbial communities such as fungi and protists with stochastic assembly are potentially more resistant to the assembly transition in alkaline soils seasonally. Thus, the fluctuation of the microbial assembly is ultimately beneficial for ecosystem stability.

Studies have determined that environmental variables (such as air temperature, soil pH, and moisture) and habitat heterogeneity are vital determinants of community assembly [4, 98–101]. We observed that soil pH was positively correlated with the soil bacterial Shannon diversity. Therefore, the decrease in the soil bacterial Shannon diversity in winter (Fig. 2) may be associated with the decrease in soil pH (Supplementary Table 1), suggesting that the fluctuation of soil pH in alkaline soil exerted substantial effects on the bacterial community assembly compared with the fungal and protist communities. Soil pH is regarded as a crucial environmental factor that shapes bacterial community assembly processes in agricultural soils [98]; specifically, acidic soil led to the stochastic assembly of the bacterial community. In contrast, it has been reported that the dominant process for the bacterial community was homogenous selection in more acidic and alkaline soils, whereas stochastic assembly processes dominated at close-to-neutral pH in nonagricultural soils [99]. We speculate that in alkaline soil with low habitat heterogeneity (monoculture plantation), the bacterial community assembly may be driven by deterministic processes with a high possibility of seasonal influence. By contrast, fungal and protist communities are more likely to be driven by stochastic processes, suggesting that stochastic processes may be more vital for soil microbial communities [96].

Conclusions

The results predicted the significant association of temperature with co-occurrence network parameters and soil enzymes, suggesting first the impact of temperature as the main aspect of seasonal variations in soil biota occurrence, and second, that in any future global change scenario such as climate warming, the microbial interactions and functioning ecosystem will likely be greatly affected, at least in alkaline soils. The protist community composition was uniquely structured with C-related functional activities (lignin-degrading enzymes, C-degradation and C-fixation) relative to bacterial and fungal β-diversity variations, which were mostly explained by seasonal variations (Supplementary Figure 36). Our study highlighted the importance of the protist phagocytosis process on soil microbial interactions through the predicted impact of protist α-diversity on microbial cooccurrence network parameters. This association might be driven by the high abundance of protist consumers as the main predator of bacterial and fungal lineages in our sampling plots. Some functional categories, such as nutrient stocks and functional groups involved in the CNPS cycling genes, were significantly associated with the microbial cooccurrence network parameters, suggesting that the abundance of this functional group can be partly driven by microbial interactions. Bacterial communities were deterministically (variable selection and homogenous selection) structured, whereas the stochastic process of dispersal limitation was mainly responsible for the assembly and turnover of the fungal and protist communities. Additionally, we showed that winter triggered an abrupt transition in bacterial community assembly from a deterministic to a stochastic process in the *Pinus* forest that was closely associated with a reduction in the bacterial Shannon diversity, with the pattern of a high level of nutrient cycling (nutrient stocks and organic matter decomposition functional categories), suggesting that the bacterial community with deterministic assembly is potentially more susceptible to the assembly transition with seasonal fluctuations in diversity and soil pH. This study contributes local-ecosystem prospects to model the behavior of soil

biota seasonally and their implied effects on soil functioning and microbial assembly processes, which will benefit global-scale afforestation programs by promoting novel, precise and rational plantation forests for future environmental sustainability and self-sufficiency.

Materials And Methods

Study area and soil sampling

The Xiong'an New Area (38°43ʹ-39°10ʹ N, 115°38ʹ-116°20ʹ E), which includes three counties (Xiong, Anxin, and Rongcheng), is located in the north of China, which was established in 2017, and it is another new national city after the Shenzhen Special Economic Zone and Shanghai Pudong New District [9] (Supplementary Figure 35). Between 2017 and 2020, Xiong'an added more than 27,000 hectares of trees, increasing its forest coverage to 30 percent (http://www.xinhuanet.com/english/2021-04/01/c_139852880.htm). This area is classified as having a warm temperate continental monsoon climate with four distinct seasons. The average annual temperature and precipitation are approximately 12.1°C and 560 mm, respectively [9, 11]. The soil samples were collected from a *P. tabulaeformis* plot (Chinese pine tree, hereinafter referred to as 'CP', with an approximate size of 3,706.01 m^2) and an *S. japonica* plot (Chinese scholar tree, hereinafter referred to as 'CS', with an approximate size of 4,114.78 m^2) in plantation forests with a history of planting either *Zea mays* L. or *Triticum aestivum* L., followed by the closing of the land for afforestation. These species were sampled one year post afforestation age, and the approximate age of the trees at the time of sampling was four years. The time frame for soil sampling was arranged considering four different seasons. Seasonal samplings were performed as follows: in July 2019 (summer: plant growth period and rhizodeposition; hereinafter referred to as 'SU'), in October 2019 (autumn; during the late phase of litterfall; hereinafter referred to as 'AU'), in January 2020 (winter; snow-covered time and carbon polymers/phenolics; hereinafter referred to as 'WI'), and in May 2020 (spring: three weeks after the emergence of leaves; hereinafter referred to as 'SP'). The time-scale sampling was selected according to the weather climate of the northern part of China. Sampling details were followed according to our previous studies [9, 102]. Briefly, three random lines were randomly selected with the distance between each line at approximately 100 m, and by walking along each line, a soil core was collected every 5 m. The resulting soil cores were mixed to yield a composite sample from each line; three samples were collected per plot for each season. Thus, we collected 120 soil subsamples at depths of 15-30 cm in the root-zone soil (24 composite soil samples) from two defined experimental plots over one year. After each set of sample collections, visible grass roots and pebbles were removed. All soil samples were divided into two parts; the first part (~ 10 g) was immediately frozen at -20°C using a portable refrigerator (Foshan Aikai Electric Appliance Co., Ltd, Guangdong, China) for DNA extraction and was stored at -80°C after the samples were transferred to the laboratory. The second part (~ 500 g) was transferred at an approximate temperature of 22 ± 2°C for samples collected in spring, 29 ± 2°C in summer, 12 ± 2°C in autumn, and 4 ± 2°C in winter and was stored at 4°C for geochemical measurements at the molecular ecology laboratory at Hebei Normal University.

