

Article

Effects of Ozone Stress on Rhizosphere Soil of Poplar Seedlings

Qin Wang ^{1,*}, Qingqing Yang ¹, Meng Zhang ¹, Jianwei Ma ¹ and Laiye Qu ^{2,*} 

¹ School of Eco-Environment, Hebei University, Baoding 071000, China; yangqqing@stumail.hbu.edu.cn (Q.Y.); zhangmeng2@stumail.hbu.edu.cn (M.Z.); mjw@hbu.edu.cn (J.M.)

² Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing 100085, China

* Correspondence: wangqin25786@hbu.edu.cn (Q.W.); lyqu@rcees.ac.cn (L.Q.)

Abstract: Near-surface O₃ has negative effects on plant productivity; however there were few studies on the effects of O₃ pollution on the belowground part of the ecosystem. The effect of O₃ stress on the belowground parts of poplar is unclear. We investigated the effects of O₃ pollution on poplar rhizosphere soil in open-top chambers (OTC). Two kinds of plants with different O₃ sensitivity were selected, i.e., high-sensitive poplar clone 546 and low-sensitive poplar clone 107. The control group and high-concentration O₃ group were set up: charcoal-filtered air, CF; unfiltered air + 60 ppb O₃, NF. Poplar rhizosphere soil was taken after 96 days (15 June to 17 September 2020) of cultivation in OTCs. O₃ stress decreased the amplicon sequence variations (ASVs) of microorganisms in poplar 107 and poplar 546 rhizosphere soil, with no significant interspecific difference. The effect of O₃ fumigation on the fungal community was greater than that on the bacterial community. The correlation between the bacterial community and rhizosphere soil physicochemical indices was closer than that of the fungal community. Some fungi, such as *Clitopilus hobsonii*, *Mortierella* sp., and *Minimedusa*, might help poplar resist the O₃ stress. O₃ stress had direct impacts on the pH, nutrients, and enzyme activities of rhizosphere soil, while it had indirect negative impacts on microbial community composition by nutrients. There was no difference in sensitivity between rhizosphere soil response to O₃ stress of poplar clone 107 and clone 546, which might take a longer accumulation time to show the effect. This study provides a certain basis for accurately evaluating the ecological effects of O₃ pollution.

Keywords: ozone stress; rhizosphere soil; microorganism; poplar



Citation: Wang, Q.; Yang, Q.; Zhang, M.; Ma, J.; Qu, L. Effects of Ozone Stress on Rhizosphere Soil of Poplar Seedlings. *Forests* **2024**, *15*, 205. <https://doi.org/10.3390/f15010205>

Academic Editors: Baojie He, Siliang Yang, K. Venkatachalam and Amos Darko

Received: 3 December 2023

Revised: 7 January 2024

Accepted: 8 January 2024

Published: 19 January 2024



Copyright: © 2024 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

Since the 20th century, the concentration of near-surface ozone (O₃) has increased sharply due to the rapid development of cities and the gradual completion of industrialization [1,2]. From June to August 2016, ozone replaced PM₁₀ and PM_{2.5} as the primary pollutant in the three urban agglomerations of Beijing-Tianjin-Hebei, Yangtze River Delta, and Pearl River Delta in China [3]. Many studies have found that near-surface O₃ could destroy photosynthetic pigments and damage chloroplasts in plants [4,5], change carbon metabolism [6,7], affect enzyme activities [8,9] and microbial communities [10,11], which might reduce production of crops and trees, and even lead to the degradation of forest ecosystem [12].

As an air pollutant with high toxicity to plants [13–15], near-surface O₃ might cause plant-soil-microbial changes by disrupting photosynthesis and reducing antioxidant activity, thus adversely affecting plant growth [16]. In recent years, the effects of O₃ on these plant processes and aboveground resource allocation have been increasingly studied [4,7,17]. However, the mechanism of how this ubiquitous pollutant changes the nutrient cycle and microbial community in subsurface soil remains to be fully clarified since there were few studies on the effects of O₃ on subsurface processes, especially in forest ecosystems [18,19]. In those limited studies, the conclusions were not consistent. In terms of nutrient uptake, Singh et al. [20] found that O₃ had a positive effect on the content of total nitrogen and organic matter in the soil of *Michelia chapensis*, while the study of Dolker et al. [8] showed

that O₃ had a negative effect on total nitrogen and phosphorus in tropical semi-natural grassland. In terms of enzyme activities, elevated ozone decreased the extracellular enzyme and antioxidant enzyme activities in the rhizosphere soil of *L. leucocephala* [20], while it increased extracellular enzyme and antioxidant enzyme activities in the rhizosphere soil of *Elaeocarpus sylvestris* [21]. Under different conditions, O₃ increased or decreased soil microbial diversity or had no effect. Elevated O₃ elicited changes in soil microbial community structure and decreased fungal diversity in both *M. ichangensis* and *T. chinensis* [22]. Elevated O₃ significantly decreased bacterial abundance of poplar saplings [23]. However, the overall functional structure of soil microbial communities of wheat Yannong 19 and wheat Yangmai 16 did not significantly change by elevated O₃ [24].

The activity of soil extracellular enzymes was closely related to microbial metabolism and the biochemical cycle of nutrients. β -Glucosidase (β G) [25], β -N-acetylglucosaminidase (NAG) [26], cellobiose hydrolase (CBH) [27], leucine aminopeptidase (LAP), and alkaline phosphatase (AKP) [28] were key soil extracellular enzymes. The ability of key extracellular enzymes to respond to the environment was an important potential indicator to evaluate soil microbial quality [29].

More than 98% of forest area in China is threatened by O₃ pollution [4]. The fast-growing poplar plantation is the second-largest plantation in China [30]. The production physiology and morphology of poplar have been studied in-depth [31]. At present, the studies on the responses of poplars to O₃ stress mainly focused on nutrient content changes in different parts of poplar [6,32,33] and photosynthesis index [4,32,34], etc. However, there were few studies on the changes in belowground parts of poplar under O₃ stress. O₃ breaks down rapidly when exposed to plants or soil; therefore, it is unlikely that O₃ reaches biologically significant depths in the soil. Thus, we hypothesized that the effect of O₃ on the belowground part of poplar may be indirect. Therefore, in this study, two poplar genotypes with different sensitivity to O₃ were selected as the research objects, and open-top chambers (OTCs) were used to simulate the condition of O₃ stress. After 96 days of cultivation, the rhizosphere soil was sampled to determine the soil physicochemical indices and enzyme activities. At the same time, MiSeq sequencing was used to analyze the changes of bacterial and fungal communities in rhizosphere soil, thus fully understanding the response of poplar to O₃ stress.

2. Materials and Methods

2.1. Soil Collection and Sample Treatment

The experimental site is located at Yanqing Ozone Research Base in Beijing (40°47' N, 116°34' E). Poplars with different sensitivity to O₃ but similar leaf morphology and phenological characteristics [33] were selected as poplar clone '107' (*Populus. euramericana* cv. '74/76', low sensitive type) and poplar clone '546' (*P. deltoides* cv. '55/56' × *P. deltoides* cv. 'Imperial', high sensitive type). Open-top chambers (OTC) were used for fumigation, and two groups of ozone treatment were set up: charcoal-filtered air, CF; unfiltered air + 60 ppb O₃, NF. The average daily O₃ concentrations for CF and NF treatments were 22.9 ± 0.72 ppb and 85.1 ± 1.88 ppb, respectively. The four groups of treatments were marked as Y107CF, Y107NF, Y546CF, and Y546NF, respectively. Each group had 3 OTC replicates of 6 saplings. The O₃ fumigation time was 10 h per day (8:00–18:00). Poplar saplings were well watered during the trial period (15 June–17 September 2020) as needed.

The root cuttings of poplar clones '107' and '546' were planted for 5 weeks and then moved to round plastic pots filled with natural brown sandy loam. After 10 days of OTCs (12.5 m², 3.0 m high, covered with toughened glass), they were randomly assigned to OTCs for 96 days of cultivation, and the rhizosphere soil was taken from the poplars after cultivation.

The roots of poplars were taken out from the potted plants and shaken to remove the loose soil at the roots. The soil that remained attached to the roots, about 1 mm thick and defined as the rhizosphere soil [35], was removed with a sterile brush and collected. The rhizosphere soil was placed on ice for transport to the laboratory. The rhizosphere soil was

divided into two parts: one was stored at 4 °C for chemical analysis, and the other was stored at −20 °C for molecular analysis.

2.2. Determination of Rhizosphere Soil Physicochemical Indices

Soil water content (SWC) was determined by the drying method. Soil pH was determined by ST3100 soil pH meter (Ohaus, Shanghai, China) (soil water ratio 2.5:1). Soil total nitrogen (TN) and alkali-hydrolyzable nitrogen (AN) were determined by Kjeldahl method and hydrochloric acid standard solution. Soil organic matter (SOM) was determined by oxidation of potassium dichromate-sulfuric acid solution for the fresh soil samples from air drying and sieving (0.25 mm). Soil total phosphorus (TP) and total potassium (TK) were determined according to molybdenum antimony resistance colorimetry and hydrofluoric acid digestion methods. Available phosphorus (AP) and available potassium (AK) were determined by the Mo-Sb colorimetric method and flame photometer, while soil enzyme activities were determined by the microplate fluorescence method, and the selection of soil enzymes and reaction substrates (RHAWN, Shanghai, China) were shown in Table S1.

