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Elevation Shapes Soil Microbial Diversity and Carbon Cycling in *Platycladus orientalis* Plantations

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Abstract: Diversified soil microbiomes are the key drivers of carbon fixation and plant residue decomposition in forest ecosystems. Revealing the elevation patterns of soil microbial carbon cycling in forests is essential for utilization of forest ecological resources. However, the soil microbial diversity and carbon cycle processes in *Platycladus orientalis* plantations across different elevations are still unclear. Here, we established a gradient with three elevations (118 m, 300 m, and 505 m) on the Beijing Ming Dynasty Tombs Forest Farm, which is located in Changping District, Beijing. The metagenomics method was applied to study the soil microbiome, with a special focus on the carbon cycle process at each elevation. We found the diversity and composition of the soil microbiomes significantly varied across the elevation gradients. The structure of bacteria and archaea was mainly driven by soil total potassium, pH and NH_4^+, but the eukaryota had no significant relationship with the environmental factors. The relative abundance of genes involved in microbial carbon fixation and decomposition of organic carbon were also significantly impacted by elevation, with the former showing increasing, u-shaped, or hump trends with increasing elevation, but the latter only showing hump trends. The rTCA cycle and 3-hydroxypropionate pathway were the dominant carbon fixation pathways in the *Platycladus orientalis* plantations. The elevation gradient shaped the microbial decomposition of plant-derived organic carbon by changing soil properties and, furthermore, led to soil organic carbon stock losses. These findings increase our understanding of soil microbial diversity and the carbon cycle across different elevations and provide a theoretical basis for the utilization of forest ecological resources to promote carbon sequestration.

Keywords: microbial diversity; carbon fixation; carbon decomposition; *Platycladus orientalis* plantation

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1. Introduction

Forests that cover a large proportion of the land surface have been identified as the largest carbon sink in terrestrial ecosystems [1–3]. Soil microorganisms are important components in forest ecosystems and play key roles in carbon cycling [4,5]. The biogeochemical cycling of soil elements is driven by the metabolic activity of microorganisms, which include the three domains of bacteria, archaea and eukaryota [6]. A large number of studies have revealed that ecosystem functions in forests are supported by plant diversity, but the importance of microbial diversity on multifunctionality has been relatively overlooked in the past decades [7]. Considering the recent advances in understanding the microbial roles in ecosystem functioning, the concept of the "forest microbiome" was recently proposed and encompasses the total structure and function of microorganisms present in all components of a forest ecosystem [8,9]. The composition and structure of soil microbiomes can reflect the stability of a forest ecosystem and indicate changes in forest ecosystem function [10]. Therefore, a better understanding of structure and function of complex forest soil microbiomes can be conducive to maintaining and improving multi-functions and services of forest ecosystems.

The organic carbon stock in forest soils is derived from plant residues, deadwood, rhizo-deposited carbon and microbial necromass [11]. The microbiomes play crucial roles in soil carbon cycling by decomposition and transformation of organic matter in forest ecosystems [12]. Soil microorganisms are the major decomposers of organic carbon stocks and annually produce 60 Pg of CO_2 in the atmosphere [13]. On the other side, some autotrophic bacteria and archaea also fix the CO_2 in the atmosphere into organic carbon forms to support their growth [14–16]. Therefore, microbial carbon fixation also constitutes a source of organic carbon pools, but it was traditionally neglected due to its small contribution [17]. Soil autotrophic microorganisms fix carbon through various pathways, including the Calvin Benson cycle, reductive tricarboxylic cycle, 3-hydroxypropionate/4-hydroxybutyrate cycle, 3-hydroxypropionate cycle and Wood–Ljungdahl pathway, and can annually fix 0.5%–4.1% of CO_2 and sequester 0.3–3.7 Pg of organic carbon [14]. However, the systematic overview of how microbial carbon cycling pathways would affect soil organic carbon stock is still incomplete, especially in forest ecosystems.

Elevation is one of the important factors that affects soil microbial diversity, community structure and metabolic function by influencing soil physicochemical properties, soil temperature and humidity, plant biomass, and local microclimate [18]. Although soil microbial diversity and community composition changes across elevations have been extensively studied, there are no consistent conclusions regarding changes in soil microbial communities and soil carbon cycling (including soil carbon fixation, carbon degradation and other processes) across elevations [19]. Previous results showed that soil microbial diversity and the dominant taxa varied, showing different trends, such as hump or u-shaped patterns, and increasing or decreasing with increasing elevation [20–22]. This lack of consistency in microbial responses to elevation may be caused by a combination of biotic and abiotic factors such as the study site, vegetation type, vegetation cover status, litter inputs and soil physicochemical properties. Therefore, conducting research on specific forest ecosystems can lead to a better understanding of the impact of elevation changes on the structure and function of soil microbial communities.

Platycladus orientalis (Oriental Arborvitae) is one of the dominant tree species in the mountainous forest ecosystems of Beijing. The *Platycladus orientalis* plantation provides multi-services, including drought resistance, wind break effects, air purification, and water retention. *Platycladus orientalis* forests cover an area of about 1.48×10^5 hm^2 in Beijing, of which the area of plantation is about 1.21×10^5 hm^2 [23–25]. Here, we used the *Platycladus orientalis* plantation as a model system to investigate the structure and function of whole microbiomes across three domains of life (bacteria, archaea and eukaryota) across the elevation gradients (118 m, 300 m and 505 m). The microbial carbon cycling processes, including two dimensions of autotrophic C-fixation, decomposition of plant-derived carbon (cellobiose, cellulose, lignin, pectin and xylan) and microbial-derived carbon (peptidoglycan and chitin), were investigated by using the metagenomic approach. The main objectives of this study are to explore (i) how microbial diversity responds to elevation gradients in *Platycladus orientalis* plantations; and (ii) whether the variations in microbial diversity and function exert significant impacts on soil organic carbon stocks.

2. Materials and Methods

2.1. Site Description

We selected the Beijing Ming Dynasty Tombs Forest Farm (115°50′17″~116°29′49″ E, 40°02′18″~40°23′13″ N) as the experimental site, which is located in Changping District, Beijing, at the junction of Yanshan Mountain and Taihang Mountain, with a total area of 8561.29 hm^2 [26]. This area has a temperate continental semi-humid and semi-arid monsoon climate, with an average annual temperature of 11.5 °C, an annual precipitation of 500–600 mm, and elevation ranging from 68.0 m to 954.2 m, with an average elevation of 400 m [27].

2.2. Experimental Design

The field survey and sampling in the *Platycladus orientalis* plantations were conducted in September 2023. Three elevations (118 m, 300 m and 505 m) were selected in the *Platycladus orientalis* plantation on the Beijing Ming Dynasty Tombs Forest Farm. Four replicated plots (20 m × 20 m) were set up at each elevation in an area with a relatively consistent slope gradient and little disturbance from anthropogenic activities. The latitude, longitude, slope, and aspect for each sample plot was collected using the Global Positioning System (GPS). All tree species with a diameter at breast height (DBH) of more than 1 cm within each sample plot were counted (Table 1). Three 5 × 5 m subplots were randomly selected within each plot to conduct the shrub survey. Additionally, three 1 × 1 m subplots were randomly selected within each plot for herb surveys. In each plot, five topsoil samples (0–10 cm) were collected randomly and pooled to make one composite sample. Topsoil was chosen because this research focused on the microbial turnover of plant litter (plant-derived carbon), which is mainly distributed in the topsoil. After removing the rocks, roots, and debris, the soil samples were passed through a 2 mm sieve. Each soil sample was divided into two parts: one part was used for the soil chemical properties measurement and the other part was frozen (−20 °C) for molecular analysis.

Table 1. Basic information of the *Platycladus orientalis* plantation sampling plots in the Beijing Ming Dynasty Tombs Forest Farm.