Soil Physicochemical Properties And Extracellular Enzyme Activities

The soil water content (SWC), total carbon (TC), soil electrical conductivity (EC), soil pH, soil organic matter (SOM), total potassium (TK), rapidly available potassium (RAK), slow-available potassium (SAK), total phosphorus (TP), total nitrogen (TN), soil hydrolyzed nitrogen (HN), inorganic phosphorus (IP), organic phosphorus (OP), available phosphorus (AP), total sulfur (TS), and available sulfur (AS) were determined, and the detailed methods can be found in Supplementary Table 18. The activities of ten soil enzymes involved in hydrolytic activities, such as carbon (β-glucosidase, BGC; β-xylosidase, BXYS; β-cellobiosidase, BCL), nitrogen (leucine aminopeptidase, LAP; ⍺-glucosidase, AGC; N-acetyl-β-D-glucosidase, NAG), phosphorus (alkaline phosphatase, ALP), sulfur (aryl sulfatase, ASF), and oxidative activities, such as phenolic compound oxidase, including polyphenol oxidase (PPO) and peroxidase (POD), were determined using soil enzyme kits purchased from Suzhou Comin Biotechnology Co., Ltd. (Suzhou, Jiangsu, China) (Supplementary Table 19). The formulas ln (BGC): ln (NAG + LAP), ln (BGC): ln (ALP), and ln (NAG + LAP): ln (ALP) were used to calculate the soil ecoenzymatic C:N, C:P, and N:P activity ratios, respectively, to gain a better understanding of possible resource shifts with seasonal variations.

Dna Extraction And Amplicon Sequencing

Soil genomic DNA extraction and quality checking were performed according to our previous publications [9, 11]. PCR amplification and sequencing were individually performed for each replicate. The abundance of soil microbial communities was estimated using PCR amplification techniques. Target genes were bacterial 16S rRNA, fungal ITS, and protist 18S rRNA. The primers 338F/806R, 1737F/2043R, and TAReuk454FWD1F/TAReukREV3R were used to amplify the V3-V4 region of the bacterial 16S rRNA gene, fungal ITS1, and 18S rRNA, respectively, by an ABI GeneAmp® 9700 PCR thermocycler (ABI, CA, USA). PCRs, amplifications, and sequencing were performed in the framework of our previous studies [9, 11, 102].

Quantitative Real-time Pcr (Qpcr) And High-throughput Quantitative Pcr (Ht-qpcr)

According to our previous protocol [11], the 7300 Real-Time PCR System (Applied Biosystems, California, USA) was used to determine the gene copy numbers of soil biotas for each soil sample. The details can be found in Supplementary Table 20. The abundance and diversity of the functional genes involved in C, N, P, and S cycling in different plantation plots and seasons were estimated using QMEC based on HT-qPCR [22], which enabled the simultaneous qualitative and quantitative determination of 72 genes (Supplementary Table 21) on the WaferGen Smart-Chip Real-time PCR system (Bio-Rad, Herculers, CA, USA).

Bioinformatics And Statistical Analysis

The details of the bioinformatics and statistical analysis are provided in the additional file1.

Abbreviations

SWC, Soil water content; TC, total carbon; EC, soil electrical conductivity, SOM, soil organic matter, TK, total potassium, RAK, rapidly available potassium, SAK, slow-available potassium, TP, total phosphorus, TN, total nitrogen, HN, soil hydrolyzed nitrogen, IP, inorganic phosphorus; OP, organic phosphorus, AP, available phosphorus, TS, total sulfur; AS, available sulfur; BGC, β-Glucosidase; BXYS, β- xylosidase; BCL, β-cellobiosidase; LAP, Leucine Aminopeptidase; AGC, ⍺-Glucosidase; NAG, N-acetyl-β-D-glucosidase; ALP, Alkaline phosphatase; ASF, Arylsulfatase; PPO, Polyphenol oxidase; POD, Peroxidase; QIIME, quantitative insight into microbial ecology; OTU, operational taxonomic units; RDP, ribosomal database project; PR2, protist ribosomal reference; BC, betweenness centrality; NMDS, non-metric multidimensional scaling analysis; ANOSIM, analysis of similarity, PERMANOVA, permutational multivariate analysis of variance; RDA, redundancy analysis; GAM, generalized additive models; PLS-PM, partial least-square path model; GoF, Goodness of fit; EEA, ecoenzymatic activity; EES: ecoenzymatic stoichiometry; LDA & LEfSe, linear discriminant analysis (LDA) effect size (LEfSe); KEGG, kyoto encyclopedia of genes and genomes; ND, network nodes; NG, network edges; AD, average degree; APL, average path length, ML, modularity; ACC, average clustering coefficient.