2.3. 16s rRNA and Fungal ITS Amplification for MiSeq Sequencing

16s rRNA gene of bacteria in V3-V4 region was amplified by primers 338F (5'-ACTCCTACGGGAGGCAGCAG-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3') [36]. The primers ITS1F (5'-CTTGGTCATTTAGAGGAA GTAA-3') and ITS2R (5'-GCTGCGTTCTTCATCGATGC-3') were selected to amplify the fungal ITS gene in the ITS1 region [37]. The sequencing was performed by the Illumina MiSeq PE250 sequencing platform by Majorbio Bio-pharm Technology Co., Ltd. (Shanghai, China). The original sequence data has been uploaded to the NCBI SRA database and can be obtained with the accession code PRJNA819584 for bacteria and PRJNA819497 for fungi.

2.4. Data Analysis

Excel 2020 and SPSS (23.0) were used for statistical analysis. The Venn diagram of the ASV table was processed on the I-SANGER <http://www.i-sanger.com/> (accessed on 21 August 2021) platform. The box diagram of the microbial diversity index and Mantel test were analyzed and plotted using R language.

A structural equation model (SEM) was used to evaluate the effects of elevated O₃ concentration and plant types on soil nutrients, pH, enzyme activities, and microbial community composition. The O₃ variable was built by assigning the value 1 to the NF treatment and 0 to the CF treatment. Bacterial and fungal communities were represented by the first axis of their corresponding nonmetric multidimensional scale analysis (NMDS). All variables were standardized by Z transformation (mean = 0, standard deviation = 1) using the “scale” function. The fitting degree of SEM was determined by a non-significant Chi-square test ($\chi^2 < 3$, $p > 0.05$), the goodness of fit index (GFI > 0.80), and root mean square error of approximation (RMSEA < 0.05).

3. Results

3.1. Physicochemical Indices and Enzyme Activities of Rhizosphere Soil

Table 1 showed that there was no significant difference in physicochemical indices of rhizosphere soil among poplar groups except TK, AN, and pH. The content of TK in the NF group was significantly higher than that in the CF group ($p = 0.006$), while the amount of AN in the NF group was significantly lower than that in the CF group ($p = 0.032$). The pH of Y107 in the NF group was lower than that in the CF group, while the pH of Y546 in the NF group was higher than that in the CF group, and there was a significant difference in the pH of the CF group with two different poplar genotypes.

Table 1. Physicochemical indices of rhizosphere soil of poplar clones 107 and 546.

Physicochemical Indices	Poplar Clone 107 CF Y107CF	Poplar Clone 107 NF Y107NF	Poplar Clone 546 CF Y546CF	Poplar Clone 546 NF Y546NF
SWC (%)	22.21% ± 2.75% a	21.29% ± 3.36% a	22.54% ± 1.59% a	19.78% ± 2.03% a
SOM (g/kg)	37.32 ± 1.40 a	36.74 ± 4.58 a	35.96 ± 4.49 a	34.01 ± 2.15 a
TN (g/kg)	1.61 ± 0.10 a	1.60 ± 0.08 a	1.58 ± 0.17 a	1.54 ± 0.09 a
TP (g/kg)	0.97 ± 0.02 a	0.96 ± 0.04 a	0.95 ± 0.03 a	0.91 ± 0.04 a
TK (g/kg)	18.53 ± 0.88 a	20.53 ± 0.55 b	18.55 ± 1.03 a	20.15 ± 0.87 b
AN (mg/kg)	139.72 ± 13.70 a	129.63 ± 12.50 b	138.60 ± 17.52 a	132.62 ± 9.40 b
AP (mg/kg)	6.80 ± 0.92 a	9.19 ± 4.48 a	6.48 ± 2.06 a	8.50 ± 0.48 a
AK (mg/kg)	153.46 ± 29.93 a	158.25 ± 12.68 a	147.06 ± 24.60 a	156.65 ± 19.95 a
pH	7.53 ± 0.02 a	7.25 ± 0.02 bc	7.15 ± 0.03 c	7.40 ± 0.19 ab

Different lowercase letters in the table indicate significant differences between groups ($p < 0.05$). Soil Water Content, SWC; Soil Organic Matter, SOM; Total Nitrogen, TN; Total Phosphorus, TP; Total Potassium, TK; Available Nitrogen, AN; Available Phosphorus, AP; Available Potassium, AK; Potential of Hydrogen, pH.

As shown in Figure 1, five rhizosphere soil extracellular enzymes were analyzed in this study, and only the activities of AKP and NAG in Y107NF were significantly lower than those in Y107CF. Among the five enzymes, NAG had the strongest activity, which was as high as $345.32 \text{ nmol} \cdot \text{g}^{-1} \cdot \text{h}^{-1}$ in Y107CF (Figure 1E). The activities of β G, CBH, and LAP in the rhizosphere soil of Y107NF were also lower than those of the CF group; however, the difference was not significant (Figure 1B–D). The above results indicated that O_3 fumigation decreased the enzyme activities in the rhizosphere soil of poplar clone 107. The five enzymes in the rhizosphere soil of poplar clone 546 showed no significant changes, indicating that O_3 fumigation had little effect on the enzyme activities in the rhizosphere soil of poplar clone 546.

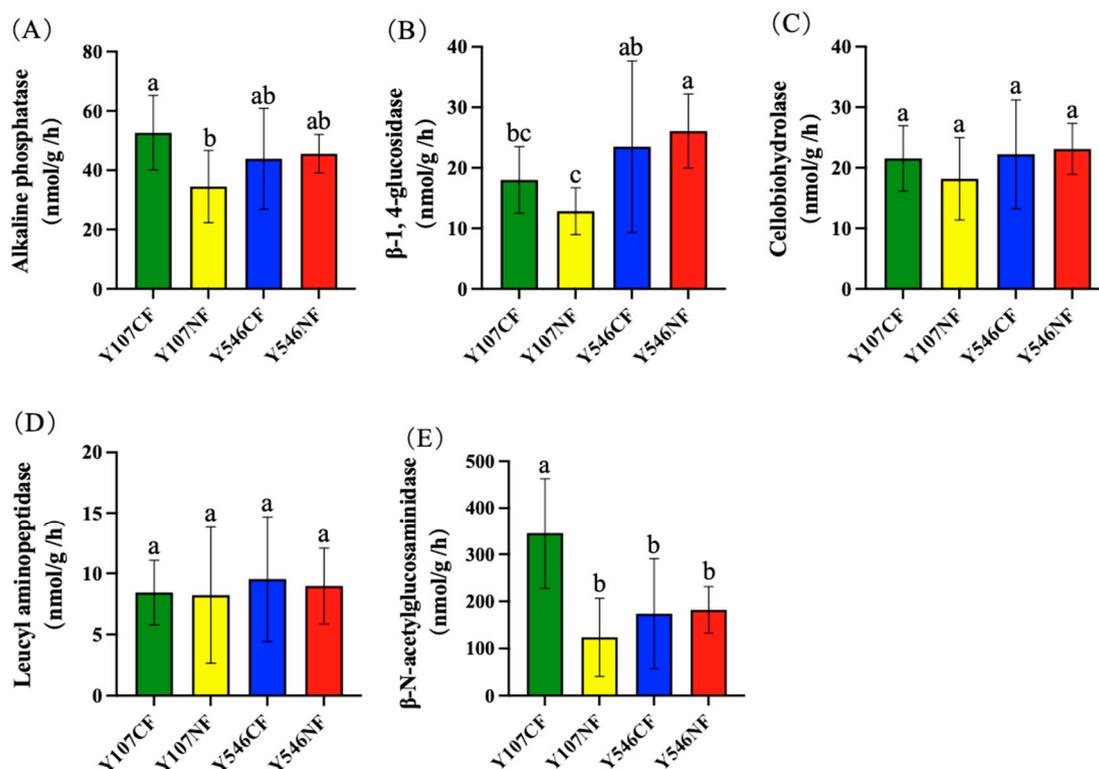


Figure 1. Activities of five enzymes in rhizosphere soil of different poplar varieties. (A) AKP, (B) β G, (C) CBH, (D) LAP, (E) NAG. Different lowercase letters in the figure indicate significant differences between groups ($p < 0.05$). The lines crossing the bars were error lines.

As illustrated in Table S2, there was a significant negative correlation between LAP and pH ($p < 0.05$) and a significant positive correlation between NAG and SOM ($p < 0.05$). There was no significant correlation between the activities of other enzymes and the measured soil physicochemical indices.

3.2. ASV Analysis of Soil Microorganisms

Amplicon sequence variations (ASVs) were widely used as the method for analyzing microbial communities, which could reach a finer classification level [38]. At the ASV level, a total of 7405 ASVs were obtained from bacterial samples after screening and flattening, and the ASVs contained in each group were 4947, 4844, 4639, and 4285, respectively (Figure 2A), while 1698 ASVs were obtained from fungal samples after screening, and the ASVs contained in each group was 1131, 1044, 1160 and 1146 respectively (Figure 2D). This showed that the increase in O₃ concentration could reduce the ASVs of poplar rhizosphere soil; however, there was no significant difference. The Venn diagram in Figure 2B showed that the four groups of bacteria shared 2331 ASVs, accounting for 31.48% of the total. The four groups of fungi shared 289 ASVs (Figure 2E), accounting for 17.02% of the total. Those shared bacterial and fungal groups played a key role in maintaining the stability of the microbial community in poplar rhizosphere soil and responding to environmental changes. The proportion of shared bacteria was one time higher than that of fungi, indicating that the effect of high concentration O₃ on bacterial community was less than that on fungal community. Among the shared ASVs, the richest 20 bacterial ASVs are shown in Figure 2C. Most of these microbial floras came from *Proteobacteria*, *Actinobacteriota*, *Acidobacteriota*, *Gemmatimonadota*, and *Nitrospirae*. The top 20 most abundant fungi ASVs are shown in Figure 2F. The above fungal communities belonged to *Ascomycota*, *Mortierellomycota*, *Rozellomycota*, and *Basidiomycota*.