Plot Number	Elevation (m)	Slope (°)	Aspect	Mean Diameter at Breast Height (cm)	Stand Density (per/hm^2)
A_100	118	26	NW	11.50	1350
B_300	300	26	NW	10.98	1375
C_500	505	27	NW	11.74	1375

Mean diameter at breast height (cm): The diameter at breast height (DBH) of each tree in the sampling plot was measured during the survey and the average DBH was calculated.

2.3. Soil Properties Characterization

Soil organic carbon (SOC) was measured by the potassium dichromate volumetric method, and soil total nitrogen (TN) was measured by the semi-micro Kjeldahl method [28,29]. Ammonium N (NH_4^+-N) and NO_3^--N concentrations were measured by flow injection analysis [6]. Soil microbial biomass carbon (SMBC) and soil microbial biomass nitrogen (SMBN) were measured by chloroform fumigation and extraction methods [30]. Soil pH was measured by the potentiometric method. Soil total phosphorus (TP) and available phosphorus (AP) were measured by the Mo-Sb colorimetric method [28]. Total potassium (TK) and available potassium (AK) were determined by flame photometry [31].

2.4. Soil DNA Extraction

The DNA samples were extracted from 0.25 g of soil using the FastDNA®Spin Kit for Soil (MP Biomedicals, Santa Ana, CA, USA) according to the standard protocols. The quality of these DNA samples (concentration and purity) was determined using Quantus Fluorometer (Promega, Madison, WI, USA) and NanoDrop2000 (Thermo Fisher Scientific, Waltham, MA, USA). The DNA samples were checked by electrophoresis in 1% agarose gels and finally stored at −80 °C for the subsequent metagenomics analysis.

2.5. Metagenomic Sequencing

The DNA extracts were fragmented into small pieces (about 400 bp) using Covaris M220 (Gene Company Limited, Shanghai, China). The paired-end libraries were constructed using NEXTFLEX Rapid DNA-Seq (Bioo Scientific, Austin, TX, USA). Adapters containing the full complement of sequencing primer hybridization sites were ligated to the blunt end of fragments. The paired-end metagenomic sequencing was conducted on Illumina NovaSeq (Illumina Inc., San Diego, CA, USA) at Majorbio Bio-Pharm Technology Co., Ltd. (Shanghai, China) using NovaSeq 6000 S4 Reagent Kit (Illumina, CA, USA) v1.5

(300 cycles) according to the manufacturer's instructions. Finally, about 12 Gb of raw sequences were yielded for each metagenomic sample.

2.6. Bioinformatics Analysis

The quality control of the raw metagenomic was performed by filtering those reads with adapter contamination and low-quality bases (length < 50 bp or with a quality value < 20 or having N bases) in fastp (https://github.com/OpenGene/fastp/, accessed on 16 October 2023). The clean reads were individually assembled into contigs by using Megahit with the default parameters [32]. The protein encoding genes in contigs were predicted by Prodigal [33]. The non-redundant gene catalogs were generated by CD-HIT (http://www.bioinformatics.org/cd-hit/, accessed on 16 October 2023) with a sequence identity threshold of 0.90 and alignment coverage of 0.9 [34]. High-quality reads were aligned to the non-redundant gene catalogs to calculate gene abundance with 95% identity using SOAPaligner (version 2.21) [35]. The non-redundant gene catalogs were aligned to the NCBI-NR database using Diamond (http://www.diamondsearch.org/index.php, version 0.8.35, accessed on 16 October 2023) [36] with an e-value cutoff of 1×10^{-5} to obtain the taxonomic profiles. The non-redundant gene catalogs were aligned to the Kyoto Encyclopedia of Genes and Genomes (KEGG) database and CAZy database using Diamond with the e-value parameter at 1×10^{-5} [36]. The microbial genes involved in the decomposition of plant-derived carbon (cellobiose, cellulose, lignin, pectin and xylan) and microbial-derived carbon (peptidoglycan and chitin) were calculated based on a previous publication [37]. The key genes in carbon fixation pathways, which include the Calvin–Benson reductive pentose phosphate cycle (CB cycle), reductive citric acid cycle (rTCA), reductive acetyl-CoA pathway (Wood–Ljungdahl), 3-hydroxypropionate pathway and 3-hydroxypropionate/4-hydroxybutyrate cycle (3HP/4HB), were used to assess the microbial carbon-fixing capacity, according to a previous publication [38].

2.7. Statistical Analysis

The statistical analysis was performed in R (R version 4.1.2). The microbial diversity, as indicated by the Shannon diversity index, was calculated using the diversity function in the R package vegan. Principal coordinate analysis (PCoA) based on Bray–Curtis distances was used to evaluate the variance of microbial community beta diversity, and the top 2 eigenvalues (the first and second axis) were selected for data visualization. Permutational multivariate analysis of variance (PERMANOVA) was also applied to test the significance of variance across the elevation gradients. Relationships between microbial carbon cycling genes and soil chemical properties were analyzed using Spearman's correlations. The Mantel test was applied to investigate the impacts of environmental factors on microbial beta diversity based on Bray–Curtis distances. Partial least squares path model analysis (PLSPM) was performed to analyze the association between soil properties, biodiversity, carbon fixation, carbon decomposition and soil organic carbon.

2.8. Data Availability

The metagenomic sequences from our *Platycladus orientalis* plantation samples have been deposited in the NCBI Sequence Read Archive (SRA) database under the accession number of Project PRJNA1089939.

3. Results

3.1. Plant Community Diversity and Soil Physiochemical Properties

With the increase in elevation, the Shannon index of the herbaceous layer decreased gradually, while the diversity of the shrub layer slightly increased, but not significantly ($p > 0.05$, Table 2). The SOC and TN contents showed consistent trends with the increasing elevation, and both were significantly higher at 500 m than those at 100 m and 300 m ($p < 0.05$). The NH_4^+ and TK contents were increased at 300 m, and were significantly higher than those at 100 m ($p < 0.05$). The soil TP reached the highest value at 500 m, and

was significantly higher than the values at 100 m and 300 m ($p < 0.05$). The C:N ratio decreased with increasing elevation, and was significantly higher at 100 m and 300 m than that at 500 m ($p < 0.05$). In contrast, the N:P ratio increased with increasing elevation, and was significantly higher at 300 m and 500 m than that at 100 m ($p < 0.05$). The pH first decreased and then increased with the increasing elevation, and was significantly higher at 100 m than that at 300 m ($p < 0.05$). There were no significant changes in NO_3^-, AK, AP, C:P, SMBC and SMBN contents across different elevations ($p > 0.05$).

Table 2. Soil properties and plant species diversity of a *Platycladus orientalis* plantation at different elevations.

Elevation/m	118	300	505
TN (g kg^{-1})	1.48 ± 0.16 b	1.65 ± 0.05 b	2.62 ± 0.27 a
SOC (g kg^{-1})	17.5 ± 2.18 b	19.2 ± 0.96 b	26.4 ± 2.00 a
TK (g kg^{-1})	18.9 ± 0.05 b	21.6 ± 0.15 a	21.2 ± 0.43 ab
TP (g kg^{-1})	0.36 ± 0.02 b	0.30 ± 0.01 b	0.43 ± 0.02 a
AK (mg kg^{-1})	178 ± 9.87 a	207 ± 16.4 a	199 ± 15.9 a
AP (mg kg^{-1})	2.42 ± 0.50 a	1.40 ± 0.15 a	2.35 ± 0.21 a
C:N	11.8 ± 0.32 a	11.7 ± 0.30 a	10.2 ± 0.36 b
C:P	48.2 ± 4.53 a	63.2 ± 5.03 a	61.7 ± 2.93 a
N:P	4.08 ± 0.30 b	5.40 ± 0.32 a	6.09 ± 0.37 a
pH	7.45 ± 0.05 a	6.87 ± 0.04 b	7.04 ± 0.15 ab
SMBC (mg kg^{-1})	462 ± 154 a	390 ± 208 a	389 ± 112 a
SMBC:SOC	0.03 ± 0.01 a	0.02 ± 0.01 a	0.01 ± 0.004 a
SMBN (mg kg^{-1})	12.1 ± 4.02 a	11.2 ± 2.01 a	8.48 ± 2.21 a
NH_4^+-N (mg kg^{-1})	2.61 ± 0.11 b	6.44 ± 0.60 a	4.45 ± 0.14 ab
NO_3^--N (mg kg^{-1})	5.95 ± 1.34 a	3.43 ± 0.90 a	7.21 ± 1.18 a
Herb Shannon	1.25 ± 0.28 a	1.12 ± 0.13 a	0.88 ± 0.32 a
Shrub Shannon	0.53 ± 0.21 a	0.74 ± 0.31 a	0.73 ± 0.11 a

TN: total nitrogen, SOC: soil organic carbon, TK: total potassium, AK: available potassium, TP: total phosphorus, AP: available phosphorus, SMBC: soil microbial biomass carbon, SMBN: soil microbial biomass nitrogen. Different letters indicate a significant difference between elevations ($p < 0.05$).