Declarations

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Authors' contributions

All authors contributed intellectual input and assistance to this study and manuscript. MW, AM, and CW designed the project, carried out the experiments, and data analysis; LZ and JY collected the samples and data analysis; MW, and AM discussed the results and wrote the manuscript; ZY, and JL designed the project, supervised the research and edited the manuscript.

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Availability of data and materials

The raw reads were deposited into the NCBI Sequence Read Archive (SRA) database under BioProject IDs PRJNA688619, PRJNA688619, and PRJNA688645 for bacteria, fungi and protists, respectively.

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Competing interests

The authors declare that have no competing interests.

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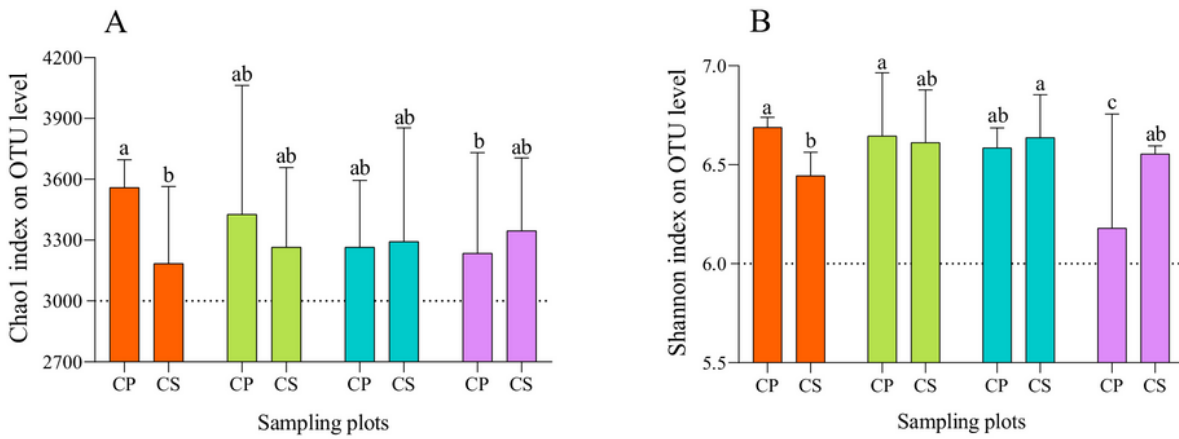
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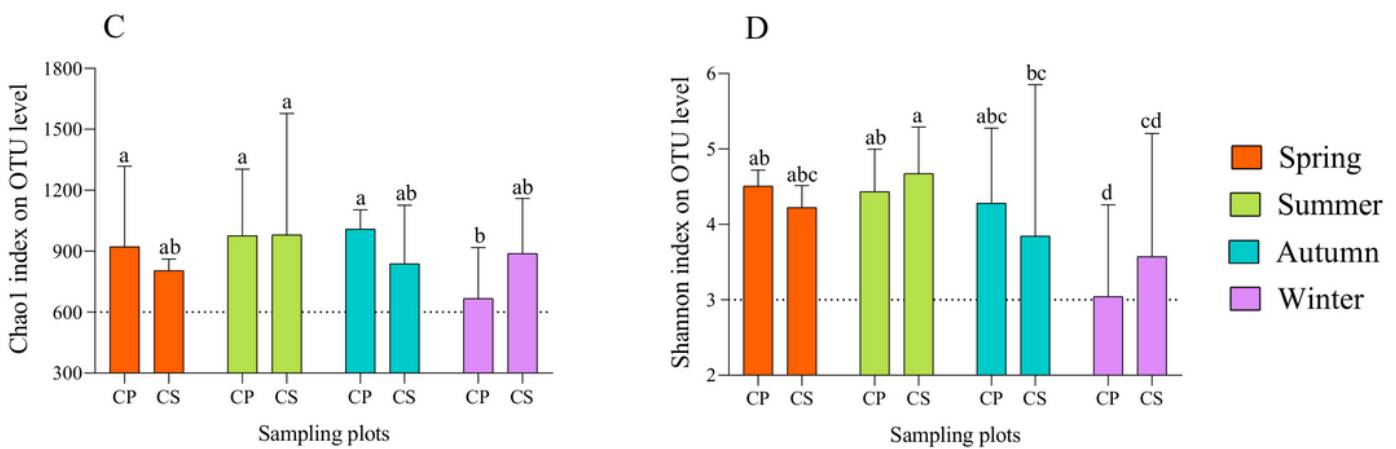
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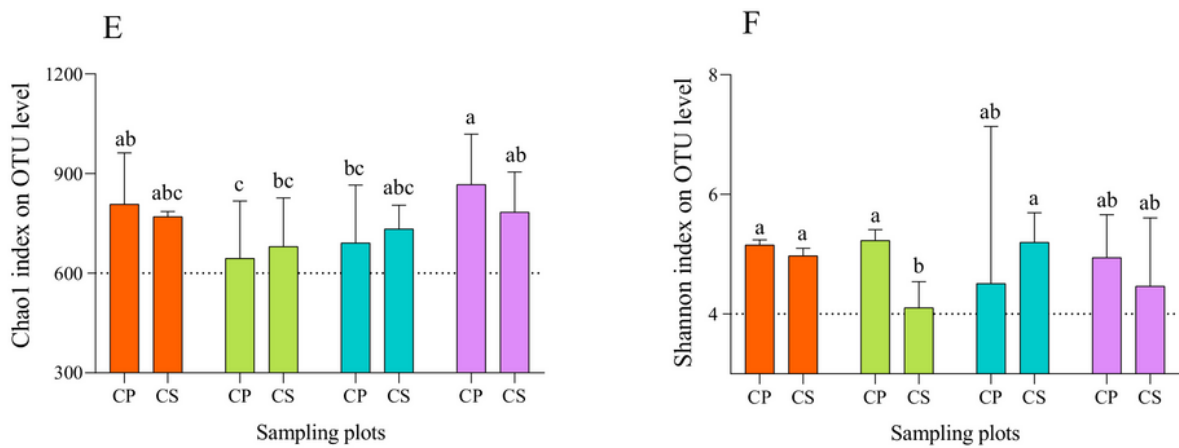
Figures