3.3. Analysis of Soil Microbial Diversity

Soil microbial diversity is one of the key factors affecting the structure and function of the belowground ecosystem [39]. The boxplot (Figure 3A) showed that there was no significant difference in the diversity, richness, and evenness of bacteria in poplar rhizosphere soil. The average levels of the Chao1 index and Shannon index of the NF group were lower than those of the CF group, while the Simpson index of the NF group was slightly higher than that of the CF group. The results indicated that the increased O₃ concentration caused a decrease in the diversity and richness of bacteria in poplar rhizosphere soil; however, with little effect.

The results of fungal α -diversity indices (Figure 3B) showed that the increase of O₃ resulted in a significant difference in the Chao1 index between Y107NF and Y546NF. The increase of O₃ concentration significantly decreased the Simpson index of highly sensitive poplar clone 546, indicating that the soil fungal diversity of poplar clone 546 increased significantly. Shannon index also showed a similar trend but with no significant difference in the results. There was no significant difference in the Shannoneven index among the four groups. The results showed that the effect of O₃ fumigation on the fungal diversity in poplar rhizosphere soil was greater than that on the bacterial diversity.

3.4. Community Composition and Species Difference of Soil Bacterial Community

At the phylum level, after averaging the relative abundance of each kind of bacteria in the sample, there were 12 phyla with a relative abundance of more than 1% (Figure 4A–L). Among them, only the relative abundance of *Methylomirabilota* changed significantly and increased with the increase of O₃ concentration ($p < 0.05$, Figure 4L). Most of the bacteria in *Methylomirabilota* are related to the nitrogen cycle. The interaction between the changes in tree species and the increase of O₃ concentration significantly affected the relative abundance of *Actinobacteriota* and *Chloroflexi* (Figure 4C,D). Overall, the O₃ concentration had little effect on the relative abundance of bacteria.

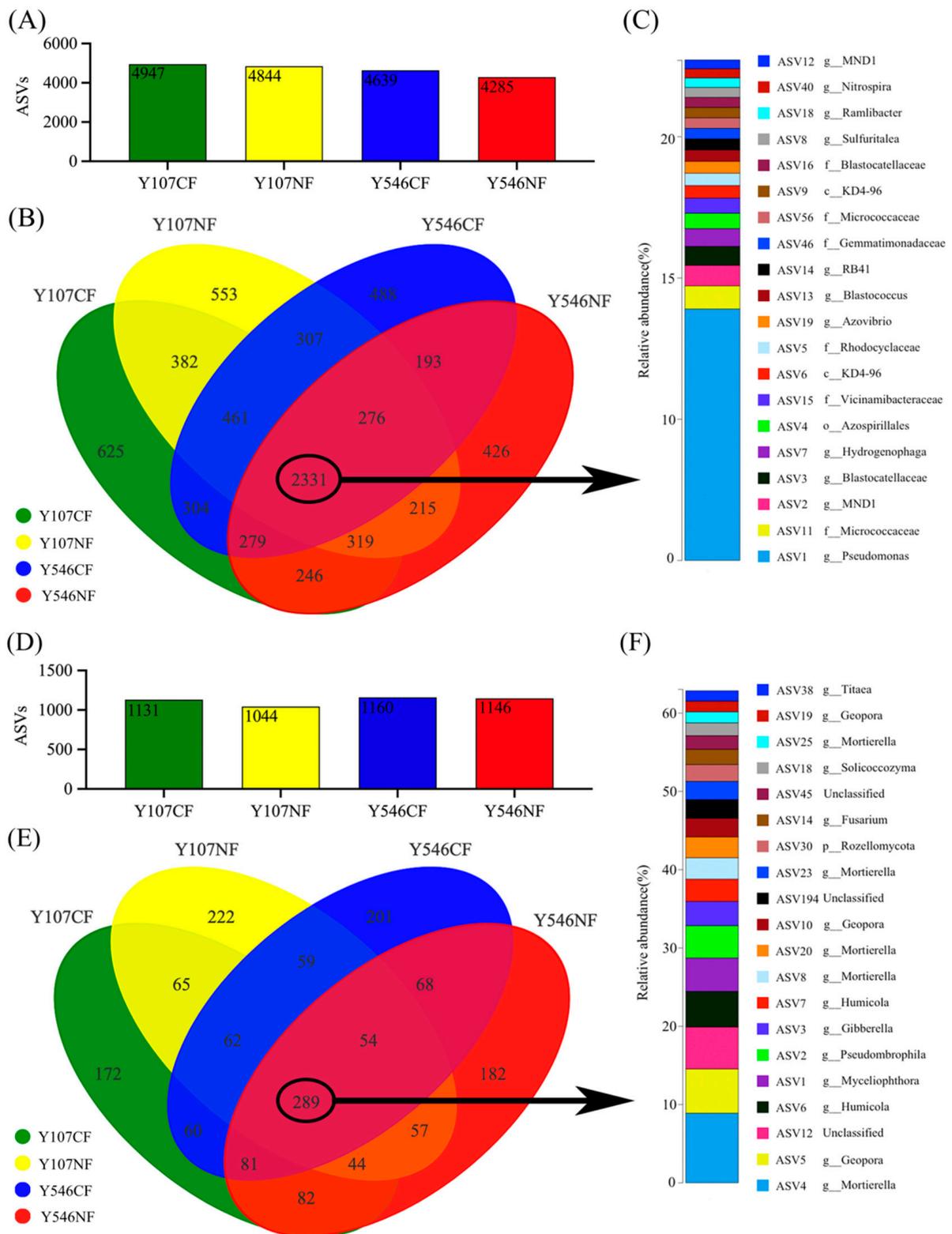


Figure 2. Venn diagrams for distribution of microbial ASV levels and the list of the top 20 ASVs shared in all treatments. (A) The number of bacterial ASVs in each group, (B) the Venn diagram of bacteria, (C) the top 20 ASVs of shared bacterial ASVs, (D) the number of fungal ASVs in each group, (E) the Venn diagram of fungi, (F) the top 20 ASVs of common fungal ASVs.

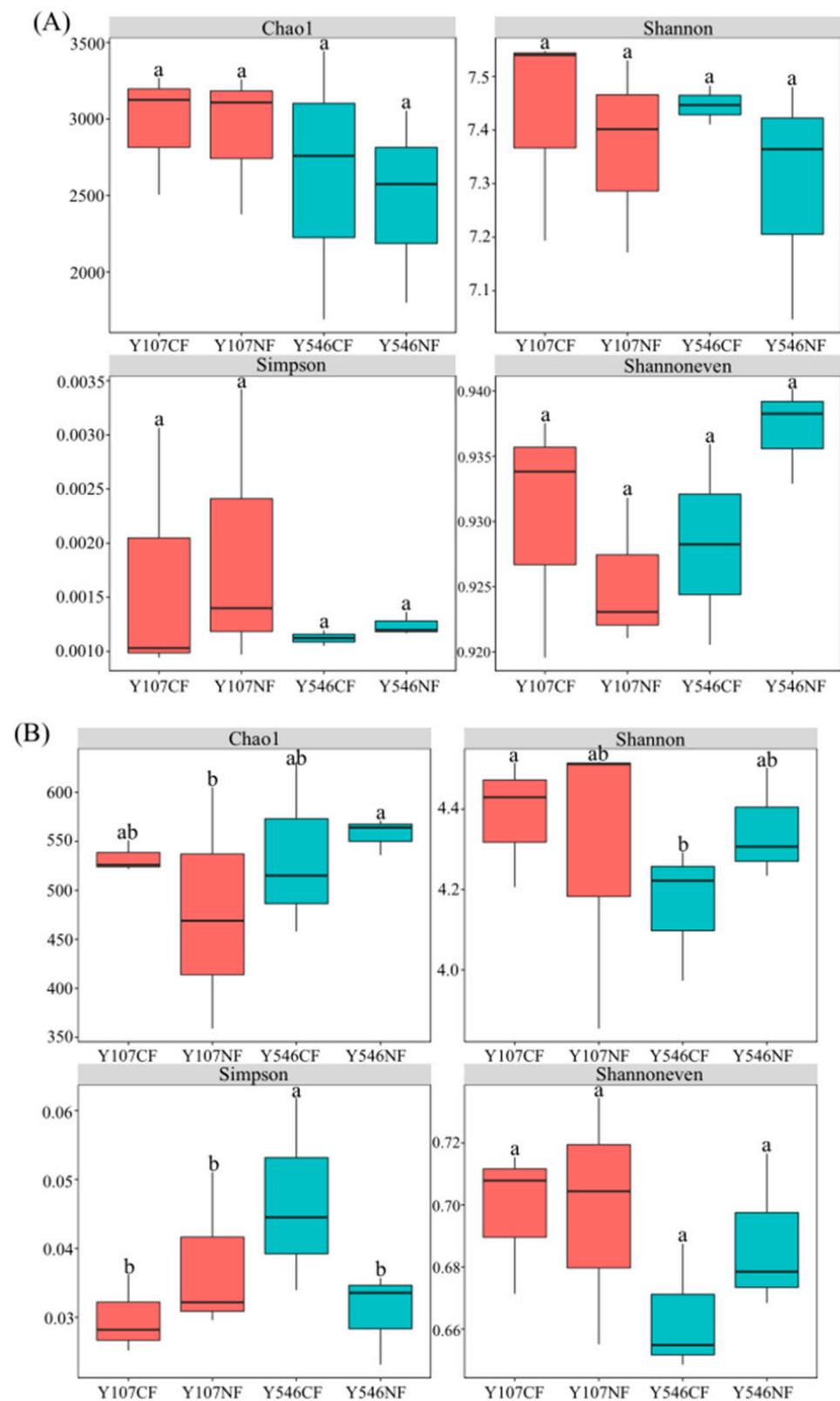


Figure 3. The α -diversity indices of soil microbial. **(A)** bacteria, **(B)** fungi. Different lowercase letters in the figure indicate significant differences between groups ($p < 0.05$).