3.2. Diversity and Structure of Soil Microbial Communities

All biota α-diversity (total micro-biomes) and bacteria α-diversity firstly increased and then decreased with the increasing elevation, but there were no significant differences among different elevations ($p > 0.05$, Figure 1a,b). Archaea α-diversity and eukaryota α-diversity peaked at 300 m. Archaea α-diversity increased by 15.14% from 100 m to 300 m ($p < 0.05$, Figure 1c), and eukaryota α-diversity was 23.37% higher at 300 m in comparison to the value at 100 m ($p < 0.05$, Figure 1d), while there were no significant differences in the values between 500 m and 300 m or between 500 m and 100 m ($p > 0.05$, Figure 1d).

The composition of microbial communities showed significant differences among the different elevations (Figure 2). In the bacteria community, the relative abundance of Actinobacteria was significantly lower at 300 m than that at 100 m, while the relative abundance of Proteobacteria and Acidobacteria firstly increased and then decreased with the increasing elevation, and the relative abundance of Verrucomicrobia increased with increasing elevation, with its abundance being significantly higher at 300 m and 500 m than that at 100 m ($p < 0.05$, Figure 2a). As for the archaea community, the relative abundance of Thaumarchaeota and Euryarchaeota was significantly lower at 300 m than that at 100 m ($p < 0.05$, Figure 2b). In the eukaryotic community, the relative abundance of Ascomycota, Mucoromycota, and Chordata firstly increased and then decreased with increasing elevation; the relative abundances of Ascomycota and Mucoromycota were significantly higher at 300 m compared to 100 m, while the relative abundance of Chordata was significantly higher at 300 m and 500 m than at 100 m ($p < 0.05$, Figure 2c).

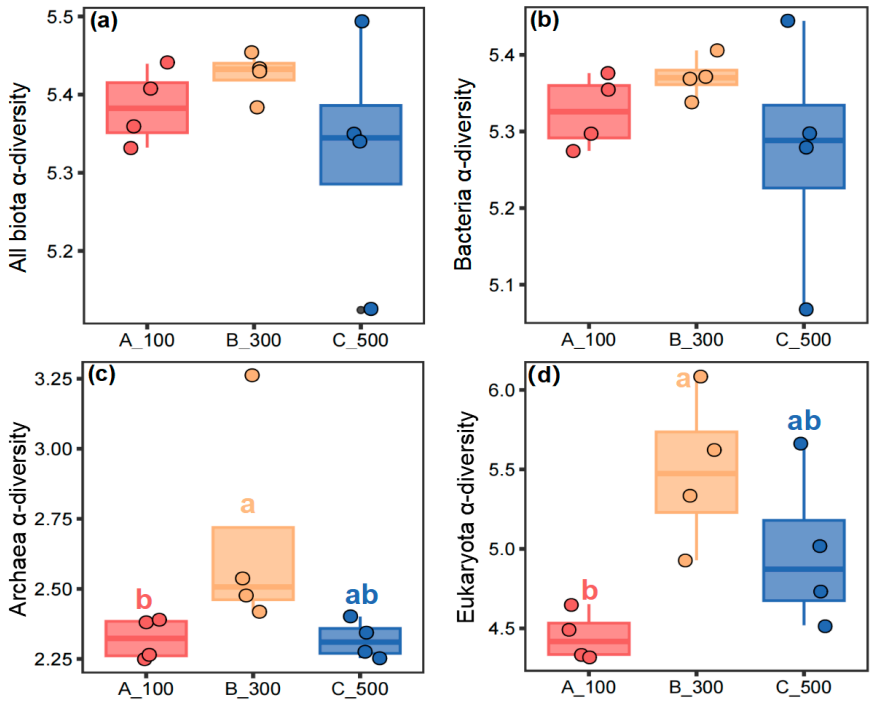

Figure 1. Alpha diversity of soil microbial communities ((**a**) total micro-biomes; (**b**) bacteria; (**c**) archaea; (**d**) eukaryota) at different elevations. The diversity is indicated by the Shannon index. Different letters indicate there was a significant difference between different elevations ($p < 0.05$).

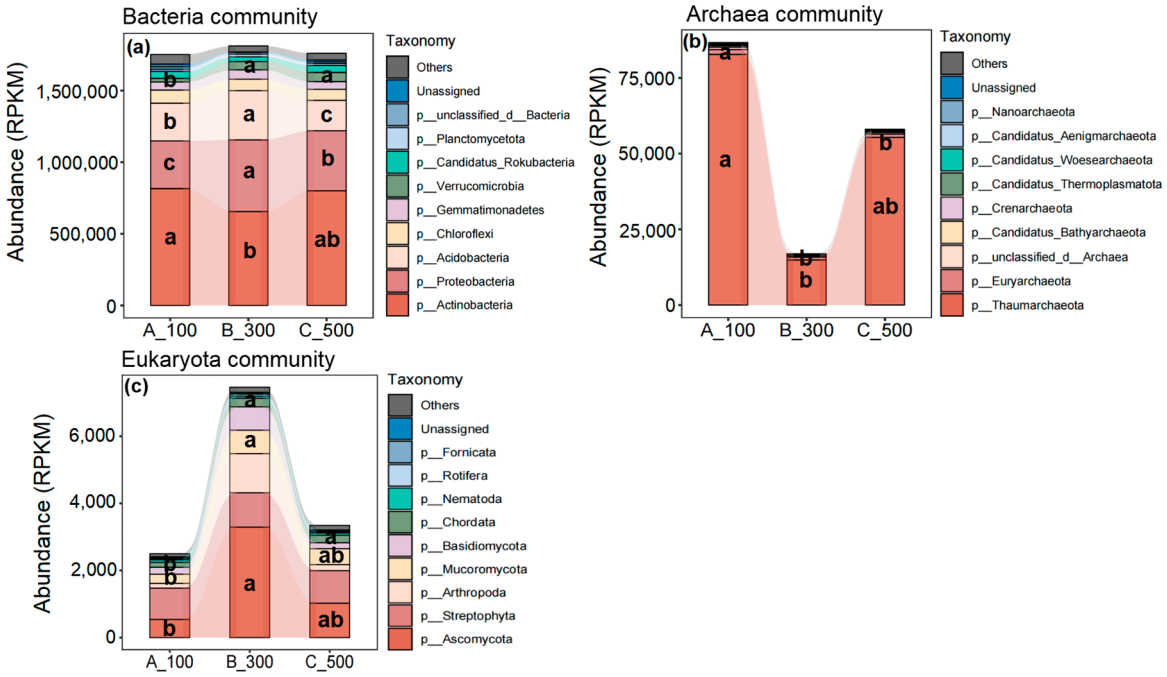

Figure 2. The relative abundance (RPKM) of soil microbial phylum levels ((**a**) bacteria community; (**b**) archaea community; (**c**) eukaryota community) at different elevations. Different letters indicate a significant difference between different elevations ($p < 0.05$). "RPKM" means "Reads Per Kilobase per Million mapped reads".