Figure 1

Alpha diversity indices of soil biota retrieved from two different forest types seasonally. Bacteria (A, B), fungi (C, D), and protists (E, F). Abbreviations: CP (*P. tabulaeformis*), and CS (*S. japonica*).
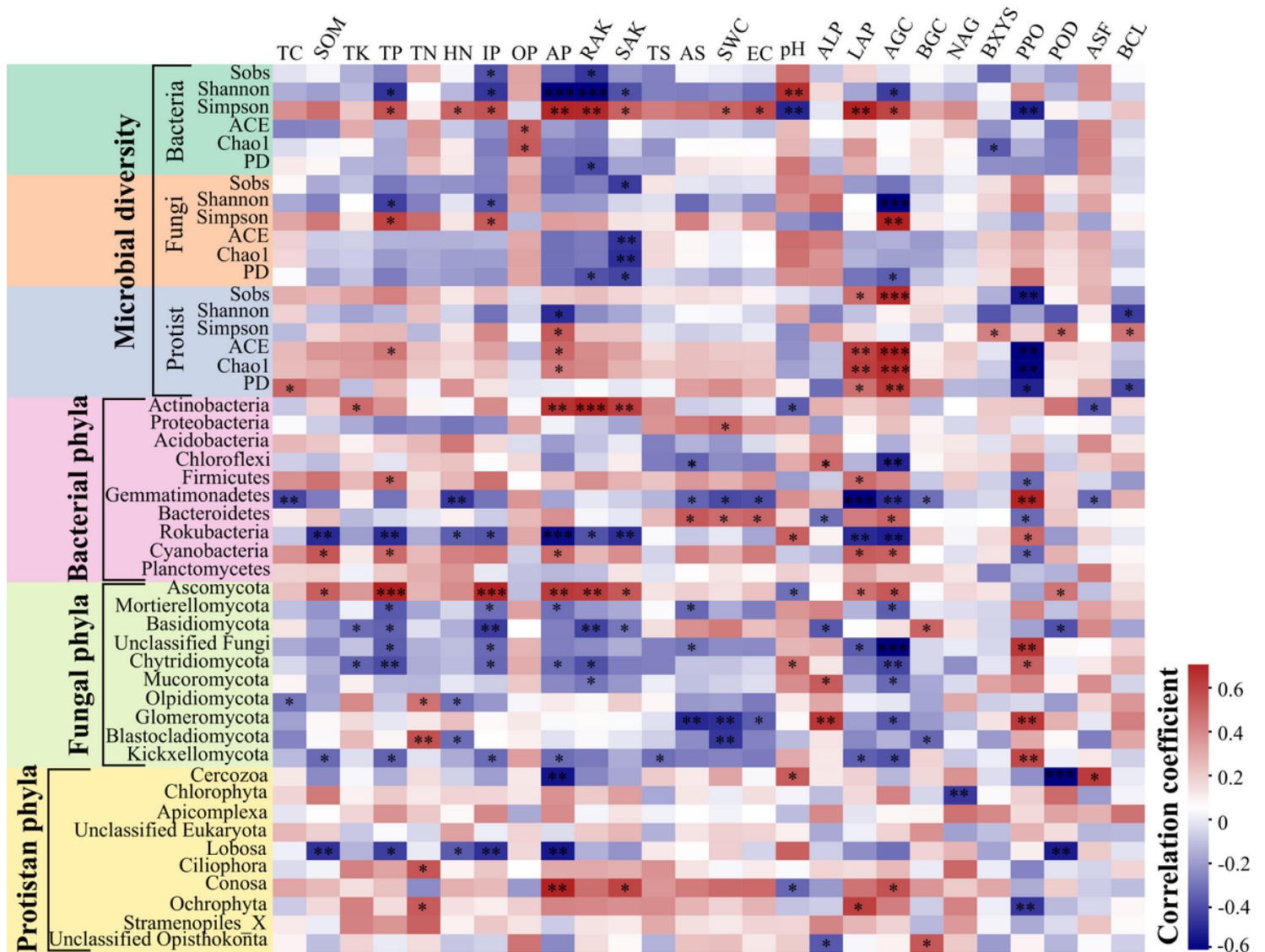
Figure 2