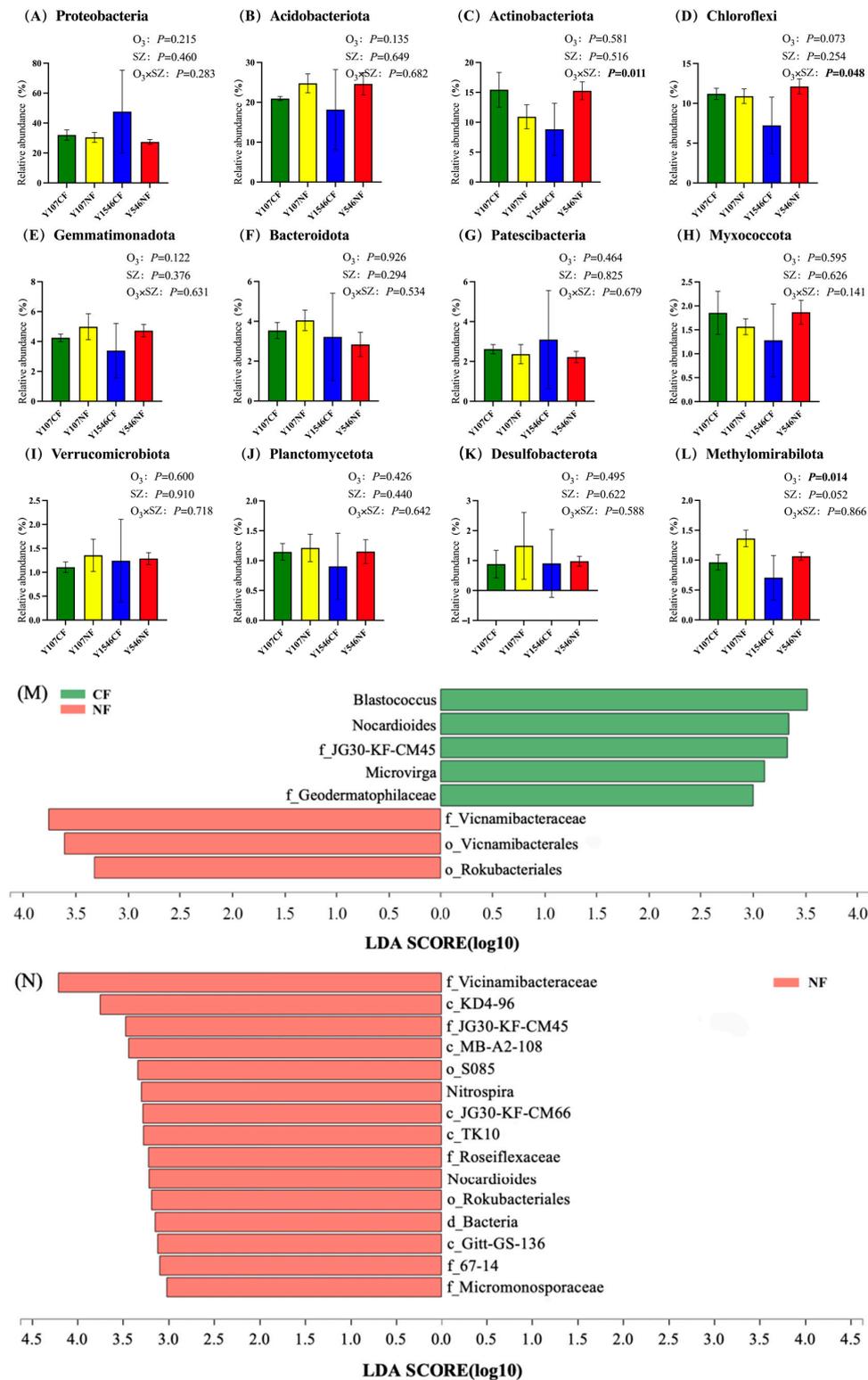


Figure 4. Relative abundance of main bacteria phyla in rhizosphere soil of each group (%) and LefSe analysis of soil bacterial community at the genus level. SZ in (A–L) represents the tree species. The boldface p values indicate significant treatment effects ($p < 0.05$) ($n = 3$ OTCs per treatment group). Error bars denote standard error. (M) poplar clone 107, (N) poplar clone 546. The lines crossing the bars were error lines.

At the genus level, LEfSe (linear discriminant analysis effect size) was used to analyze the differences among different treatments. Compared with the CF group, the relative abundances of *Vicinamibacteraceae*, *Vicinamibacterales*, and *Rokubacteriales* in the high-concentration O₃ treatment (NF) increased significantly in poplar clone 107 (Figure 4M). In the NF group of poplar clone 546, the relative abundance of *Vicinamibacteraceae*, KD4-96, JG30-KF-CM45, MB-A2-108, S085, *Nitrospira*, JG30-KF-CM66, TK10, *Roseiflexaceae*, *Nocardiodioides*, *Rokubacteriales*, Gitt-GS-136, 67-14, and *Micromonosporaceae* increased (Figure 4N).

3.5. Community Composition and Species Difference of Soil Fungal Community

In the phylum level of fungi, there were 8 phyla, which made up more than 90% of the total sequence. The results showed that the relative abundance of *Ascomycota* in the rhizosphere soil of poplar clone 546 was significantly higher than that of poplar clone 107 (Figure 5A). The relative abundance of *Basidiomycota* changed significantly with the increase of O₃ concentration and different tree species (Figure 5D). However, the O₃ concentration change did not cause a significant increase or decrease in these eight fungal phyla (Figure 5A–H), indicating that the increase of O₃ concentration had no significant effect on fungi communities at the phylum level in poplar rhizosphere soil.

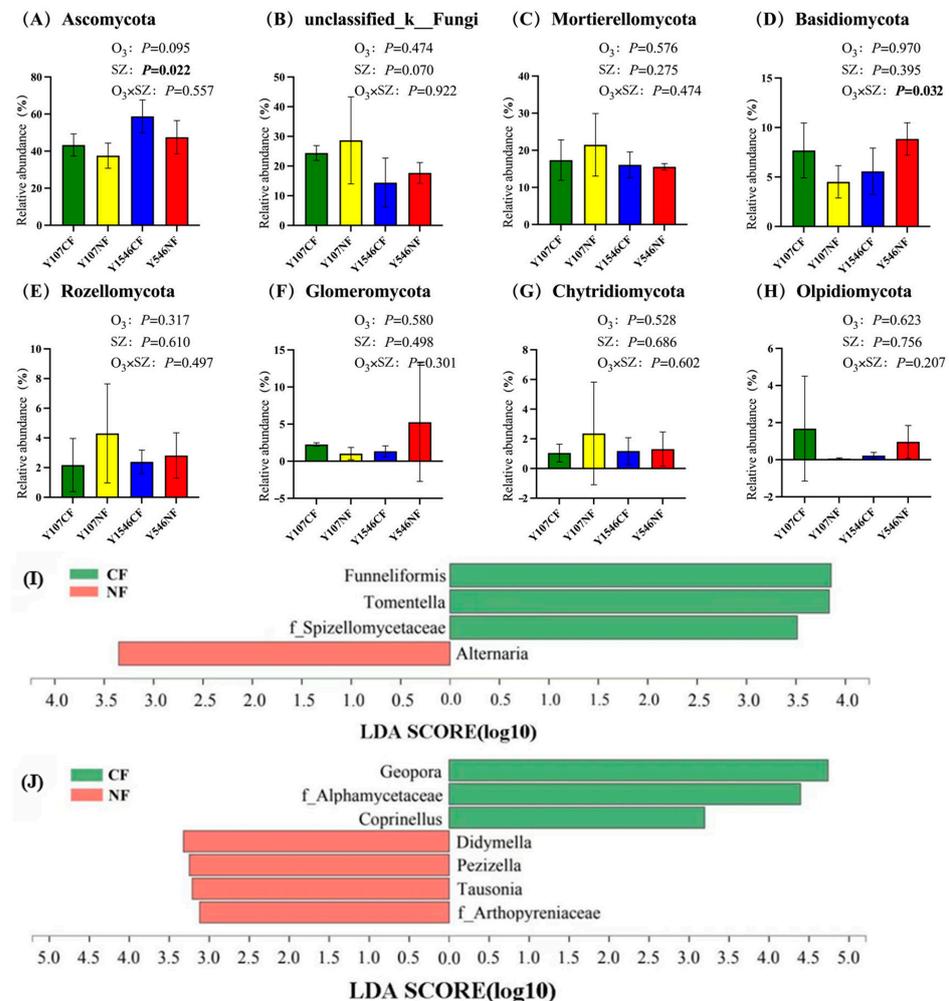


Figure 5. Relative abundance of main fungal phyla in rhizosphere soil of each group (%) and LEfSe analysis of soil fungal community at the genus level. SZ in (A–H) represents tree species. The boldface *p* values indicate significant treatment effects (*p* < 0.05) (*n* = 3 OTCs per treatment group). Error bars denote standard error. (I) poplar clone 107, (J) poplar clone 546. The lines crossing the bars were error lines.