The community biodiversity of bacteria, archaea and eukaryota showed significant variance across different elevations ($p < 0.01$). Across different elevations, the PCoA1 and PCoA2 explained 59% and 17%, respectively, of the variation in biodiversity structure

(R^2 = 0.70, p = 0.001, Figure 3a); 57% and 18% of the variation in bacteria community structure (R^2 = 0.70, p = 0.001, Figure 3b); 74% and 19% of the archaea community structure variation (R^2 = 0.62, p = 0.004, Figure 3c); and 52% and 12% of the variation in the eukaryota community structure (R^2 = 0.49, p = 0.001, Figure 3d).

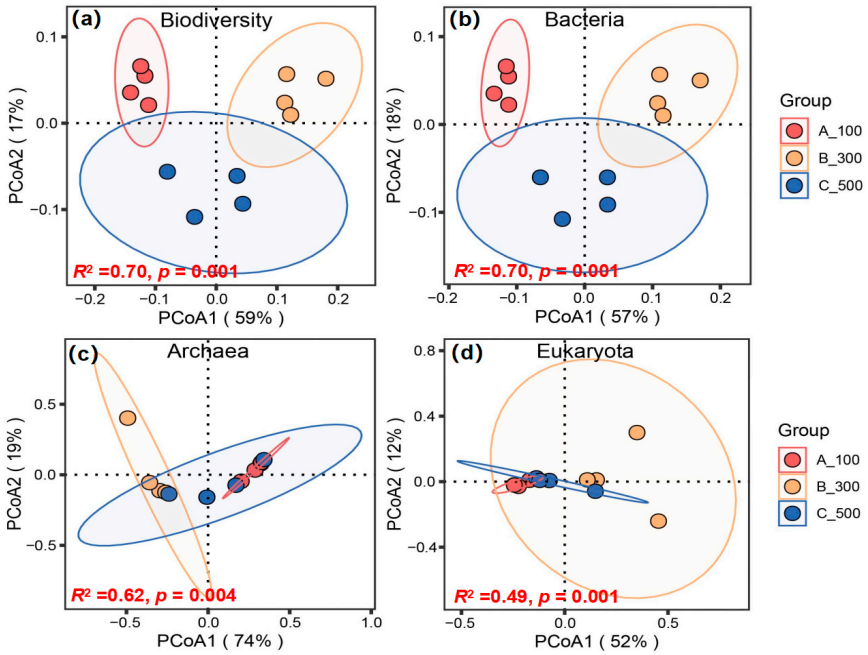

Figure 3. Shifts in soil microbial community beta diversity ((**a**) total micro-biomes; (**b**) bacteria; (**c**) archaea; (**d**) eukaryota) at different elevations. The significance of variation across the different elevations was tested by the PERMANOVA analysis.

The Mantel's test showed that TK, pH, NH_4^+, and TP were the environmental driving factors that significantly affected total biodiversity and bacterial communities ($p < 0.05$, Figure 4a,b). TK, pH, and NH_4^+ were the environmental driving factors that significantly affected the archaea community ($p < 0.05$, Figure 4c), but the eukaryota community was not significantly affected by the environmental factors ($p > 0.05$, Figure 4d).

3.3. Microbial Genetic Capacity for Carbon Fixation and Decomposition

The relative abundance of the Calvin cycle genes increased significantly with the increasing elevation, and was significantly higher at 300 m and 500 m in comparison to the levels at 100 m ($p < 0.05$, Figure 5a). The relative abundance of the 3HP/4HB pathway genes had the lowest abundance at 300 m, which at 37.94%, was significantly reduced compared to 100 m ($p < 0.05$, Figure 5b). The relative abundance of the 3-hydroxypropionate pathway genes firstly increased and then decreased with the increasing elevation, and was 14.56% and 10.47% higher at 300 m compared to 100 m and 500 m ($p < 0.05$, Figure 5c). The relative abundance of rTCA cycle and W-L pathway genes firstly decreased and then increased with the increasing elevation, but not significantly ($p > 0.05$, Figure 5d,e).

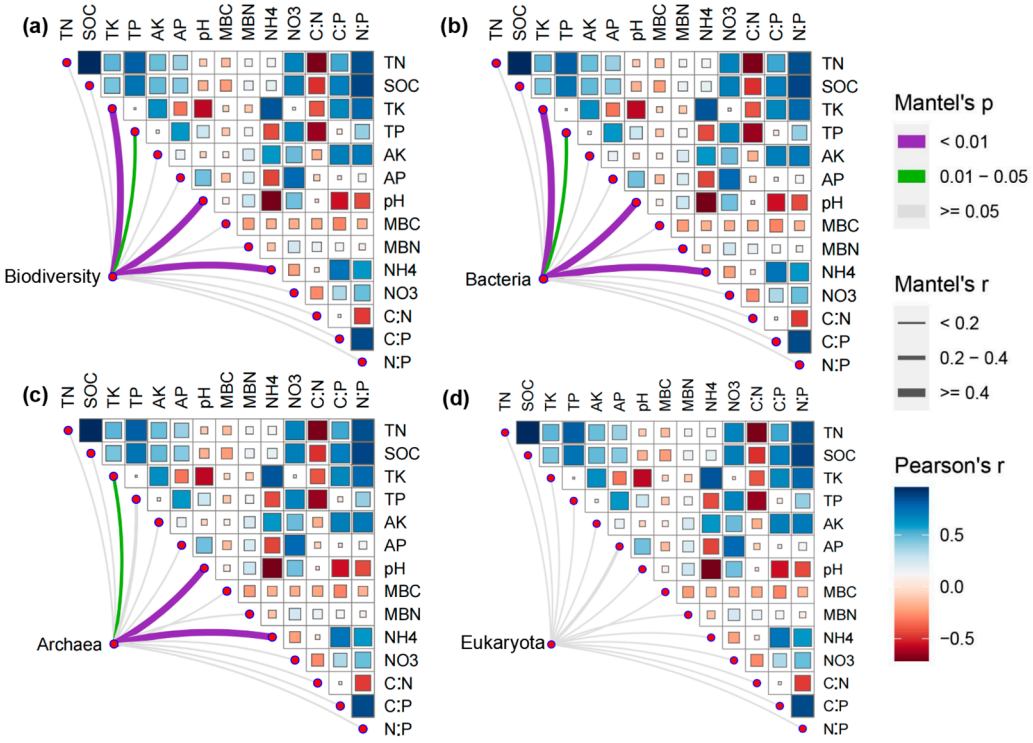

Figure 4. Environmental drivers of microbial beta-biodiversity ((**a**) total micro-biomes; (**b**) bacteria; (**c**) archaea; (**d**) eukaryota) in a *Platycladus orientalis* plantation. The Mantel test was applied to test the significance of each environmental factor on microbial beta-biodiversity.

