Structural and Functional Characteristics of Soil Microbial Community in a Pinus Massoniana Forest at Different Elevations

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Abstract

Background

In spite of the ecological importance of soil microbial communities, very little data is available on different soil microorganism types along altitudinal gradients on a mountain system with a forest ecosystem to identify the distribution patterns of soil microbial diversity and function. This study aimed to determine the diversity patterns of different soil microorganism types (archaea, bacteria, fungi, viruses) along an altitudinal gradient on Mt. Leigong, a typical mid-subtropical mountain forest ecosystem.

Results

The richness of bacterial, fungal, and virus community changed in a unimodal pattern, while archaeal community changed in a bimodal pattern with increasing altitude. Euryarchaeota and Thaumarchaeota, Proteobacteria and Acidobacteria, Ascomycota and Basidiomycota, Myoviridae and Podoviridae were abundant taxa in archaeal, bacterial, fungal, and virus community, respectively. Amino acid transport and metabolism, and energy production and conversion were the predominant categories as per Nonsupervised Orthologous Groups (NOG) function gene-annotation. Carbohydrate metabolism, global and overview map, and amino acid metabolism, were predominant categories in the Kyoto encyclopedia of genes and genomes-based (KEGG) pathways. Glycosyl transferase and glycoside hydrolase were predominant categories among carbohydrate enzyme-functional genes. Cluster analysis, redundancy analysis, and Network analysis showed obvious differences in composition, structure, and function of different soil microorganism types in the *P. massoniana* forest on Mt. Leigong.

Conclusions

These results indicate that different soil microorganism types (archaea, bacteria, fungi, viruses) along the altitudinal gradient have an obvious different distribution patterns in the typical mid-subtropical mountain coniferous forest soil.

1. Introduction

Natural vertical gradients associated with altitude in mountain ecosystems have attracted great interest from ecologists around the world because these habitats show characteristics of altitudinal gradients, whereby rapid changes in climate and biological characteristics within a short geographic range are commonly observed (Siles & Margesin 2016). For example, if ambient temperature decreases with increasing altitude, precipitation, solar radiation, and atmospheric pollution deposits change due to the changing elevation of the terrain, whereby complex ecological changes occur along the altitude gradient (Körner 2007). Therefore, the mechanism underlying changes in species richness along an elevation gradient has always been a controversial issue in ecological and biogeographic studies. Although there are reports of diminishing patterns or single-peak patterns for animal and plant mechanisms responsive to elevation, such differences may show stronger variation among different regions in the case

microorganisms, including no trend, a decline, a single peak, or a concave or other distribution models associated with changes in altitude (Siles & Margesin 2016; Liu et al. 2018). In recent years, research on altitudinal patterns of microbial diversity (i.e., bacteria, fungi, ectomycorrhizal fungi, etc.) has gradually increased by using high-throughput sequencing technology (Jarvis et al. 2015; Wang et al. 2015; Geml et al. 2017; Liu et al. 2018; Schoen et al. 2018).

Soil microorganisms are important biological components associated with aboveground interactions of terrestrial ecosystems (Bahram et al. 2018), with microorganisms being key drivers of litter decomposition, plant growth, soil nutrient cycling and biogeochemical process (van der Heijden & Wagg 2013; Bardgett & van der Putten 2014; Schlöter et al. 2018; Thakur & Geisen 2019). Due to the importance of forest soils as both sinks and potential sources of carbon and their predicted sensitivity to climate change, microbial ecologists have been struggling to indicate that what is the interaction mechanism between environment factors (topography, climate, vegetation and soil) and soil microbial communities (diversity and function) in forest ecosystem (Lladó et al. 2018). Topography, as a long-term constant factor, have a significant impact on ecosystem dynamics, which may lead to changes in soil microbial communities (Qiu et al. 2012; Zhang et al. 2013; Tajik et al. 2020). From a biological perspective, topographic parameters can provide accurate and valuable information that is useful for predicting the distribution of biodiversity of soil microbial communities (Lladó et al. 2018; Tajik et al. 2020). Therefore, promoting the study of the mechanism of distribution of soil microorganisms along altitude gradients in mountain ecosystems should prove useful for a scientifically based management practice of mountain ecosystems.