At the fungal genus level, the LEfSe analysis showed that the relative abundance of *Alternaria* in the NF group of poplar clone 107 increased significantly (Figure 5I), and the relative abundance of *Didymella*, *Pezizella*, *Tausonia*, and *Arthopyreniaceae* in the NF group of poplar clone 546 also increased significantly (Figure 5J). During the data processing, it was found that the relative abundance of *Minimedusa* in poplar clone 107 was about 1.97% in the CF group, but it was 0 in the NF group. In addition, *Clitopilus* has not been detected in the soil of poplar clone 107, but its proportion in the CF group and the NF group of poplar clone 546 was about 1.61% and 1.04%, respectively.

3.6. Correlation Analysis of Soil Indices and Microbial Composition

Redundancy analysis (RDA) on the soil indices was carried out at the genus level of bacteria and fungi. The results showed that the first two ranking axes of bacteria explained 84.70% (84.30%, 0.40%, Figure 6A) of the total variance. The main factor affecting the change in bacterial community composition was TP ($p = 0.014$). The first two ranking axes of fungi explained 57.25% of the total variance (35.36%, 21.89%, Figure 6B), but the soil indices that affected the composition of fungal communities did not change significantly, which was consistent with the results of Mental experiment (Table S3). Spearman’s correlation analysis (Figure 6C) showed that most of the dominant bacterial genera were positively correlated with soil indices, except for TP with *Vicinamibacterales* and *Rhodocyclaceae*, while there was a significant negative correlation between AP and *Pseudomonas*. Among which SOM with *Ellin6067* and *Comamonadaceae*, TP with *Pseudomonas* and *Blastococcus*, TK with *Vicinamibacteraceae* and *Rokubacteriales*, AP with *Vicinamibacterales*, MND1, *Rhodocyclaceae* and TRA3-20, AK with *Rmlibacter* and *Comamonadaceae*, pH with *Gaiella* and *Blastococcus*, were all positively correlated. In the fungal genres (Figure 6D), unclassified_k_Fungi with about 20% (14.32%~28.60%) relative abundance had a significant positive correlation with TP, a negative correlation for *Titaea* with TP and a positive correlation for *Titaea* with AP, and a positive correlation for *Tausonia* with TK and pH.

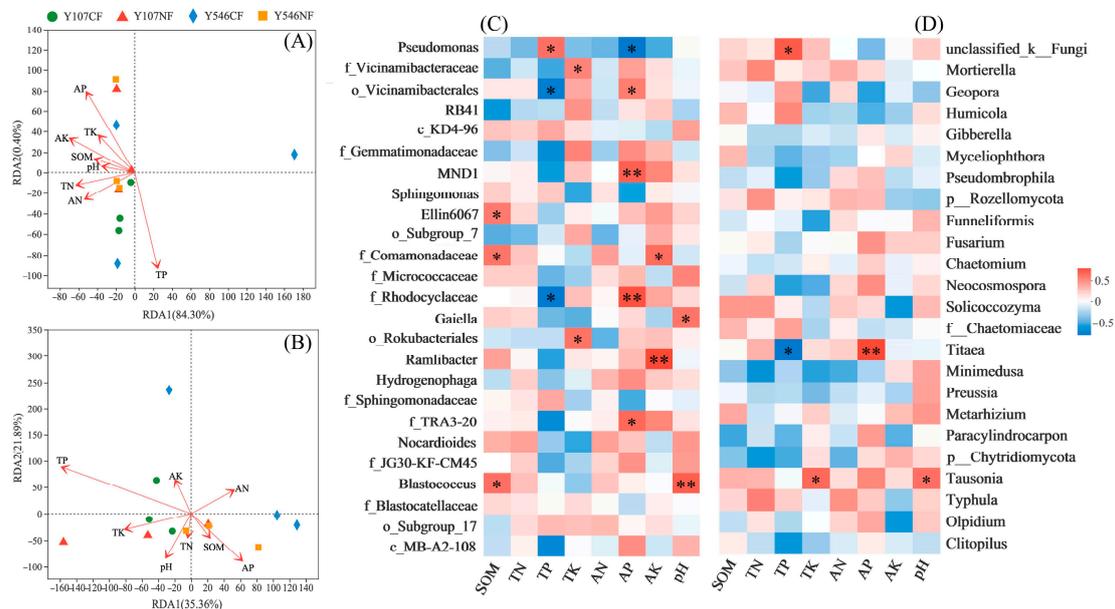


Figure 6. Correlation analysis between the rhizosphere soil indices and the microbial composition. (A) RDA analysis for the soil indices and the bacteria, (B) RDA analysis for the soil indices and the fungi, (C) Spearman correlation heatmap for the bacterial community composition and the soil indices, (D) Spearman correlation heatmap for the fungal community composition and the soil indices. * $p < 0.05$, ** $p < 0.01$.

3.7. Structural Equation Model

As shown in Figure 7, the increase in O₃ pollution had a positive effect on pH (path coefficient = 0.77, $p < 0.001$) and enzyme activity (path coefficient = 1.15, $p < 0.001$); however, it had a significantly negative effect on soil nutrients (path coefficient = -0.64 , $p < 0.001$). Soil nutrients had significantly positive effects on pH (path coefficient = 1.29, $p < 0.001$) and enzyme activity (path coefficient = 1.88, $p < 0.001$), while pH had significantly negative effects on enzyme activity (path coefficient = -1.40 , $p < 0.001$). Soil nutrients had significantly negative effects on the community composition of bacteria (path coefficient = -1.12 , $p < 0.05$) and fungi (path coefficient = -1.26 , $p < 0.05$). The type of vegetation had a negative effect on soil nutrients (path coefficient = -0.47 , $p < 0.05$), and the composition of the bacterial community had a negative effect on soil enzyme activity (path coefficient = -0.46 , $p < 0.05$).

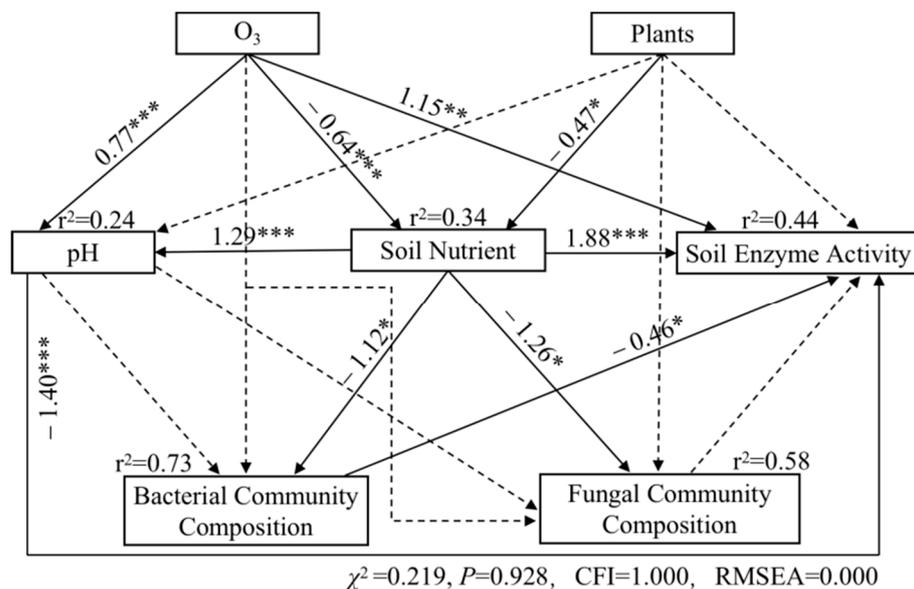


Figure 7. Structural equation model of O₃ affected poplar rhizosphere soils. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Lines illustrate all of the potential variable interactions; solid lines were significant, and dashed were not.

4. Discussion

4.1. Effects of Ozone on Physicochemical Indices and Enzyme Activities of Rhizosphere Soil

It has been confirmed that O₃ significantly reduced the biomass and net photosynthetic rate of poplar clone 107 and clone 546 [40]. In this study, the AN content of the rhizosphere soil of two poplar clones decreased significantly after O₃ fumigation ($p < 0.05$), which was consistent with the results of soil nitrogen dynamics of the studies on *T. chinensis* [22] and on *L. leucocephala* [20]. It might be due to the decrease of root biomass and root carbohydrate concentration with the increase of O₃ concentration, which resulted in the effect of photosynthate transportation to the belowground part [41]. After the O₃ fumigation, the pH changed differently.

The results of enzyme activities showed that the activity of NAG was the highest among the five enzymes, indicating that the content of available nitrogen in the soil was insufficient, and the microorganisms accelerated the decomposition of soil organic nitrogen to meet their needs [42]. The results of β G:NAG (or β G:(NAG + LAP)), β G:AKP measured the enzymatic resources directed towards the acquisition of nitrogen (N) and phosphorus (P) relative to C, reflecting the limitation of C, N, P on microbial survival [43,44]. In this study, although O₃ had no significant influence on some enzymes, the ratio of $\ln\beta$ G/ \ln (LAP + NAG) in soil decreased significantly ($p < 0.01$), indicating that the increase of O₃ could stimulate the production of more enzymes in rhizosphere soil to obtain organic nitrogen [43]. There are relatively few studies on the response of extracellular enzyme

activity to the increase of O₃ concentration; however, the results are not the same. In the study of poplar and birch forest ecosystems by [45], it was reported that the increase of O₃ had no effect on enzyme activity in the second year of treatment. Ten years later, cellobiohydrolase activity on the forest floor was affected, but *N*-acetyl-glucosaminidase was still not affected [46]. In addition, Williamson et al. [47] measured the decomposition rate of wetland plants exposed to high concentrations of O₃ and found that the response of hydrolase activity to O₃ was related to plant species. Therefore, the effects of O₃ on extracellular enzymes still need further study.