Spearman analysis. This analysis evaluated correlation ships between the relative abundance of dominated phyla, and microbial diversity indices with edaphic physicochemical variables and soil enzymes. The red and blue colors indicate positive and negative correlations, respectively. The significances of correlation analyses are marked with asterisks (*) at different significance levels (*P < 0.05, **P < 0.01, and ***P < 0.001).
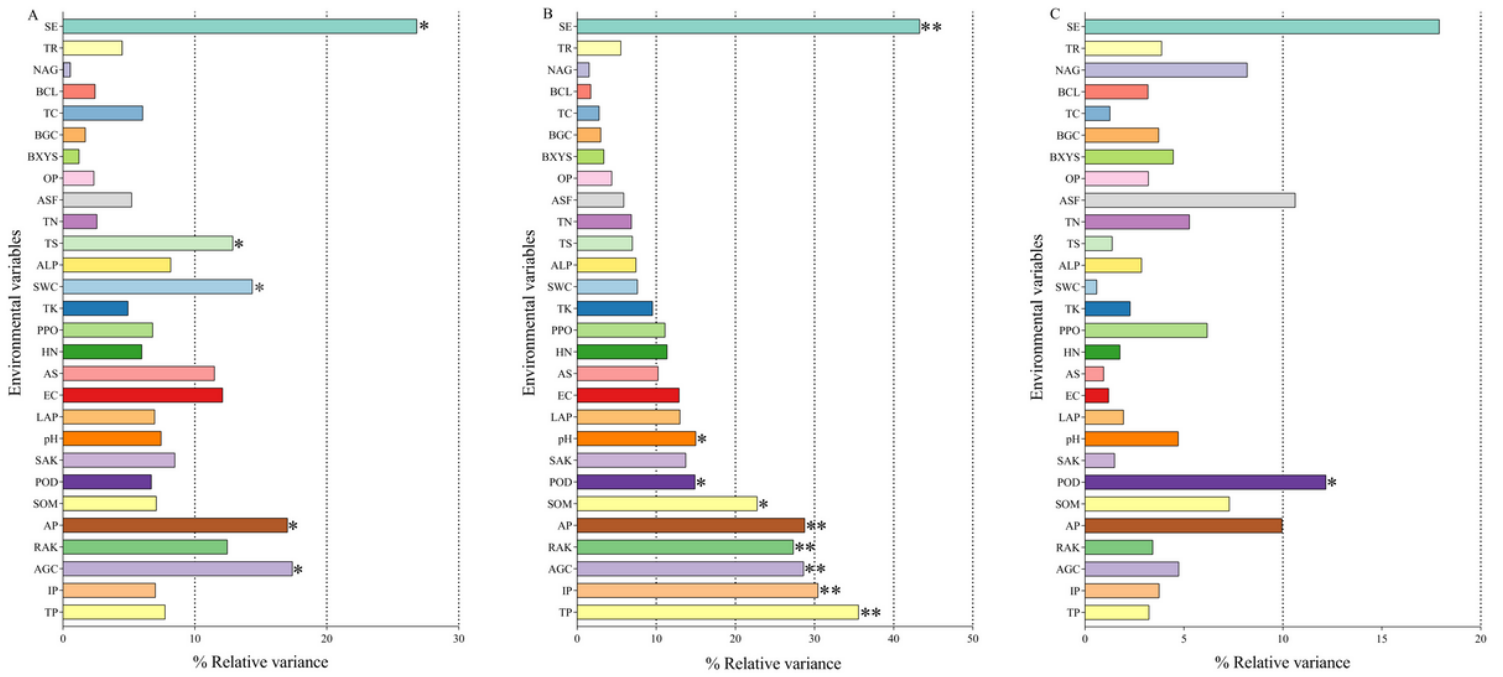
Figure 3