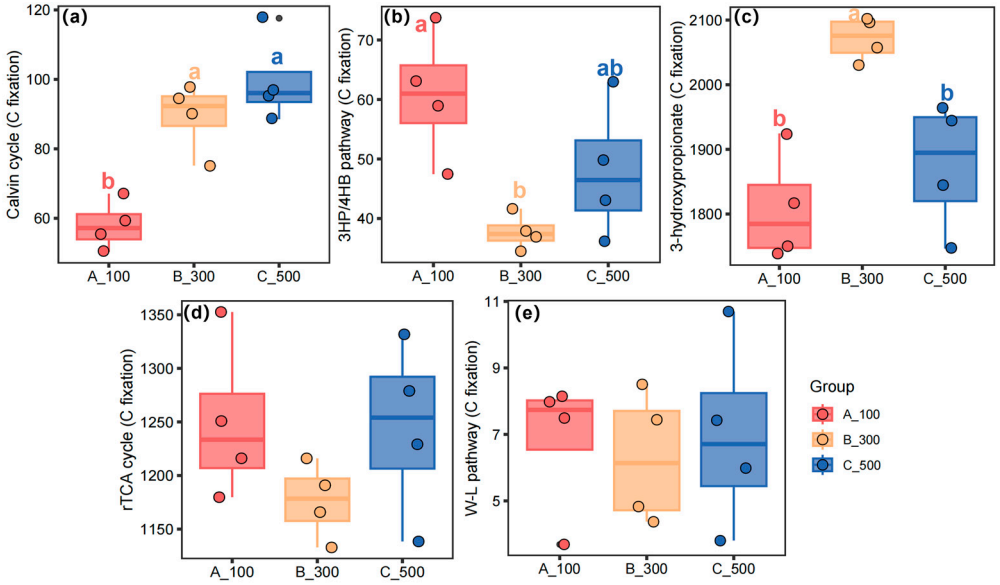

Figure 5. Microbial carbon fixation pathways at different elevations. The key marker genes responsible for the Calvin cycle (**a**), 3HP/4HB (**b**), 3-hydroxypropionate (**c**), reductive citric acid cycle (rTCA) (**d**) and Wood–Ljungdahl (**e**) pathways were used to assess the microbial capacity for carbon fixation. Different letters indicate a significant difference between different elevations ($p < 0.05$).

The relative abundance of the microbial-derived carbon decomposition genes and plant-derived carbon decomposition genes firstly increased and then decreased with increasing elevation (Figure 6), with the former being significantly higher at 300 m than that at 100 m and 500 m ($p < 0.05$, Figure 6a), and the latter being significantly higher at 300 m than at 500 m ($p < 0.05$, Figure 6b).

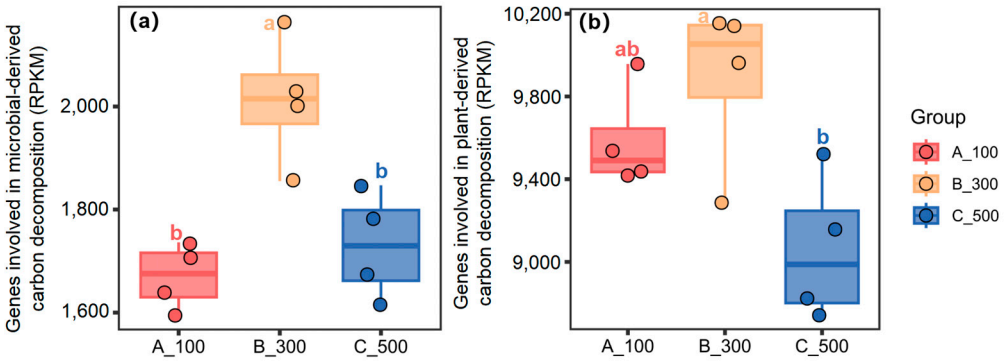

Figure 6. Genes involved in microbial-derived (**a**) and plant-derived (**b**) carbon decomposition at different elevations. The microbial genes involved in the decomposition of plant-derived carbon (cellobiose, cellulose, lignin, pectin and xylan) and microbial-derived carbon (peptidoglycan and chitin) were calculated based on the methods of a previous publication [37]. Different letters indicate there is a significant difference between different elevations ($p < 0.05$). "RPKM" means "Reads Per Kilobase per Million mapped reads".

3.4. Mechanism Underlying Changes in Soil Organic Carbon Stock across Different Elevations

We finally explored how biotic and abiotic factors drive carbon cycling processes and affect organic carbon stocks using PLS-PM (Figure 7). The biodiversity (biotic factors) had a significant path effect on carbon fixation (path coefficient = 0.686, $p < 0.05$), but not for carbon decomposition ($p > 0.05$). In contrast, the soil properties (abiotic factors) exerted a negative effect on both plant-derived carbon decomposition (path coefficient = −0.718, $p < 0.01$) and microbial-derived carbon decomposition (path coefficient = −0.659, $p < 0.05$). Additionally, the microbial carbon fixation had a positive impact on soil organic carbon stock (path coefficient = 0.469, $p = 0.06$), but the microbial decomposition of plant-derived carbon decomposition negatively contributed to the soil organic carbon stock (path coefficient = −0.650, $p < 0.05$) (Figure 7).

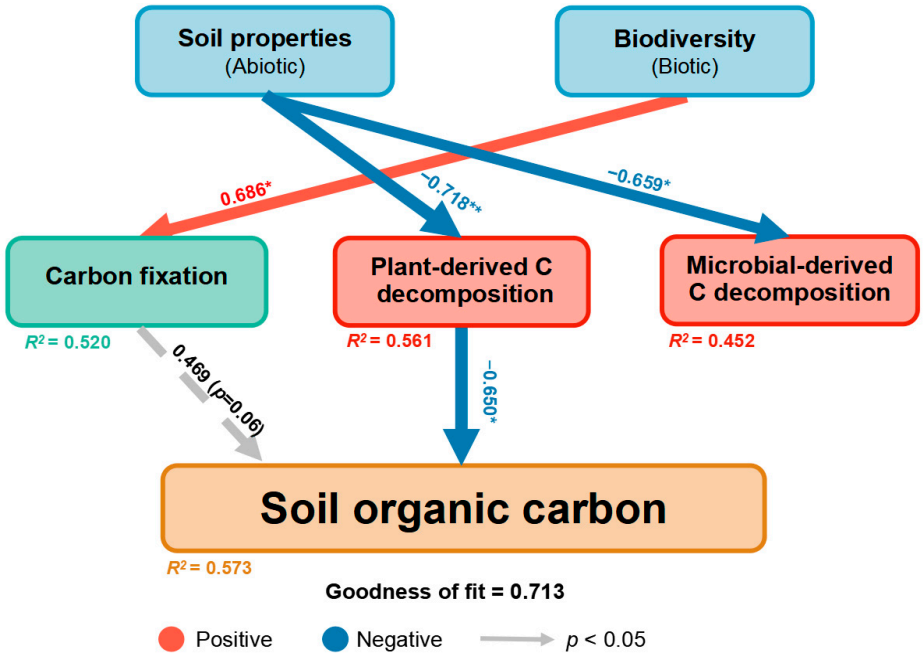

Figure 7. The partial least squares path modeling (PLS-PM) showing the mechanism underlying changes in soil organic carbon stocks across different elevations. Asterisks indicate that the path is significant (* $p < 0.05$, ** $p < 0.01$). The significant or near-significant paths were visualized in the PLS-PM.

4. Discussion

The diversity of the herbaceous layer gradually decreases and the diversity of the shrub layer increases with elevation because soil TN and SOC contents increase with elevation and the shrub layer plants are highly efficient in utilizing soil nutrients. This leads to the shrub layer species being the dominant species and occupying the dominant ecological niche, and contributes to the increase in diversity and biomass of the shrub species. As a result, herbaceous plants are gradually eliminated with the increase in elevation, resulting in a decrease in herbaceous diversity [23].