Mountainous landforms are very common in Southwest China, which increases habitat heterogeneity while providing an important foundation for biodiversity richness (Myers et al. 2000). *Pinus massoniana* is an important native tree species and the most widely distributed pine tree in Southern China, with strong adaptability and tolerance to harsh environments (Lei et al. 2018). *P. massoniana* forest is the main vegetation type with key ecological and economic importance (such as vegetation construction, timber, resin, etc.) in Southern China. Masson pine forests are distributed along elevation gradients which provide natural experimental locations for trying to elucidate the elevation-associated mechanism of soil microbial-communities distribution. In this paper, soil microorganisms of the *P. massoniana* forest at different altitudes in Leigong Mountain, Guizhou Province, China, were selected as the research object. Species diversity composition and function of soil microbial communities were analyzed by Illumina Meseq metagenome sequencing technology in order to provide a scientific basis for the ecological management and utilization of Masson pine forests in the region.

2. Materials And Methods

2.1 Site Description

Leigong Mountain Nature Reserve (26°20'25" - 26°25'00" N; 108°12'00" - 108°20'00" E) was established as a provincial Nature Reserve in 1982, and was promoted to National Nature Reserve in 2001. Leigong

Mountain (2178.8 m a.s.l.) is the main peak among the Miaoling Mountains, which constitute the drainage divide between the Pearl River basin and the Yangtze River basin. With 83% forest coverage, this mountain range is an ideal area to study the typical mid-subtropical mountain forest ecosystem (Zhang et al. 2020). Leigong Mountain belongs to the humid climatic region of the mid-subtropical monsoon mountainous region, with a characteristic warm and humid climate with abundant rainfall, many rain and fog days, and low light levels. The annual rainfall is 1300~1600 mm; the annual average temperature is about 9.2 and 16.3 °C at the top and at the foot of the mountain, respectively, with an annual average temperature drop rate of 0.46 °C per 100 m. The soils are mainly acidic mountain yellow soils and mountain yellow brown soils, with a deep soil profile. The boundary between these two soil types lies at approximately 1300 m a.s.l. The zonal vegetation belongs to the humid evergreen broad-leaved forest in the eastern mid-subtropical zone, where it is distributed below 1350 m, while the mountain evergreen deciduous broad-leaved mixed forest is distributed between 1350 and 2100 m a.s.l., above which the alpine shrub is observed.

2.2 Study Site and Soil Sampling

In June 2019, four similar points were selected along an altitudinal gradient across the *P. massoniana* forest in Leigong Mountain: namely, 1000, 1200, 1400, and 1600 m a.s.l.—Mp1000, Mp1200, Mp1400, and Mp1600, respectively. An artificial tea garden (Tea-garden) was selected as control at 1000 m a.s.l. One vegetation survey plot with 20 m × 20 m in size were set at each altitude. Our survey included information on site conditions, plant communities, and litter in the sample plot. Soil samples were excavated from the 0-20 cm topsoil layer at six points located along an “S” path drawn within each plot, and thoroughly mixed. Detailed information about these samples is listed in Table 1. For each sample, 500 g of soil was sieved through a 2-mm mesh and stored at 4 °C for physicochemical analyses; additionally, 50 g of soil was encapsulated in a plastic tube and kept at -80 °C before microbial DNA extraction.

2.3 Soil Analysis and Microbial DNA Extraction

Soil pH was determined at a ratio of 1:2.5 (soil to water, w/w). Soil organic carbon (SOC) was determined using the $K_2Cr_2O_7$ titration method (Walkley–Black method). Total nitrogen (TN) was measured using the semi-micro Kjeldahl method. Total phosphorus (TP) was determined colorimetrically after wet digestion with $H_2SO_4 + HClO_4$ (Xu, et al., 2014).

Total genomic DNA was isolated from 0.50 g of soil using the MoBio Ultraclean Soil DNA Isolation Kit (MoBio laboratories, Carlsbad, California, USA) following the instructions of the manufacturer. Extracted DNA was stored at -80 °C, and tenfold diluted DNA samples were used for metagenome analyses on an Illumina MiSeq platform (Illumina, San Diego, USA) according to the standard protocols of Majorbio Bio-Pharm Technology Co. Ltd. (Shanghai, China).