The PERMANOVA analysis. The impact of environmental variables on bacterial (A), fungal (B), and protist community compositions using the PERMANOVA test (Bray-Curtis distance matrix). Values less than 0.05 were considered significant.
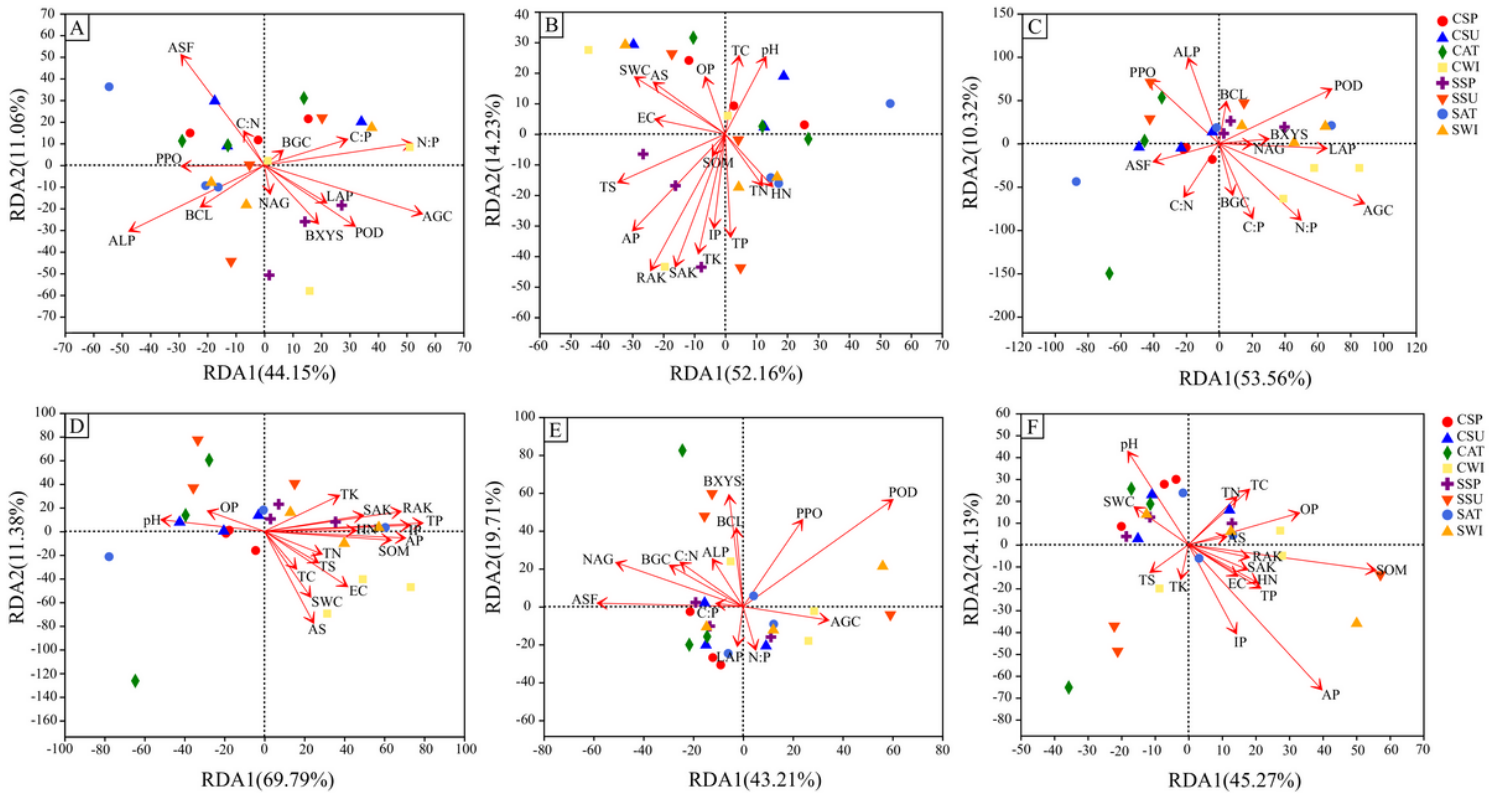

Figure 4

Redundancy analysis (RDA) of microbiota compositions at the phylum level. RDA used to establish the linkage of bacterial (A: soil enzymes, B: soil properties), fungal (C: soil enzymes, B: soil properties), and protist (E: soil enzymes, F: soil properties) community compositions with soil enzymes and soil properties.
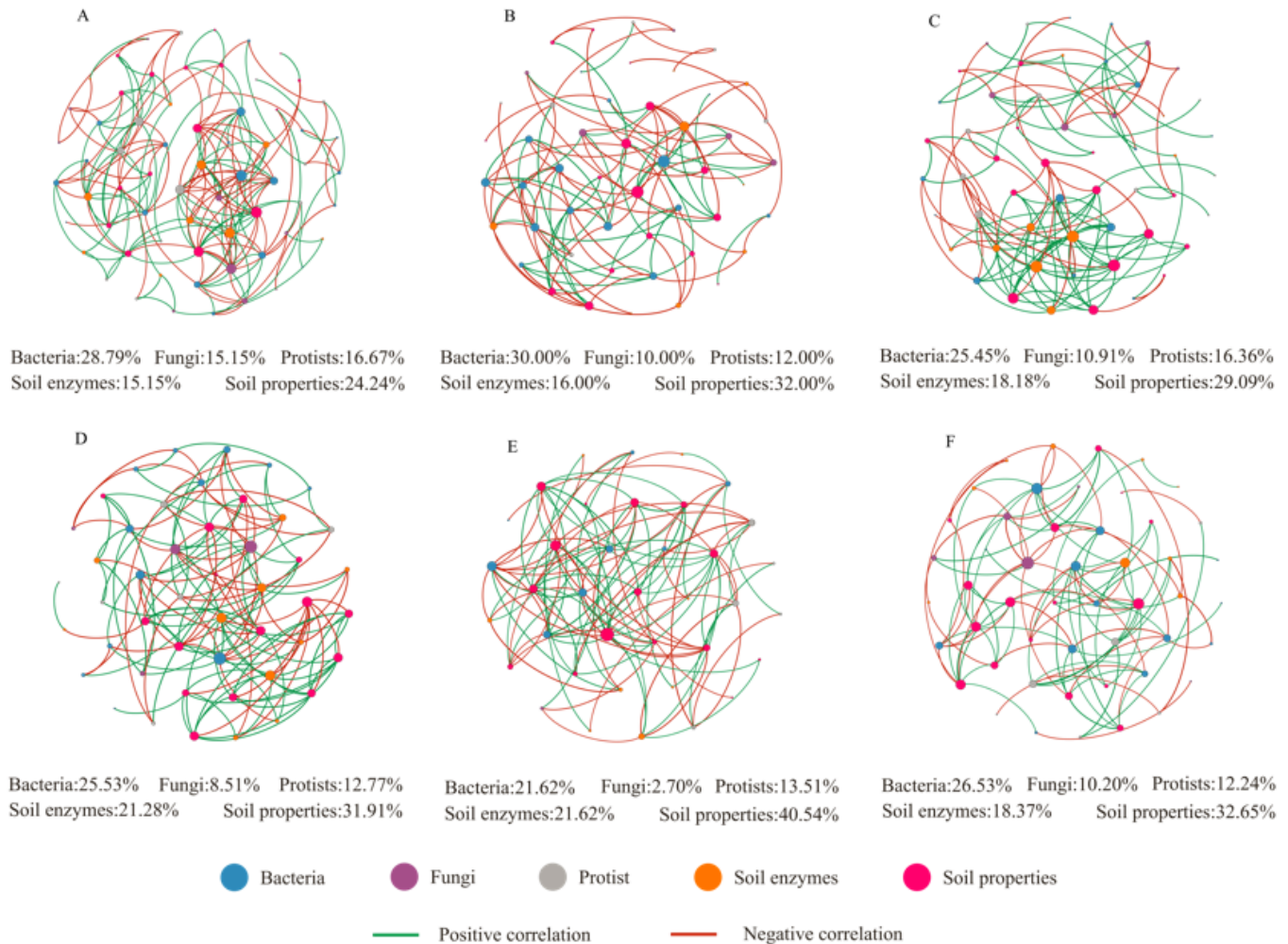