The present study revealed that bacteria diversity increases and then decreases with increasing elevation, while archaea diversity and eukaryotic diversity reach a maximum at 300 m, suggesting that these groups may have more ecological functions at the middle elevation. However, in terms of the overall soil microbial diversity and function, there was no consistent relationship with increasing elevation, which may be attributed to different factors such as microclimate, litter, and soil physiochemical properties at the different study sites [20,39,40]. We found that different elevations significantly affected the microbial community structure, which is similar to the results of other studies, due to the fact that changes in vegetation cover and litter with increasing elevation affect the input of nutrients into the soil, which in turn, affects the microbial community structure [41–43]. As an important factor influencing the structure and diversity of soil microbial communities, elevation may regulate soil microbial diversity by changing soil physicochemical properties, thus indirectly influencing the structural and functional diversity of soil microbial communities [44,45]. Our results showed that soil pH, TP, TK and NH_4^+ were major environmental drivers that significantly affected total microbial diversity and bacterial diversity, illustrating that an increase or decrease in bacterial diversity is closely related to changes in soil nutrient content and pH [4,20,46]. In the present study, pH, TK, and NH_4^+ were environmental drivers that significantly affected archaea biodiversity, suggesting that archaea biodiversity was also highly dependent on soil nutrients and pH [46–48]. In contrast, there was no significant correlation between eukaryotic diversity and environmental factors, suggesting that eukaryote biodiversity remains relatively stable under different environmental conditions due to their larger genomes and stress-tolerance capacity [49].

To provide a theoretical basis for the increasing carbon sinks in forest ecosystems, it is important to understand the microbial carbon cycle processes, which are the basis of key ecosystem services [15,50]. Microbial autotrophic carbon fixation, which directly fixes atmospheric CO_2 into organic forms, is one of the most important sources of soil organic carbon pools in forest ecosystems [47]. Here, our PLSPM demonstrated the positive impact of microbial carbon fixation on soil organic carbon, but this effect was not significant. This slight positive effect of microbial carbon fixation on soil organic carbon pools can be well explained by the scarcity of autotrophic bacteria, which only accounted 2%–11% of the total microbiome [51]. The 3-hydroxypropionate pathway, the dominant carbon fixation pathway in our study site, showed an increasing and then decreasing trend with increasing elevation, indicating that the abundance of microorganisms (e.g., *Chloroflexus aurantiacus*) that encode for 3-hydroxypropionate had the highest abundance at mid-elevations [52]. Their higher abundance at mid-elevation might be attributed to the significantly lower pH in mid-elevation soils, which has been reported to be an important driver of autotrophic CO_2-fixing microbes [53]. Previous studies also identified 3-hydroxypropionate a dominant CO_2 fixation pathway in semiarid grassland soils, suggesting its wide importance in promoting soil carbon sinks [38].

The changes in soil organic carbon stocks across different elevations were associated with the microbial decomposition of soil organic carbon compounds, as evidenced by our PLS-PM analysis. Microorganisms are involved in soil organic carbon decomposition and release of CO_2 to the atmosphere through catabolism, which has been reported to cause an annual loss of soil organic carbon as high as 58 Pg [54,55]. Elevation indirectly leads to changes in the inputs of plant litter and root debris, and thus, may alter microbial preference in utilization of organic carbon forms [56]. The present study found that

microbial-derived carbon decomposition and plant-derived carbon decomposition genes showed an increasing and then decreasing trend with the increasing elevation, suggesting that microorganisms had a high decomposition capacity when utilizing diversified soil organic carbon forms at mid-elevation, which might have contributed to the loss of soil organic carbon pools in mid-elevation sites [57]. This elevation pattern of microbial decomposition genes was consistent with the previous research, which found that carbohydrate esters, chitin and pectin showed hump trends along elevation climosequences [19]. Soil pH was the important driver that shaped the abundance patterns of genes involved in carbon decomposition [19]. This was evidenced by our PLS-PM, as the genes involved in decomposition of plant-derived carbon significantly affected soil organic carbon stocks. Thus, our study provided evidence for the links between microbial functional genes and soil carbon cycling in forest soils, which has implications for maintaining multi-services in forest ecosystem [58]. Soil chemical properties (nutrient availability) had a significant impact on microbial decomposition of organic carbon, which might be associated with microbial maintenance of the stoichiometric balance [59]. Soil microorganisms degrade organic carbon to acquire nutrients, such as nitrogen and phosphorus for cell growth, and this explains why soil chemical properties are important factors in determining the source of microbial-degraded carbon [60]. Overall, these findings increase the mechanistic understanding of microbial regulation of soil carbon cycling in forest soils from a metagenomic perspective.

5. Conclusions

Elevation was an important influencing factor on the structure and function of soil microbial communities in *Platycladus orientalis* plantations. Soil microbial α-diversity tended to increase and then decrease with increasing elevation, and microbial community structure showed significant variation between elevations, which was driven by soil TK, TP, pH and NH_4^+. The 3-hydroxypropionate pathway was the most important carbon fixation pathway in the *Platycladus orientalis* plantations. The genes involved in decomposition of plant-derived carbon had negative effects on soil carbon stocks across the elevation gradient. Our study increases the understanding of soil microbial community structure and function across different elevations, providing a theoretical basis for the utilization of forest ecological resources to mitigate climate change. Further studies are needed to explore microbial functions across larger elevation gradients to provide more convincing results.

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Data Availability Statement: The raw/processed data required to reproduce these findings cannot be shared at this time as the data also form part of an ongoing study.

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References

1. Crowther, T.W.; Glick, H.B.; Covey, K.R.; Bettigole, C.; Maynard, D.S.; Thomas, S.M.; Smith, J.R.; Hintler, G.; Duguid, M.C.; Amatulli, G.; et al. Mapping tree density at a global scale. *Nature* **2015**, *525*, 201–205. [CrossRef] [PubMed]
2. Hui, D.; Deng, Q.; Tian, H.; Luo, Y. Climate change and carbon sequestration in forest ecosystems. *Handb. Clim. Change Mitig. Adapt.* **2016**, *555*, 594.
3. Onyango, L.A.; Ngonga, F.A.; Karanja, E.N.; Kuja, J.O.; Boga, H.I.; Cowan, D.A.; Mwangi, K.W.; Maghenda, M.W.; Marinho, L.P.; Kambura, A.K. The soil microbiomes of forest ecosystems in Kenya: Their diversity and environmental drivers. *Sci. Rep.* **2023**, *13*, 7156. [CrossRef] [PubMed]