2.4 Metagenome Sequencing

The Illumina sequencing platform was used to perform parallel mixing and sequencing of multiple samples. The sequence in each sample was introduced with an Index tag sequence indicating sample source information. Software "Seqprep" was used to cut the 3' end and 5' the adapter sequence at the end. Software "Sickle" was used to remove the cut length less than 50 bp; the average quality value was below a certain threshold (default 20) and N-base reads; high-quality pair-end reads and single-end reads were retained (Pabinger et al. 2014; chirmer, et al. 2015). We then used fastp (<https://github.com/OpenGene/fastp>) software to perform quality control processing on the original sequencing data to obtain high-quality control data (clean data), and Megahit (<https://github.com/voutcn/megahit>) software to assemble sequences with different sequencing depths (Li et al. 2015), and to preserve sequences with assembly contigs > 300 bp. MetaGene (<http://metagene.cb.ku-tokyo.ac.jp/>) was then used to predict Open Reading Frames (ORF) of contigs $\geq$ 100 bp in the stitched result, and CD-HIT software (<http://www.bioinformatics.org/cd-hit/>) for clustering (identity $\geq$ 90% and coverage $\geq$ 90%), taking the longest gene in each class as a representative sequence to construct a non-redundant gene set (Noguchi et al. 2006). SOAPaligner software (<http://soap.genomics.org.cn/>) was used to compare the high-quality reads of each sample with the non-redundant gene set (identity $\geq$ 95%), and to count gene abundance information in the corresponding samples (Luo et al. 2012). In turn, BLASTP software (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>) was used to separate non-redundant gene sets with NR database (Non-Redundant Protein Sequence Database) (Altschul et al. 1997) and eggNOG database (Evolutionary genealogy of genes: Nonsupervised Orthologous Groups, <http://egglog.embl.de/>) (Powell et al. 2012), KEGG's Gene Database (GENES) and CAZy (Carbohydrate-active enzymes, <http://www.cazy.org/>, Version 5.0) Annotate species and functions, and set the expected e-value to $1e-5$ (Du et al. 2014; Lombard et al. 2014).

Subsequently, R language was used to count the total and unique species, functions or genes of archaea, bacteria, fungi, and virus groups in different sample-soil microbial communities, and to draw a Wayne diagram; count the archaea, bacteria, fungi, and viruses in the soil microbial community. The horizontal community histogram was based on cluster analysis of the similarity between the samples and the soil microbial archaea, bacteria, fungi, and virus groups. Similarity results of the soil microbial community among different soil samples are presented by a heatmap. Test analyses were performed to test the correlation between the soil microbial community matrix and the function matrix among different samples. The soil microbial community species-level was based on the Bray-Curtis distance algorithm for RDA analysis. The networks software was used for co-occurrence network analysis (Barberan et al. 2012). Soil environmental factors were analyzed using SPSS 22.5 One-way ANOVA statistics. Finally, we used Sigma Plot 12.5 and Adobe Illustrator software for drawing processing details.

3. Results

3.1 Composition and Structure of Soil Microbial Communities

A total of 452 526 750 original sequences were obtained from the metagenomic sequencing of soil microorganisms in the *P. massoniana* forest along the altitudinal gradient established. After quality

control, a total of 447 074 316 effective sequences were generated, and the sub-effective sequences were selected for different soil microbial groups, including archaea, bacteria, fungi, and viruses. There were 62 208 980 effective sequences and a single sample produced 9 960 236~16 161 802 effective sequences. After metagenomic sequencing and NR (non-redundant protein sequences) database species-annotation, a total of 13 307 soil microorganisms were obtained. These included 912 archaea, 11 576 bacteria, 417 fungi, and 402 virus species (Table 2).

The changes in richness and Shannon index of soil microbial groups at the different altitudes selected along the altitudinal gradient marked across the *P. massoniana* forest at the study site showed different trends (Table 3). Specifically, in terms of species richness, the abundance of soil bacteria, fungi, and viruses exhibited a "unimodal" pattern with altitude, while archaea showed a significant peak at Mp1200 (752) and a secondary peak at Mp1600 (716). The Shannon index for archaea and fungi increased with increasing altitude, whereas the Shannon index for bacteria decreased with increasing altitude. The Shannon index for viruses did not show any obvious pattern of change with altitude.