4.2. Effects of Ozone on Microorganisms in Rhizosphere Soil

According to the SEM analysis, O₃ had a significant negative effect on soil nutrients. Soil nutrients also had significant negative effects on the composition of the rhizosphere bacterial and fungal communities. Thus, the elevated O₃ had indirect negative effects on bacterial and fungal communities by soil nutrients. The interactions among plants, soils, and microorganisms played a key role in the biogeochemical cycles [48], the soil microorganisms supporting plant fitness and sustainable ecosystems [49]. A recent review by Agathokleous et al. [16] showed that O₃ altered plant properties (such as leaf and root litter input, plant secretions, and root turnover) and soil processes (such as soil enzyme activity and decomposition rate). These processes defined plant-soil-microbial interactions and plant-soil feedback, thus threatening the function of soil ecosystems. As shown in Figure 2, the increase of O₃ concentration would decrease the ASVs of poplar rhizosphere soil, however, with no significant difference. Some studies have shown that the O₃ stress could inhibit the resource allocation of plant roots [50,51], thereby limiting the level of nutrients available to microorganisms to change belowground microbial processes [52,53].

This study also found that the proportion of shared bacteria in rhizosphere soil of low-sensitive poplar clone 107 and high-sensitive poplar clone 546 was one time higher than that of shared fungi before and after O₃ fumigation, respectively. There were significant differences in the partial diversity of the fungal community. However, there were no significant differences in bacterial community diversity. Many studies [22,51,54] also found that O₃ stress could change microbial diversity. Results in this study indicated that the stability of the bacterial community in poplar rhizosphere soil was very high. The response of the fungal community to O₃ stress was greater than that of the bacterial community, which was consistent with the results of Kanerva [55], Li [24], and Phillips [56]. The increase of O₃ concentration significantly increased the fungal diversity of high-sensitive poplar clone 546, which might be due to the decrease of soil organic carbon storage caused by O₃ exposure [6,24,57], since fungi could assimilate more C into biomass than bacteria [58]. Therefore, the increase in fungal diversity might be a survival strategy to cope with limited carbon resources.

The microorganisms with high relative abundance shared among all samples (Figure 2) were insensitive to the rise of O₃ concentration, suggesting that they might play a key role in maintaining community stability and responding to environmental changes. They might promote plant growth through the production of various plant growth-promoting (PGP) molecules (such as siderophores, nitrogenases, hormones, etc.) [59,60] in order to better adapt to environmental stresses. Among them, *Pseudomonas* of *Proteobacteria* occupied many ecological niches, and its strains could survive in different environments, which could promote plant growth and improve disease resistance [61]. Strains of *Arthrobacter*, *Blastococcus*, and *Gaiella* of *Actinobacteriota* played an important role in the direct antagonism of antibiotics against fungal pathogens [60,62,63]. Strains of *Methylomirabilota*, *Azospirillales*, *Azovibrio*, and *Sphingomonas* participated in the nitrogen cycle and were related to soil nitrogen fixation and denitrification [64–69]. In the fungal community, *C. hobsonii*, a common saprophytic fungus, could promote the development of seedling roots and potassium uptake and accumulation [70]. *Mortierella* sp. could release a variety of organic acids to dissolve soil phosphorus [71]. After double inoculation with AMF in saline-alkali soil, it could enhance soil phosphatase activity and promote plant growth [72]. *Minimedusa* could

transport nutrients and concentrate trace elements such as N, P, S, K, and Ca from biological origin [73]. *Fusarium* and *Gibberella* have been proven to be capable of denitrification [74]. These fungi could help poplars to resist the O₃ stress.

The results of SEM (Figure 7) showed that O₃ fumigation had direct impacts on pH, nutrients, and enzyme activities of poplar rhizosphere soil, while O₃ fumigation had an indirect negative impact on microbial community composition due to the changed nutrients. A study by Li et al. [11] has shown that increasing O₃ indirectly affected the microbial communities of plant rhizosphere soil by increasing soil pH rather than by reducing available plant carbon sources. Therefore, the effects of O₃ on the composition and structure of microbial communities need to be further explored. In this study, the effects of O₃ on rhizosphere soil physicochemical indices and microbial community of low-sensitive poplar clone 107 and high-sensitive poplar clone 546 were not significant, which might be caused by the low O₃ fumigation level (such as shorter exposure time). The effect of O₃ on plants is a cumulative process, which may take a longer time to verify. The microbial responses to elevated O₃ were phylogenetically conserved [75]. Therefore, long-term experimental studies need to be carried out in the future, which is of great significance for the scientific evaluation of the ecological effect of O₃ on poplar.

5. Conclusions

In this study, O₃ fumigation reduced the ASVs of microorganisms in poplar rhizosphere soil. The response of the fungal community in poplar rhizosphere soil to O₃ stress was greater than that of the bacterial community. The stability of the bacterial community was very high. In general, there was no obvious difference in sensitivity between the response of poplar clone 107 and 546 rhizosphere soil to O₃ after a high concentration of O₃ fumigation for 96 days. In the fungal community, *C. hobsonii*, *Mortierella* sp., and *Minimedusa* might help poplar resist O₃ stress. O₃ stress had direct impacts on pH, nutrients, and enzyme activities of the rhizosphere soil while having an indirect negative impact on microbial community composition due to the changed nutrient. Therefore, it is of great significance to continue the study of the ecological effects of O₃ on plants. This study could provide a scientific basis for the subsequent accumulation process of O₃ influence on plants and provide some help for scientific evaluation of the ecological effect of O₃ on poplar.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/f15010205/s1>, Table S1: Measured soil enzymes and corresponding substrates. Table S2: Correlation between soil enzyme activities and soil physicochemical indices. Table S3: Mental analysis of the relationship between microbial community composition and soil indices.

Author Contributions: Q.W.: designed the experiment. Q.Y.: completed the biochemical assays, analyzed the data. M.Z.: analyzed the data. J.M.: completed parts of biochemical assays. L.Q.: designed the experiment, ran the OTCs. All authors contributed to completion of the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: This work was financially supported by the National Key Research and Development Program of China (No. 2017YFE0127700).

Data Availability Statement: Data are available upon request to the corresponding author.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Young, P.J.; Naik, V.; Fiore, A.M.; Gaudel, A.; Guo, J.; Lin, M.Y.; Neu, J.L.; Parrish, D.D.; Rieder, H.E.; Schnell, J.L.; et al. Tropospheric Ozone Assessment Report: Assessment of global-scale model performance for global and regional ozone distributions, variability, and trends. *Elem. Sci. Anthr.* **2018**, *6*, 10. [[CrossRef](#)]
2. Ziemke, J.R.; Oman, L.D.; Strode, S.A.; Douglass, A.R.; Olsen, M.A.; McPeters, R.D.; Bhartia, P.K.; Froidevaux, L.; Labow, G.J.; Witte, J.C.; et al. Trends in global tropospheric ozone inferred from a composite record of TOMS/OMI/MLS/OMPS satellite measurements and the MERRA-2 GMI simulation. *Atmos. Chem. Phys.* **2019**, *19*, 3257–3269. [[CrossRef](#)]