A

Bacteria:28.79% Fungi:15.15% Protists:16.67%
Soil enzymes:15.15%   Soil properties:24.24%

B

Bacteria:30.00% Fungi:10.00% Protists:12.00%
Soil enzymes:16.00%   Soil properties:32.00%

C

Bacteria:25.45% Fungi:10.91% Protists:16.36%
Soil enzymes:18.18%   Soil properties:29.09%

D

Bacteria:25.53% Fungi:8.51% Protists:12.77%
Soil enzymes:21.28%   Soil properties:31.91%

E

Bacteria:21.62% Fungi:2.70% Protists:13.51%
Soil enzymes:21.62%   Soil properties:40.54%

F

Bacteria:26.53% Fungi:10.20% Protists:12.24%
Soil enzymes:18.37%   Soil properties:32.65%

● Bacteria   ● Fungi   ● Protist   ● Soil enzymes   ● Soil properties

— Positive correlation   — Negative correlation

Figure 5

Microbial co-occurrence networks. Bacterial, fungal and protistan co-occurrence networks (at the family level) with environmental variables in the Pinus forest (A), in the Sophora forest (B), at spring (C), at summer (D), at autumn (E), and winter (F). The lines indicate interlinkage between OTUs. The green lines indicate a positive linkage and the red lines indicate a negative linkage between OTUs.
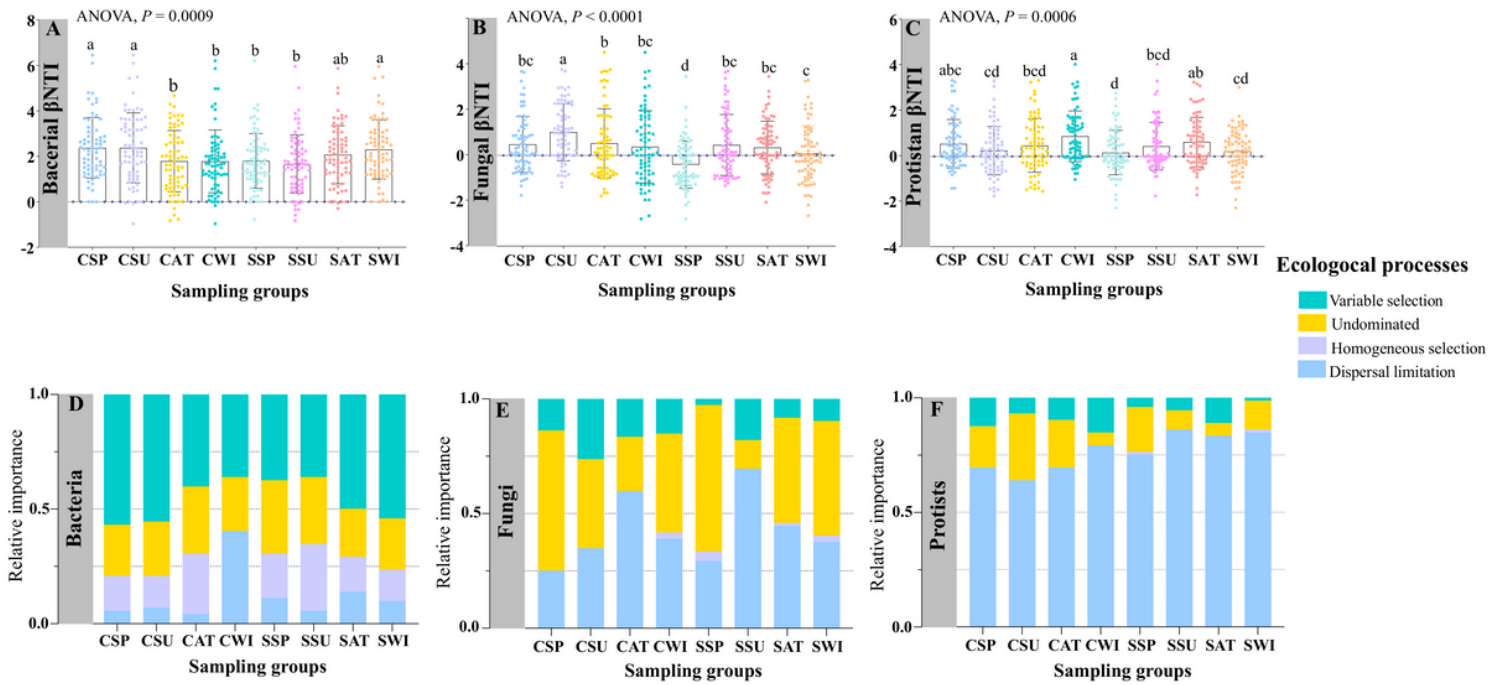
Figure 6