The distribution of common species and endemic species of different soil microbial groups in different sites showed significantly different profiles (Fig. 1). Common species of archaea, bacteria, fungi, and viruses accounted for 58.6%, 75.1%, 50.4%, and 7.5% of the soil microbial groups, respectively. Whereas endemic species of archaea, bacteria, fungi, and viruses accounted for 12.3%, 6.6%, 15.6%, and 54.5%, respectively. These results indicated that the spatial distribution pattern of different soil microbial groups across altitudes differed significantly. Thus, for example, archaea and fungi showed similar patterns in their distribution but there were obvious differences between those of bacteria and viruses.

Euryarchaeota and Thaumarchaeota were the predominant groups of soil archaea in the *P. massoniana* forest across the altitudinal gradient surveyed. The order of relative abundance of these two groups at the five sites was: Tea-garden (84.1%) > Mp1000 (80.3%) > Mp1200 (75.8%) > Mp1400 (75.6%) > Mp1600 (75.5%). Euryarchaeota and Thaumarchaeota were followed in relative abundance by Candidatus Bathyarchaeota, Crenarchaeota, Candidatus Thorarchaeota, Candidatus Lokiarchaeota, Candidatus Micrarchaeota, Candidatus Korarchaeota, Candidatus Parvarchaeota, Candidatus Nanohaloarchaeota and Nanoarchaeota (Fig. 2a).

Proteobacteria and Acidobacteria were the predominant groups of bacteria in the soils of the *P. massoniana* forest across the altitudinal gradient established in this study. The order of relative abundance of these two bacterial groups at the five sites was: Mp1000 (73.1%) < Tea-garden (74.0%) < Mp1200 (74.3%) < Mp1600 (76.0 %) < Mp1400 (76.2 %), with a slightly increasing trend with increasing altitude. The bacterial groups following in relative abundance were Actinobacteria, Verrucomicrobia, Chloroflexi, Planctomycetes, Cyanobacteria, Firmicutes, Bacteroidetes, Gemmatimonadetes and other bacteria (Fig. 2b).

Ascomycota and Basidiomycota were the predominant groups in the *P. massoniana* forest across the altitudinal gradient. The order of relative abundance of these two fungal groups at the five sites was: Tea-garden (89.4%) < (92.2%) < Mp1200 (93.3%) < Mp1400 (94.2 %) < Mp1600 (94.4%), with a slightly

increasing trend with increasing altitude. Thus, ascomycetes increased with elevation and their relative abundance decreased significantly; while basidiomycete showed an obvious increase with elevation; and the relative abundance of the two phylum groups has a stable upward trend with increasing altitude. The fungal groups following in relative abundance were Chytridiomycota, Glomeromycota, Blastocladiomycota, Entomophthoromycota, Cryptomycota, Microsporidia, Neocallimastigomycota and other fungi (Fig. 2c).

Myoviridae, Podoviridae, and Siphoviridae were the predominant virus groups in the *P. massoniana* forest across the altitudinal gradient established for this survey. The order of relative abundance of these viral groups at the five sites was: Mp1600 (42.1%) < Tea-garden (48.7%) < Mp1000 (49.6%) < Mp1200 (50.8%) < Mp1400 (54.5%). The groups following in relative abundance were Phycodnaviridae, Tectiviridae, Mimiviridae, unclassified_d_virus, Caudovirales and other viruses (Fig. 2d).

Clustering of different soil microbial groups (archaea, bacteria, fungi and viruses) at genus level showed significant differences and most genera present in different soil microbial groups changed highly significantly with altitude. Overall, Candidatus_Nitrosotalea, unclassified_p_Candidatus_Bathyarchaeota, Methanosarcina, Nitrososphaera, and unclassified_d_Archaea were the predominant archaeal genera (Fig. 3a), while unclassified_f_Acidobacteriaceae, Candidatus_Solibacter, Candidatus_Koribacter and Bradyrhizobium were the predominant bacterial genera (Fig. 3b). In turn, Moniliophthora, Penicillium, Oidiodendron, Colletotrichum, Aspergillus and Cladophialophora were the predominant fungal genera (Fig. 3c). Unclassified_d_Viruses, unclassified_f_Myoviridae, unclassified_f_Siphoviridae and unclassified_f_Podoviridae were the predominant virus genera (Fig. 3d). Archaea and fungal genera present in soil microbial communities showed a relatively more similar trend of change along altitude.