3. China National Environmental Monitoring Center (CNEMC). *Monthly/Quarterly Report of Air Quality of 74 Cities*; China National Environmental Monitoring Centre: Beijing, China, 2016.
4. Feng, Z.; De Marco, A.; Anav, A.; Gualtieri, M.; Sicard, P.; Tian, H.; Fornasier, F.; Tao, F.; Guo, A.; Paoletti, E. Economic losses due to ozone impacts on human health, forest productivity and crop yield across China. *Environ. Int.* **2019**, *131*, 104996. [[CrossRef](#)]
5. Li, Q.; Yang, Y.; Bao, X.; Liu, F.; Liang, W.; Zhu, J.; Bezemer, T.M.; van der Putten, W.H. Legacy effects of elevated ozone on soil biota and plant growth. *Soil Biol. Biochem.* **2015**, *91*, 50–57. [[CrossRef](#)]
6. Shang, B.; Yuan, X.Y.; Li, P.; Xu, Y.S.; Feng, Z.Z. Effects of elevated ozone and water deficit on poplar saplings: Changes in carbon and nitrogen stocks and their allocation to different organs. *For. Ecol. Manag.* **2019**, *441*, 89–98. [[CrossRef](#)]
7. Feng, Z.; Shang, B.; Gao, F.; Calatayud, V. Current ambient and elevated ozone effects on poplar: A global meta-analysis and response relationships. *Sci. Total Environ.* **2019**, *654*, 832–840. [[CrossRef](#)]
8. Dolker, T.; Mukherjee, A.; Agrawal, S.B.; Agrawal, M. Responses of a semi-natural grassland community of tropical region to elevated ozone: An assessment of soil dynamics and biomass accumulation. *Sci. Total Environ.* **2020**, *718*, 137141. [[CrossRef](#)]
9. Kou, T.J.; Lam, S.K.; Chen, D.L.; Yu, W.W. Soil urease and catalase responses to ozone pollution are affected by the ozone sensitivity of wheat cultivars. *J. Agron. Crop Sci.* **2018**, *204*, 424–428. [[CrossRef](#)]
10. Chen, Z.; Wang, X.; Shang, H. Structure and function of rhizosphere and non-rhizosphere soil microbial community respond differently to elevated ozone in field-planted wheat. *J. Environ. Sci.* **2015**, *32*, 126–134. [[CrossRef](#)]
11. Li, P.; Yin, R.; Zhou, H.; Yuan, X.; Feng, Z. Soil pH drives poplar rhizosphere soil microbial community responses to ozone pollution and nitrogen addition. *Eur. J. Soil Sci.* **2022**, *73*, 13186. [[CrossRef](#)]
12. Ashmore, M.R. Assessing the future global impacts of ozone on vegetation. *Plant Cell Environ.* **2005**, *28*, 949–964. [[CrossRef](#)]
13. Mills, G.; Hayes, F.; Simpson, D.; Emberson, L.; Norris, D.; Harmens, H.; Bueker, P. Evidence of widespread effects of ozone on crops and (semi-)natural vegetation in Europe (1990–2006) in relation to AOT40-and flux-based risk maps. *Glob. Chang. Biol.* **2011**, *17*, 592–613. [[CrossRef](#)]
14. Sicard, P.; Anav, A.; De Marco, A.; Paoletti, E. Projected global ground-level ozone impacts on vegetation under different emission and climate scenarios. *Atmos. Chem. Phys.* **2017**, *17*, 12177–12196. [[CrossRef](#)]
15. Burkey, K.; Agathokleous, E.; Saitanis, C.J.; Mashhaeet, A.; Hung, Y.T. Chapter 10: Ozone effects on vegetation: A walk from cells to ecosystems. In *Handbook of Environment and Waste Management*; World Scientific Publishing Co. Inc.: Singapore, 2020; pp. 357–396.
16. Agathokleous, E.; Feng, Z.; Oksanen, E.; Sicard, P.; Wang, Q.; Saitanis, C.J.; Araminiene, V.; Blande, J.D.; Hayes, F.; Calatayud, V.; et al. Ozone affects plant, insect, and soil microbial communities: A threat to terrestrial ecosystems and biodiversity. *Sci. Adv.* **2020**, *6*, eabc1176. [[CrossRef](#)] [[PubMed](#)]
17. Li, P.; Feng, Z.; Calatayud, V.; Yuan, X.; Xu, Y.; Paoletti, E. A meta-analysis on growth, physiological, and biochemical responses of woody species to ground-level ozone highlights the role of plant functional types. *Plant Cell Environ.* **2017**, *40*, 2369–2380. [[CrossRef](#)]
18. Kasurinen, A.; Keinänen, M.M.; Kaipainen, S.; Nilsson, L.O.; Vapaavuori, E.; Kontro, M.H.; Holopainen, T. Below-ground responses of silver birch trees exposed to elevated CO₂ and O₃ levels during three growing seasons. *Glob. Chang. Biol.* **2005**, *11*, 1167–1179. [[CrossRef](#)]
19. Matyssek, R.; Wieser, G.; Calfapietra, C.; de Vries, W.; Dizengremel, P.; Ernst, D.; Jolivet, Y.; Mikkelsen, T.N.; Mohren, G.M.J.; Le Thiec, D.; et al. Forests under climate change and air pollution: Gaps in understanding and future directions for research. *Environ. Pollut.* **2012**, *160*, 57–65. [[CrossRef](#)]
20. Singh, P.; Tewari, A.; Pandey, V. Changes in growth pattern and rhizospheric soil biochemical properties of a leguminous tree species *Leucaena leucocephala* under long-term exposure to elevated ozone. *3 Biotech* **2022**, *12*, 152. [[CrossRef](#)]
21. Pan, L.; Lin, W.-q.; Yu, M.; Lie, G.-w.; Xue, L.; Chen, H.-y. Effects of Elevated Ozone Concentrations on Root Characteristics and Soil Properties of *Elaeocarpus sylvestris* and *Michelia chapensis*. *Bull. Environ. Contam. Toxicol.* **2020**, *104*, 682–688. [[CrossRef](#)]
22. Chen, Z.; Maltz, M.R.; Cao, J.; Yu, H.; Shang, H.; Aronson, E. Elevated O₃ alters soil bacterial and fungal communities and the dynamics of carbon and nitrogen. *Sci. Total Environ.* **2019**, *677*, 272–280. [[CrossRef](#)]
23. Wang, Q.; Li, Z.; Li, X.; Ping, Q.; Yuan, X.; Agathokleous, E.; Feng, Z. Interactive effects of ozone exposure and nitrogen addition on the rhizosphere bacterial community of poplar saplings. *Sci. Total Environ.* **2021**, *754*, 142134. [[CrossRef](#)] [[PubMed](#)]
24. Li, X.; Deng, Y.; Li, Q.; Lu, C.; Wang, J.; Zhang, H.; Zhu, J.; Zhou, J.; He, Z. Shifts of functional gene representation in wheat rhizosphere microbial communities under elevated ozone. *Isme J.* **2013**, *7*, 660–671. [[CrossRef](#)]
25. Turner, B.L.; Hopkins, D.W.; Haygarth, P.M.; Ostle, N. β -Glucosidase activity in pasture soils. *Appl. Soil Ecol.* **2002**, *20*, 157–162. [[CrossRef](#)]
26. Olander, L.P.; Vitousek, P.M. Regulation of soil phosphatase and chitinase activity by N and P availability. *Biogeochemistry* **2000**, *49*, 175–190. [[CrossRef](#)]
27. Burke, D.J.; Weintraub, M.N.; Hewins, C.R.; Kalisz, S. Relationship between soil enzyme activities, nutrient cycling and soil fungal communities in a northern hardwood forest. *Soil Biol. Biochem.* **2011**, *43*, 795–803. [[CrossRef](#)]
28. Allison, V.J.; Condron, L.M.; Peltzer, D.A.; Richardson, S.J.; Turner, B.L. Changes in enzyme activities and soil microbial community composition along carbon and nutrient gradients at the Franz Josef chronosequence, New Zealand. *Soil Biol. Biochem.* **2007**, *39*, 1770–1781. [[CrossRef](#)]