The microbial community assembly processes across two forest types seasonally. The values of the beta nearest taxon index (βNTI) for soil bacterial (A), fungal (B), and protistan (C) communities present. The upper and lower significance thresholds for the βNTI index was + 2 and - 2, respectively. The relative turnover in soil bacterial (D), fungal (E), and protistan (F) community assemblies, governed principally by deterministic processes (homogeneous and variable selections), stochastic processes (dispersal limitation and homogenizing dispersal), or undominated process. Bars with different lowercase letters indicate significant differences within the microbial taxa phyla (P < 0.05) across two forest types with seasonal variations, as revealed by one-way ANOVA with Turkey's post hoc test.
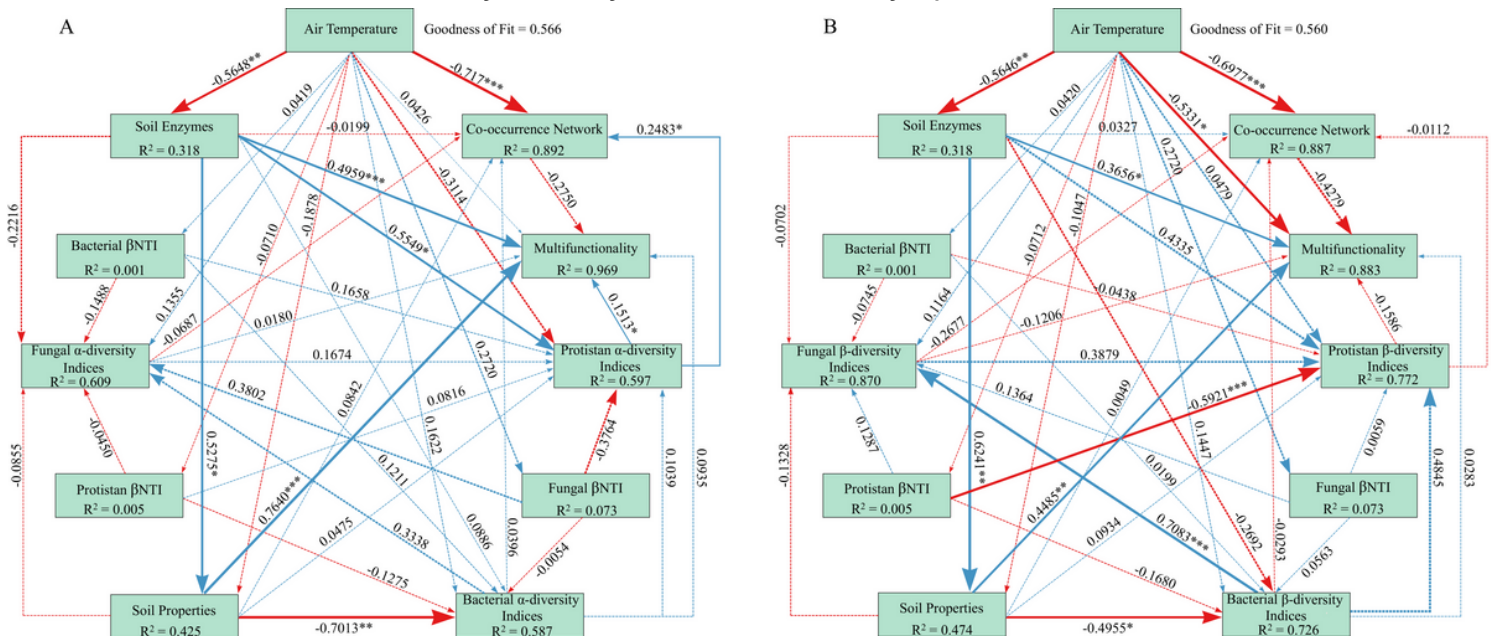

Figure 7

PLS-PM diagram indicating the model behind the relation among latent variables and their manifest variables. 'temperature', 'soil enzymes', soil properties', microbial α-diversity indices', 'microbial co-occurrence network parameters', microbial βNTI values, and multifunctionality indices refer to the latent variables (inner model), and small rectangles refer to manifest variables (outer model) based on microbial α-diversity indices (A), and microbial β-diversity indices. Blue and red arrows represent positive and negative path coefficients, respectively. The width of arrows is proportional to the strength of path coefficients. Continuous and dashed arrows indicate significant and non-significant associations, respectively. Numbers associated with lines indicate path coefficients. R2 denotes the proportion of variance explained, which was calculated after 999 bootstraps. The significances of correlation analyses are marked with asterisks (*) at different significance levels (*P < 0.05, **P < 0.01, and ***P < 0.001).

Supplementary Files

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