4. Zhou, Z.; Wang, C.; Luo, Y. Meta-analysis of the impacts of global change factors on soil microbial diversity and functionality. *Nat. Commun.* **2020**, *11*, 3072. [CrossRef] [PubMed]
5. Cong, J.; Yang, Y.; Liu, X.; Lu, H.; Liu, X.; Zhou, J.; Li, D.; Yin, H.; Ding, J.; Zhang, Y. Analyses of soil microbial community compositions and functional genes reveal potential consequences of natural forest succession. *Sci. Rep.* **2015**, *5*, 10007. [CrossRef] [PubMed]
6. Wu, X.; Peng, J.; Liu, P.; Bei, Q.; Rensing, C.; Li, Y.; Yuan, H.; Liesack, W.; Zhang, F.; Cui, Z. Metagenomic insights into nitrogen and phosphorus cycling at the soil aggregate scale driven by organic material amendments. *Sci. Total Environ.* **2021**, *785*, 147329. [CrossRef] [PubMed]
7. Lang, A.K.; LaRue, E.A.; Kivlin, S.N.; Edwards, J.D.; Phillips, R.P.; Gallion, J.; Kong, N.; Parker, J.D.; McCormick, M.K.; Domke, G.; et al. Forest structural diversity is linked to soil microbial diversity. *Ecosphere* **2023**, *14*, 4702. [CrossRef]
8. Baldrian, P.; Banin, E. Forest microbiome: Diversity, complexity and dynamics. *FEMS Microbiol. Rev.* **2017**, *41*, 109–130. [CrossRef] [PubMed]
9. Baldrian, P.; López-Mondéjar, R.; Kohout, P. Forest microbiome and global change. *Nat. Rev. Microbiol.* **2023**, *21*, 487–501. [CrossRef] [PubMed]
10. Anthony, M.A.; Tedersoo, L.; De Vos, B.; Croisé, L.; Meesenburg, H.; Wagner, M.; Andreae, H.; Jacob, F.; Lech, P.; Kowalska, A.; et al. Fungal community composition predicts forest carbon storage at a continental scale. *Nat. Commun.* **2024**, *15*, 2385. [CrossRef] [PubMed]
11. Qian, Z.; Gu, R.; Gao, K.; Li, D. High plant species diversity enhances lignin accumulation in a subtropical forest of southwest China. *Sci. Total Environ.* **2023**, *865*, 161113. [CrossRef] [PubMed]
12. Li, T.; Yuan, Y.; Mou, Z.; Li, Y.; Kuang, L.; Zhang, J.; Wu, W.; Wang, F.; Wang, J.; Lambers, H.; et al. Faster accumulation and greater contribution of glomalin to the soil organic carbon pool than amino sugars do under tropical coastal forest restoration. *Glob. Change Biol.* **2023**, *29*, 533–546. [CrossRef] [PubMed]
13. Li, J.; Baath, E.; Pei, J.; Fang, C.; Nie, M. Temperature adaptation of soil microbial respiration in alpine, boreal and tropical soils: An application of the square root (Ratkowsky) model. *Glob. Change Biol.* **2021**, *27*, 1281–1292. [CrossRef] [PubMed]
14. Jiang, P.; Xiao, L.Q.; Wan, X.; Yu, T.; Liu, Y.F.; Liu, M.X. Research progress on microbial carbon sequestration in soil: A review. *Eurasian Soil Sci.* **2022**, *55*, 1395–1404. [CrossRef]
15. Wu, H.; Cui, H.; Fu, C.; Li, R.; Qi, F.; Liu, Z.; Yang, G.; Xiao, K.; Qiao, M. Unveiling the crucial role of soil microorganisms in carbon cycling: A review. *Sci. Total Environ.* **2024**, *909*, 168627. [CrossRef] [PubMed]
16. Liu, X.; Huang, L.; Rensing, C.; Ye, J.; Nealson, K.H.; Zhou, S. Syntrophic interspecies electron transfer drives carbon fixation and growth by rhodopseudomonas palustris under dark, anoxic conditions. *Sci. Adv.* **2021**, *7*, eabh1852. [CrossRef]
17. Yuan, H.; Ge, T.; Chen, C.; O'Donnell, A.G.; Wu, J. Significant role for microbial autotrophy in the sequestration of soil carbon. *Appl. Environ. Microbiol.* **2012**, *78*, 2328–2336. [CrossRef] [PubMed]
18. Shigyo, N.; Umeki, K.; Hirao, T. Seasonal dynamics of soil fungal and bacterial communities in cool-temperate montane forests. *Front. Microbiol.* **2019**, *10*, 1944. [CrossRef]
19. Dai, Z.; Zang, H.; Chen, J.; Fu, Y.; Wang, X.; Liu, H.; Shen, C.; Wang, J.; Kuzyakov, Y.; Becker, J.N.; et al. Metagenomic insights into soil microbial communities involved in carbon cycling along an elevation climosequences. *Environ. Microbiol.* **2021**, *23*, 4631–4645. [CrossRef] [PubMed]
20. Shen, C.; Gunina, A.; Luo, Y.; Wang, J.; He, J.Z.; Kuzyakov, Y.; Hemp, A.; Classen, A.T.; Ge, Y. Contrasting patterns and drivers of soil bacterial and fungal diversity across a mountain gradient. *Environ. Microbiol.* **2020**, *22*, 3287–3301. [CrossRef]
21. Nottingham, A.T.; Fierer, N.; Turner, B.L.; Whitaker, J.; Ostle, N.J.; McNamara, N.P.; Bardgett, R.D.; Leff, J.W.; Salinas, N.; Silman, M.R.; et al. Microbes follow Humboldt: Temperature drives plant and soil microbial diversity patterns from the Amazon to the Andes. *Ecology* **2018**, *99*, 2455–2466. [CrossRef] [PubMed]
22. Singh, D.; Lee-Cruz, L.; Kim, W.; Kerfahi, D.; Chun, J.; Adams, J.M. Strong elevational trends in soil bacterial community composition on Mt. Halla, South Korea. *Soil Biol. Biochem.* **2014**, *68*, 140–149. [CrossRef]
23. Cui, R.; Qi, S.; Wu, B.; Zhang, D.; Zhang, L.; Zhou, P.; Ma, N.; Huang, X. The influence of stand structure on understory herbaceous plants species diversity of *Platycladus orientalis* plantations in Beijing, China. *Forests* **2022**, *13*, 1921. [CrossRef]
24. Deng, W.; Jia, G.; Liu, Y.; Chen, Q.; Huang, J.; Wen, L.; Zhang, L.; Liu, X.; Jia, J.; Peng, S. Long-term study on the seasonal water uptake of *Platycladus orientalis* in the Beijing mountain area, northern China. *Agric. For. Meteorol.* **2021**, *307*, 108531.
25. Zhang, L.; Qi, S.; Li, P.; Zhou, P. Influence of stand and environmental factors on forest productivity of *Platycladus orientalis* plantations in Beijing's mountainous areas. *Ecol. Indic.* **2024**, *158*, 111385. [CrossRef]
26. Zhang, H.; Zhang, Y.M.; Sun, H.N.; Li, J.; Zheng, J.P.; Zhang, P. Analysis on the characteristics of forest resources in the Beijing Shisanling Forest Farm. *South China Agric.* **2023**, *17*, 88–90.
27. Luo, M.; Zheng, X.; Du, Y. Natural regeneration of an artificial *Platycladus orientalis* stand in Beijing. *Nat. Environ. Pollut. Technol.* **2017**, *16*, 287.
28. Liu, G.S.; Jiang, N.H.; Zhang, L.D.; Liu, Z.L. *Soil Physical and Chemical Analysis and Description of Soil Profiles*; China Standard Methods Press: Beijing, China, 1996; Volume 24, p. 266.
29. Baral, S.; Katzensteiner, K. Impact of biomass extraction on soil properties and foliar nitrogen content in a community forest and a semi-protected natural forest in the central mid-hills of Nepal. *Trop. Ecol.* **2015**, *56*, 323–333.