3.2 Functional Gene Composition and Structure of Soil Microbial Communities

The BLASTP function prediction based on eggNOG database was used to determine the soil microbial community functions in a *P. massoniana* forest at different altitudes. The main groups of identified genes corresponded to those with functions involved in amino acid transport and metabolism (7.51-7.98%), energy production and transformation (6.62-7.46%), signal transduction mechanisms (6.76-7.24%), and DNA replication, recombination, and repair (6.46-7.16%). These were the dominant gene categories and, clearly, these functions are important for energy production and cell biology. In addition, a group of genes with unknown functions accounted for 30% (28.58-31.16%) of all genes classified (Fig. 4).

A quantitatively high representation of carbohydrate metabolism (12.2-13.0%), global and overview map (11.5-11.6%), amino acid metabolism (10.2-10.7%), and energy metabolism (7.5-7.8%) was observed when the KEGG database was applied to the classification of the identified genes into putative pathways (Fig. 5a). Glycosyl transferase (GT, 34.59-38.58%) and glycoside hydrolase (GH, 28.98%-33.59%) were the highest proportions in carbohydrate active enzymes (CAZy), followed by carbohydrate esterase (CE, 15.91-16.28%) and coenzyme activity (AA, 10.43-10.92%), and polysaccharide lyase (PL, 3.00-3.64%), combined with the carbohydrate Module (CBM, 1.98-2.14%)(Fig. 5b).

3.3 Similarity of Species and Functions Among Soil Microbial Communities

The results of similarity and dissimilarity of species as per NR, NOG, KO and CAZy functional genes among all sample sites were compared to results obtained from similarity clustering analysis and redundancy analysis (RDA). Figure 6 indicated that the pattern of similarity of species and functions of soil microbial communities along an altitudinal gradient in a *P. massoniana* forest was basically the same as described above. RDA ordination of soil microbial communities at different altitudes based on functional gene composition showed a good level of consistency, which was slightly different from the ordination based on NR species. The distribution of NR species, NOG, KO and CAZy functional genes of soil microbial community at MP1000, MP1400, and MP1600 were clearly separated from those observed at MP1200. Furthermore, a significant difference was observed between the *P. massoniana* forest and the tea garden surveyed.

3.4 Co-occurrence Patterns of Different Soil Microbial Groups

We detected significant differences among different soil microbial groups (archaea, bacteria, fungi, and viruses) and their corresponding random networks (Fig. 7) in terms of average degree, modularity, number of communities, and degree centrality (Table 4). Modularity ranked as follows: archaea (0.866) > bacteria (0.794) > fungi (0.698) > viruses (0.539), while degree centrality showed the following ranking: bacteria (0.181) > viruses (0.155) > fungi (0.087) > archaea (0.048) (Table 4). Overall, the co-occurrence network of archaeal and fungal species showed a relatively higher similarity, which may be related to the origin of the relationship between archaea and fungi. In turn, the co-occurrence network of bacterial and viral species showed a relatively higher similarity, which may be due to the closer relationship between them in the life history of the individuals. Overall, these data indicate a challenging and complex ecological network of relationships among different groups of soil microorganisms that warrants further in-depth study.

4. Discussion

The relationship between altitudinal distribution patterns and maintenance of biodiversity is currently a highly controversial topic in the field of biodiversity and ecosystem functions. The Leigong Mountain belongs to a typical mid-subtropical mountain forest ecosystem in southwestern China declared a National Nature Reserve, whose ecological environment is well preserved, thus providing an ideal site for the "an ideal site for the in-situ experiment" reported herein. In addition, previous studies at the site provided us with important and useful information on soil microorganism diversity and vegetation types along the elevation gradient of Leigong Mountain. Such information revealed great differences in soil microbiome and vegetation diversity at each elevation step. Therefore, we hypothesized that the variation in soil microorganism communities at different elevations may be affected by the variation in vegetation types.

P. massoniana is a native and widely distributed tree species that is well adapted to the regional climatic conditions; this ensured the selection of representative *P. massoniana* forest plots along the altitudinal

gradient established for conducting the survey reported herein. At the same time, soil microbial communities can be diagnosed by metagenome technology, which can annotate microorganisms to the species level, and detect archaea, bacteria, fungi, and viruses in soil microbiomes; further, this technology can perform genetic function annotations, thereby providing richer biological information for the structural and functional characteristics of microbial communities among different elevations.