30. Inubushi, K.; Brookes, P.C.; Jenkinson, D.S. Soil microbial biomass C, N and ninhydrin-N in aerobic and anaerobic soils measured by the fumigation-extraction method. *Soil Biol. Biochem.* **1991**, *23*, 737–741. [CrossRef]
31. Das, D.; Nayak, A.K.; Thilagam, V.K.; Chatterjee, D.; Shahid, M.; Tripathi, R.; Mohanty, S.; Kumar, A.; Lal, B.; Gautam, P.; et al. Measuring potassium fractions is not sufficient to assess the long-term impact of fertilization and manuring on soil's potassium supplying capacity. *J. Soils Sediments* **2018**, *18*, 1806–1820. [CrossRef]
32. Li, D.; Liu, C.M.; Luo, R.; Sadakane, K.; Lam, T.W. MEGAHIT: An ultra-fast single-node solution for large and complex metagenomics assembly via succinct de Bruijn graph. *Bioinformatics* **2015**, *31*, 1674–1676. [CrossRef] [PubMed]
33. Hyatt, D.; Chen, G.L.; Locascio, P.F.; Land, M.L.; Larimer, F.W.; Hauser, L.J. Prodigal: Prokaryotic gene recognition and translation initiation site identification. *BMC Bioinform.* **2010**, *11*, 119. [CrossRef] [PubMed]
34. Fu, L.; Niu, B.; Zhu, Z.; Wu, S.; Li, W. CD-HIT: Accelerated for clustering the next-generation sequencing data. *Bioinformatics* **2012**, *28*, 3150–3152. [PubMed]
35. Li, R.; Li, Y.; Kristiansen, K.; Wang, J. SOAP: Short oligonucleotide alignment program. *Bioinformatics* **2008**, *24*, 713–714. [CrossRef] [PubMed]
36. Buchfink, B.; Xie, C.; Huson, D.H. Fast and sensitive protein alignment using DIAMOND. *Nat. Methods* **2015**, *12*, 59–60. [CrossRef] [PubMed]
37. Zifcakova, L.; Vetrovsky, T.; Lombard, V.; Henrissat, B.; Howe, A.; Baldrian, P. Feed in summer, rest in winter: Microbial carbon utilization in forest topsoil. *Microbiome* **2017**, *5*, 122. [CrossRef] [PubMed]
38. Huang, Q.; Huang, Y.; Wang, B.; Dippold, M.A.; Li, H.; Li, N.; Jia, P.; Zhang, H.; An, S.; Kuzyakov, Y. Metabolic pathways of CO_2 fixing microorganisms determined C-fixation rates in grassland soils along the precipitation gradient. *Soil Biol. Biochem.* **2022**, *172*, 108764. [CrossRef]
39. Singh, D.; Takahashi, K.; Kim, M.; Chun, J.; Adams, J.M. A Hump-backed trend in bacterial diversity with elevation on Mount Fuji, Japan. *Microb. Ecol.* **2012**, *63*, 429–437. [CrossRef] [PubMed]
40. Zhang, Y.; Aaron Hogan, J.; Crowther, T.W.; Xu, S.; Zhao, R.; Song, P.; Cui, M.; Song, X.; Cao, M.; Yang, J. Drivers and mechanisms that contribute to microbial β-diversity patterns and range sizes in mountains across a climatic variability gradient. *Ecography* **2024**, *2024*, e07049. [CrossRef]
41. Corneo, P.E.; Pellegrini, A.; Cappellin, L.; Roncador, M.; Chierici, M.; Gessler, C.; Pertot, I. Microbial community structure in vineyard soils across altitudinal gradients and in different seasons. *FEMS Microbiol. Ecol.* **2013**, *84*, 588–602. [CrossRef] [PubMed]
42. Siles, J.A.; Cajthaml, T.; Minerbi, S.; Margesin, R.; Max, H. Effect of altitude and season on microbial activity, abundance and community structure in Alpine forest soils. *FEMS Microbiol. Ecol.* **2016**, *92*, 1. [CrossRef] [PubMed]
43. Zhao, Y.; Zhou, Y.; Jia, X.; Han, L.; Liu, L.; Ren, K.; Ye, X.; Qu, Z.; Pei, Y. Soil characteristics and microbial community structure on along elevation gradient in a *Pinus armandii* forest of the Qinling Mountains, China. *For. Ecol. Manag.* **2022**, *503*, 119793. [CrossRef]
44. Ivashchenko, K.; Sushko, S.; Selezneva, A.; Ananyeva, N.; Zhuravleva, A.; Kudeyarov, V.; Makarov, M.; Blagodatsky, S. Soil microbial activity along an altitudinal gradient: Vegetation as a main driver beyond topographic and edaphic factors. *Appl. Soil Ecol.* **2021**, *168*, 104197. [CrossRef]
45. Xie, L.; Li, W.; Pang, X.; Liu, Q.; Yin, C. Soil properties and root traits are important factors driving rhizosphere soil bacterial and fungal community variations in alpine *Rhododendron nitidulum* shrub ecosystems along an altitudinal gradient. *Sci. Total Environ.* **2023**, *864*, 161048. [CrossRef] [PubMed]
46. Zhalnina, K.; Dias, R.; de Quadros, P.D.; Davis-Richardson, A.; Camargo, F.A.; Clark, I.M.; McGrath, S.P.; Hirsch, P.R.; Triplett, E.W. Soil pH determines microbial diversity and composition in the park grass experiment. *Microb. Ecol.* **2015**, *69*, 395–406. [CrossRef] [PubMed]
47. Lynn, T.M.; Ge, T.; Yuan, H.; Wei, X.; Wu, X.; Xiao, K.; Kumaresan, D.; Yu, S.S.; Wu, J.; Whiteley, A.S. Soil Carbon-fixation rates and associated bacterial diversity and abundance in three natural ecosystems. *Microb. Ecol.* **2017**, *73*, 645–657. [CrossRef] [PubMed]
48. Liu, S.; Wang, H.; Tian, P.; Yao, X.; Sun, H.; Wang, Q.; Delgado-Baquerizo, M. Decoupled diversity patterns in bacteria and fungi across continental forest ecosystems. *Soil Biol. Biochem.* **2020**, *144*, 107763. [CrossRef]
49. Rappaport, H.B.; Oliverio, A.M. Extreme environments offer an unprecedented opportunity to understand microbial eukaryotic ecology, evolution, and genome biology. *Nat. Commun.* **2023**, *14*, 4959. [CrossRef] [PubMed]
50. Nielsen, U.N.; Ayres, E.; Wall, D.H.; Bardgett, R.D. Soil biodiversity and carbon cycling: A review and synthesis of studies examining diversity-function relationships. *Eur. J. Soil Sci.* **2011**, *62*, 105–116. [CrossRef]
51. Fang, Y.; Liu, J.; Yang, J.; Wu, G.; Hua, Z.; Dong, H.; Hedlund, B.P.; Baker, B.J.; Jiang, H. Compositional and metabolic responses of autotrophic microbial community to salinity in lacustrine environments. *Msystems* **2022**, *7*, e0033522. [CrossRef] [PubMed]
52. Zarzycki, J.; Brecht, V.; Müller, M.; Fuchs, G. Identifying the missing steps of the autotrophic 3-hydroxypropionate CO_2 fixation cycle in *Chloroflexus aurantiacus*. *Proc. Natl. Acad. Sci. USA* **2009**, *106*, 21317–21322. [CrossRef] [PubMed]
53. Liao, H.; Hao, X.; Qin, F.; Delgado Baquerizo, M.; Liu, Y.; Zhou, J.; Cai, P.; Chen, W.; Huang, Q. Microbial autotrophy explains large-scale soil CO_2 fixation. *Glob. Change Biol.* **2023**, *29*, 231–242. [CrossRef] [PubMed]
54. Hartmann, M.; Six, J. Soil structure and microbiome functions in agroecosystems. *Nat. Rev. Earth Environ.* **2023**, *4*, 4–18. [CrossRef]
55. Schimel, J.P.; Schaeffer, S.M. Microbial control over carbon cycling in soil. *Front. Microbiol.* **2012**, *3*, 348. [CrossRef] [PubMed]
56. Feng, J.; He, K.; Zhang, Q.; Han, M.; Zhu, B. Changes in plant inputs alter soil carbon and microbial communities in forest ecosystems. *Glob. Change Biol.* **2022**, *28*, 3426–3440. [CrossRef] [PubMed]

57. Trivedi, P.; Anderson, I.C.; Singh, B.K. Microbial modulators of soil carbon storage: Integrating genomic and metabolic knowledge for global prediction. *Trends Microbiol.* **2013**, *21*, 641–651. [CrossRef] [PubMed]
58. Tian, J.; Dungait, J.A.J.; Lu, X.; Yang, Y.; Hartley, I.P.; Zhang, W.; Mo, J.; Yu, G.; Zhou, J.; Kuzyakov, Y.; et al. Long-term nitrogen addition modifies microbial composition and functions for slow carbon cycling and increased sequestration in tropical forest soil. *Glob. Change Biol.* **2019**, *25*, 3267–3281. [CrossRef] [PubMed]
59. Hartman, W.H.; Ye, R.; Horwath, W.R.; Tringe, S.G. A genomic perspective on stoichiometric regulation of soil carbon cycling. *ISME J.* **2017**, *11*, 2652–2665. [CrossRef] [PubMed]
60. Hicks, L.C.; Lajtha, K.; Rousk, J. Nutrient limitation may induce microbial mining for resources from persistent soil organic matter. *Ecology* **2021**, *102*, e03328. [CrossRef] [PubMed]