Euryarchaeota and Thaumarchaeota were the predominant phylum of soil archaea in the P. massoniana forest across different altitude gradients. A large number of reports have pointed that they are the dominant phylum of soil archaea, making important contributions to the biogeochemical cycles of soil carbon, nitrogen, and hydrogen, among many other elements. A good example of this paramount ecological function is the ammonia oxidation by autotrophic ammonia-oxidizing archaea as a key step in the nitrogen digestion process in soils (Leininger, et al. 2006; Zhang et al. 2012). In addition, this study found that the abundance of archaea increased with increasing altitude in the range of 1000-1600 m a.s.l., on Leigong Mountain. This finding is consistent with results reported for the range of 1000-1500 m a.s.l., on Mount Fuji, in Japan (Singh et al. 2012).

Bacteria are the largest and most diverse and versatile of soil microbial groups (Delgado-Baquerizo et al. 2018). Consistently with the general consensus that they are the most common phylum among soil bacteria (Wang et al. 2015), in this study Proteobacteria and Acidobacteria were the overwhelmingly predominant soil bacterial groups, both playing a very important role in the soil-matter cycle (carbon, nitrogen, sulphur, and other elements) and in the construction of the ecological environment (Barns et al. 2007, Spain et al. 2009).

Fungi are another important component of soil microbiome, as they play a crucial role in nutrient cycling, as well as in promoting plant growth and vegetation community succession. Consistently with previous reports (Matsuoka et al. 2019), in this study Ascomycota and Basidiomycota were the predominant fungal groups across the altitudinal gradient under study; however, they showed opposite trends in relative abundance along the altitude gradient; thus, the relative abundance of Ascomycota decreased with increasing elevation (from 66.9% at Mp1000 to 46.3% at Mp1600), whereas Basidiomycota increased with increasing elevation (from 25.2% at Mp1000 to 48.1% at Mp1600); still, the overall relative abundance of both Ascomycota and Basidiomycota together also increased slightly with elevation (92.2% at Mp1000 to 94.4% at Mp1600). The increasing trend in relative abundance observed herein for Basidiomycota is consistent with the increasing trend observed for the same group in the altitude range of 950-1700 m a.s.l., in Norikura Mountain, in Japan (Ogwu et al. 2019). Furthermore, the change trend observed here for Ascomycota was also similar in both studies. Therefore, the specific set of predominant fungal groups may serve as a more sensitive indicator of environmental gradients determined by differences in altitude.

Viruses are ubiquitous and play important roles in regulating their hosts' mortality and community structure, the genetic landscape, and the nutrient turnover in ecosystem (Anderson et al. 2011; Paez-Espino et al. 2016; Narr et al. 2017). Although soil is the most important habitat for virus distribution,

there is currently a very limited number of reports on soil viruses (Paez-Espino et al. 2016; Narr et al. 2017), which may be due to the challenges involved in the detection, isolation, and classification of unknown viruses (Narr et al. 2017); therefore, viruses constitute what is referred to as, "the dark matter" of soil microbial communities (Paez-Espino et al. 2016). In this study, Myoviridae, Podoviridae and Siphoviridae, were the predominant virus groups in the soils of the *P. massoniana* forest of Leigong Mountain. Although they are reportedly the most common virus groups in a wide range of environments (Chen et al. 2014); we found no obvious trend in viral community-composition changes along between 1000 and 1600 m a.s.l., in our study. This result likely reflects the fact that virus dynamics may be affected by multiple factors, including host and environmental factors. The results of our symbiotic network analysis further intuitively reflect the differences in interaction patterns among species within different groups of the microbial community in a soil.

The gene functional composition of the soil microbial community at different altitudes was predicted by NOG, KEGG, and CAZ functional gene annotations. The results showed a relatively consistent trend among these different functional gene analyses. Thus, there were no obvious differences at the high category level in functional gene detection along the elevation gradient surveyed, a finding that may be related to the phenomenon of "gene redundancy" (Fierer et al. 2013); which consists in soil microbial communities containing similar functional genes despite even significant differences in community species composition in response to environmental changes. Nonetheless, our comparison between species composition and functional gene composition matrix of the soil microbial communities along the altitudinal gradient surveyed showed a significant correlation between the two, which supports our hypothesis that soil microbiome diversity can influence multiple ecosystem functions, while the elevation factor may indirectly influence soil microbiome functions by shaping soil microbiome composition.

5. Conclusions

Our findings suggest that different types of soil microorganisms over the altitudinal gradient have different distribution patterns in a *P. massoniana* forest. However, Leigong Mountain is a typical mid-subtropical mountain forest ecosystem that shows an overall altitude gradient from 650-2179 m a.s.l., within which our study focused the range from 1000 to 1600 m a.s.l. In the future, studies on the pattern of soil microbial distribution should disentangle the inner links between the environmental variables (vegetation types, edaphic factor, microclimate, etc.) and the detailed functions of the microbial communities along wider elevation gradients in this typical sub-tropical, non-karst mountain subtropical forest ecosystem.

Abbreviations

NOG: nonsupervised orthologous groups; KEGG: the Kyoto encyclopedia of genes and genomes-based pathways; SOC: soil organic carbon; TN: total nitrogen; TP: total phosphorus; DNA: deoxyribonucleic acid; ORF: open reading frames; NR: non-redundant protein sequences; CAZy: carbohydrate-active enzymes; FUNGuild—an open annotation tool for parsing fungal community datasets by ecological guild; RDA

redundancy analysis; GT: glycosyl transferase; GH: glycoside hydrolase; CE: carbohydrate esterase; AA: coenzyme activity; PL: polysaccharide lyase; CBM: carbohydrate module

Declarations

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Authors' contributions

Jian Zhang, Ming Xu and Xiao Zou conceived the study; Jian Zhang performed the analyses and wrote the first draft; all authors contributed critically to successive drafts and gave final approval for publication.

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Availability of data and materials

The datasets used and/or analyzed in during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

No applicable.

Consent for publication

No applicable.

Competing interests

The authors declare that they have no competing interests.

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Tables

Table 1 Basic information of study sites

Sites	Latitude and longitude	Altitude m	Slope °	Aspect °	Dominant species
Mp1000	108°06'42.08", 26°23'00.72"	1008	12	121	<i>Pinus massoniana</i> + <i>Cunninghamia Lanceolata</i>
Mp1200	108°10'02.83", 26°21'29.72"	1198	10	160	<i>P. massoniana</i>
Mp1400	108°10'04.86", 26°21'40.68"	1394	15	141	<i>P. massoniana</i>
Mp1600	108°11'30.85", 26°22'27.92"	1596	7	165	<i>P. massoniana</i> + <i>C. Lanceolata</i>
Tea-garden	108°06'42.21", 26°23'01.73"	991	3	120	<i>Camellia sinensis</i>

Table 2 Statistics of metagenomic sequencing by NR species annotation

Microbe	Phylum	Class	Order	Family	Genus	Species
Aracheae	12	24	38	60	144	912
Bacteria	82	135	251	466	1864	11576
Fungi	10	33	74	154	254	417
Viruses	1	1	4	17	60	402
Total	105	193	367	697	2322	13307

Table 3 Alpha diversity of soil microbial community

Sites	Richness				Shannon index			
	Archaea	Bacteria	Fungi	Viruses	Archaea	Bacteria	Fungi	Viruses
Mp1000	689	9993	292	125	7.29	9.08	7.3	4.72
Mp1200	752	10172	297	166	7.64	8.62	7.16	4.45
Mp1400	698	10247	318	138	7.85	8.97	7.29	4.91
Mp1600	716	10114	297	118	7.86	8.9	7.12	4.06
Tea-garden	697	10348	354	225	7.03	8.45	7.51	5.7

Table 4 Topological properties of soil microbiome co-occurrence network

Network properties	Archaea	Bacteria	Fungi	Viruses
Number of nodes	527	748	399	236
Positive edges	2589	12308	1762	6952
Negative edges	494	663	144	15
Average degree	11.7	34.7	9.6	37.1
Modularity	0.866	0.794	0.698	0.539
Number of communities	23	53	38	34
Degree centralization	0.048	0.181	0.087	0.155

Figures

Figure 1

Figure 4

NOG functional gene composition of soil microbial communities at different elevations in a *Pinus massoniana* forest.

Figure 5

KEGG-based CAZy functional gene classification for soil microbial communities at different elevations in a *Pinus massoniana* forest.

Figure 6

(a) NR species, (b) NOG, (c) KO, and (d) CAZy classification of functional genes of soil microbial communities by clustering tree and RDA analyses

(a) Archaea

(b) Bacteria

(c) Fungi

(d) Viruses

Figure 7

Species co-occurrence networks of different groups of soil microorganisms (a. archaea; b. bacteria; c. fungi; d. viruses)