29. Doran, J.W.; Coleman, D.C.; Bezdicek, D.F.; Stewart, B.A.; Dick, R.P. Soil enzyme activities as indicators of soil quality. *Soil Sci. Soc. Am. J.* **1994**, *58*, 107–124. [[CrossRef](#)]
30. Fang, S.-Z. Silviculture of poplar plantation in China: A review. *Ying Yong Sheng Tai Xue Bao = J. Appl. Ecol.* **2008**, *19*, 2308–2316.
31. Scarascia-Mugnozza, G.E.; Hinckley, T.M.; Stettler, R.F.; Heilman, P.E.; Isebrands, J.G. Production physiology and morphology of Populus species and their hybrids grown under short rotation. III. Seasonal carbon allocation patterns from branches. *Can. J. For. Res.* **1999**, *29*, 1419–1432. [[CrossRef](#)]
32. Shang, B.; Xu, Y.; Dai, L.; Yuan, X.; Feng, Z. Elevated ozone reduced leaf nitrogen allocation to photosynthesis in poplar. *Sci. Total Environ.* **2019**, *657*, 169–178. [[CrossRef](#)]
33. Shang, B.; Feng, Z.; Li, P.; Calatayud, V. Elevated ozone affects C, N and P ecological stoichiometry and nutrient resorption of two poplar clones. *Environ. Pollut.* **2018**, *234*, 136–144. [[CrossRef](#)] [[PubMed](#)]
34. Xu, W.; Shang, B.; Xu, Y.; Yuan, X.; Dore, A.J.; Zhao, Y.; Massad, R.-S.; Feng, Z. Effects of elevated ozone concentration and nitrogen addition on ammonia stomatal compensation point in a poplar clone. *Environ. Pollut.* **2018**, *238*, 760–770. [[CrossRef](#)]
35. Edwards, J.; Johnson, C.; Santos-Medellín, C.; Lurie, E.; Podishetty, N.K.; Bhatnagar, S.; Eisen, J.A.; Sundaresan, V. Structure, variation, and assembly of the root-associated microbiomes of rice. *Proc. Natl. Acad. Sci. USA* **2015**, *112*, E911–E920. [[CrossRef](#)] [[PubMed](#)]
36. Lv, X.C.; Jia, R.B.; Li, Y.; Chen, F.; Chen, Z.C.; Liu, B.; Chen, S.J.; Rao, P.F.; Ni, L. Characterization of the dominant bacterial communities of traditional fermentation starters for Hong Qu glutinous rice wine by means of MALDI-TOF mass spectrometry fingerprinting, 16S rRNA gene sequencing and species-specific PCRs. *Food Control* **2016**, *67*, 292–302. [[CrossRef](#)]
37. Adams, I.P.; Miano, D.W.; Kinyua, Z.M.; Wangai, A.; Kimani, E.; Phiri, N.; Reeder, R.; Harju, V.; Glover, R.; Hany, U.; et al. Use of next-generation sequencing for the identification and characterization of *Maize chlorotic mottle virus* and *Sugarcane mosaic virus* causing maize lethal necrosis in Kenya. *Plant Pathol.* **2013**, *62*, 741–749. [[CrossRef](#)]
38. Schloss, P.D. Amplicon Sequence Variants Artificially Split Bacterial Genomes into Separate Clusters. *Mosphere* **2021**, *6*, e0019121. [[CrossRef](#)]
39. Yang, Y.; Li, T.; Wang, Y.; Cheng, H.; Chang, S.X.; Liang, C.; An, S. Negative effects of multiple global change factors on soil microbial diversity. *Soil Biol. Biochem.* **2021**, *156*, 108229. [[CrossRef](#)]
40. Yin, R.; Hao, Z.; Yuan, X.; Wang, M.; Li, S.; Zhang, X.; Chen, B. Arbuscular mycorrhizal symbiosis alleviates ozone injury in ozone-tolerant poplar clone but not in ozone-sensitive poplar clone. *Sci. Total Environ.* **2023**, *894*, 165023. [[CrossRef](#)]
41. Burns, R.G. Enzyme activity in soil: Location and a possible role in microbial ecology. *Soil Biol. Biochem.* **1982**, *14*, 423–427. [[CrossRef](#)]
42. Kuzyakov, Y.; Xu, X. Competition between roots and microorganisms for nitrogen: Mechanisms and ecological relevance. *New Phytol.* **2013**, *198*, 656–669. [[CrossRef](#)]
43. Sinsabaugh, R.L.; Lauber, C.L.; Weintraub, M.N.; Ahmed, B.; Allison, S.D.; Crenshaw, C.; Contosta, A.R.; Cusack, D.; Frey, S.; Gallo, M.E.; et al. Stoichiometry of soil enzyme activity at global scale. *Ecol. Lett.* **2008**, *11*, 1252–1264. [[CrossRef](#)] [[PubMed](#)]
44. Waring, B.G.; Weintraub, S.R.; Sinsabaugh, R.L. Coenzymatic stoichiometry of microbial nutrient acquisition in tropical soils. *Biogeochemistry* **2014**, *117*, 101–113. [[CrossRef](#)]
45. Larson, J.L.; Zak, D.R.; Sinsabaugh, R.L. Extracellular Enzyme Activity Beneath Temperate Trees Growing Under Elevated Carbon Dioxide and Ozone. *Soil Sci. Soc. Am. J.* **2002**, *66*, 1848–1856. [[CrossRef](#)]
46. Edwards, I.P.; Zak, D.R. Fungal community composition and function after long-term exposure of northern forests to elevated atmospheric CO₂ and tropospheric O₃. *Glob. Chang. Biol.* **2011**, *17*, 2184–2195. [[CrossRef](#)]
47. Williamson, J.; Mills, G.; Freeman, C. Species-specific effects of elevated ozone on wetland plants and decomposition processes. *Environ. Pollut.* **2010**, *158*, 1197–1206. [[CrossRef](#)] [[PubMed](#)]
48. Tai, A.P.K.; Martin, M.V.; Heald, C.L. Threat to future global food security from climate change and ozone air pollution. *Nat. Clim. Change* **2014**, *4*, 817–821. [[CrossRef](#)]
49. Zhang, K.; Zentella, R.; Burkey, K.O.; Liao, H.-L.; Tisdale, R.H. Microbial community dynamics responding to nutrient allocation associated with soybean cultivar ‘Jake’ ozone adaptation. *Sci. Total Environ.* **2023**, *864*, 161008. [[CrossRef](#)]
50. Grantz, D.A.; Gunn, S.; Vu, H.B. O₃ impacts on plant development: A meta-analysis of root/shoot allocation and growth. *Plant Cell Environ.* **2006**, *29*, 1193–1209. [[CrossRef](#)]
51. Li, P.; Yin, R.; Shang, B.; Agathokleous, E.; Zhou, H.; Feng, Z. Interactive effects of ozone exposure and nitrogen addition on tree root traits and biomass allocation pattern: An experimental case study and a literature meta-analysis. *Sci. Total Environ.* **2020**, *710*, 136379. [[CrossRef](#)]
52. Agathokleous, E.; Saitanis, C.J.; Wang, X.; Watanabe, M.; Koike, T. A Review Study on Past 40 Years of Research on Effects of Tropospheric O₃ on Belowground Structure, Functioning, and Processes of Trees: A Linkage with Potential Ecological Implications. *Water Air Soil Pollut.* **2016**, *227*, 33. [[CrossRef](#)]
53. Andersen, C.P. Source-sink balance and carbon allocation below ground in plants exposed to ozone. *New Phytol.* **2003**, *157*, 213–228. [[CrossRef](#)] [[PubMed](#)]
54. Ueda, Y.; Frindte, K.; Knief, C.; Ashrafuzzaman, M.; Frei, M. Effects of Elevated Tropospheric Ozone Concentration on the Bacterial Community in the Phyllosphere and Rhizosphere of Rice. *PLoS ONE* **2016**, *11*, e0163178. [[CrossRef](#)] [[PubMed](#)]
55. Kanerva, T.; Palojarvi, A.; Ramo, K.; Manninen, S. Changes in soil microbial community structure under elevated tropospheric O₃ and CO₂. *Soil Biol. Biochem.* **2008**, *40*, 2502–2510. [[CrossRef](#)]

56. Phillips, R.L.; Zak, D.R.; Holmes, W.E.; White, D.C. Microbial community composition and function beneath temperate trees exposed to elevated atmospheric carbon dioxide and ozone. *Oecologia* **2002**, *131*, 236–244. [[CrossRef](#)] [[PubMed](#)]
57. Wang, J.; Hayes, F.; Turner, R.; Chadwick, D.R.; Mills, G.; Jones, D.L. Effects of four years of elevated ozone on microbial biomass and extracellular enzyme activities in a semi-natural grassland. *Sci. Total Environ.* **2019**, *660*, 260–268. [[CrossRef](#)]
58. Six, J.; Frey, S.D.; Thiet, R.K.; Batten, K.M. Bacterial and Fungal Contributions to Carbon Sequestration in Agroecosystems. *Soil Sci. Soc. Am. J.* **2006**, *70*, 555–569. [[CrossRef](#)]
59. Manzanera, M.; Narvaez-Reinaldo, J.J.; Garcia-Fontana, C.; Vilchez, J.I.; Gonzalez-Lopez, J. Genome Sequence of *Arthrobacter koreensis* 5J12A, a Plant Growth-Promoting and Desiccation-Tolerant Strain. *Genome Announc.* **2015**, *3*, e00648-15. [[CrossRef](#)]
60. Barreiro, C.; Martinez-Castro, M. Regulation of the phosphate metabolism in *Streptomyces* genus: Impact on the secondary metabolites. *Appl. Microbiol. Biotechnol.* **2019**, *103*, 1643–1658. [[CrossRef](#)]
61. Jun, S.-R.; Wassenaar, T.M.; Nookaew, I.; Hauser, L.; Wanchai, V.; Land, M.; Timm, C.M.; Lu, T.-Y.S.; Schadt, C.W.; Doktycz, M.J.; et al. Diversity of *Pseudomonas* Genomes, Including *Populus*-Associated Isolates, as Revealed by Comparative Genome Analysis. *Appl. Environ. Microbiol.* **2016**, *82*, 375–383. [[CrossRef](#)]
62. Zhao, F.; Zhang, Y.; Dong, W.; Zhang, Y.; Zhang, G.; Sun, Z.; Yang, L. Vermicompost can suppress *Fusarium oxysporum* f. sp. *lycopersici* via generation of beneficial bacteria in a long-term tomato monoculture soil. *Plant Soil* **2019**, *440*, 491–505. [[CrossRef](#)]
63. Gamalero, E.; Bona, E.; Novello, G.; Boatti, L.; Mignone, F.; Massa, N.; Cesaro, P.; Berta, G.; Lingua, G. Discovering the bacteriome of *Vitis vinifera* cv. Pinot Noir in a conventionally managed vineyard. *Sci. Rep.* **2020**, *10*, 6453. [[CrossRef](#)] [[PubMed](#)]
64. Zhang, X.; Liao, X.; Huang, L.; Shan, Q.; Hu, A.; Yan, D.; Zhang, J.; Long, X.-E. Soil profile rather than reclamation time drives the mudflat soil microbial community in the wheat-maize rotation system of Nantong, China. *J. Soils Sediments* **2021**, *21*, 1672–1687. [[CrossRef](#)]
65. Felizardo, J.P.; Muniz, M.C.; Vezzoni, M.; Cardoso, R.P.; Wasserman, J.; Padilla, R.; Migliori, A.; Anjos, R.M. Sources of sedimentary organic matter and assessment of heavy-metal levels in estuarine sediments after Funda similar to o dam breach. *Estuar. Coast. Shelf Sci.* **2021**, *261*, 107507. [[CrossRef](#)]
66. Steenhoudt, O.; Vanderleyden, J. *Azospirillum*, a free-living nitrogen-fixing bacterium closely associated with grasses: Genetic, biochemical and ecological aspects. *FEMS Microbiol. Rev.* **2000**, *24*, 487–506. [[CrossRef](#)] [[PubMed](#)]
67. Fernandez-Gonzalez, A.J.; Martinez-Hidalgo, P.; Cobo-Diaz, J.F.; Villadas, P.J.; Martinez-Molina, E.; Toro, N.; Tringe, S.G.; Fernandez-Lopez, M. The rhizosphere microbiome of burned holm-oak: Potential role of the genus *Arthrobacter* in the recovery of burned soils. *Sci. Rep.* **2017**, *7*, 6008. [[CrossRef](#)] [[PubMed](#)]
68. Patureau, D.; Zumstein, E.; Delgenes, J.P.; Moletta, R. Aerobic Denitrifiers Isolated from Diverse Natural and Managed Ecosystems. *Microb. Ecol.* **2000**, *39*, 145–152. [[CrossRef](#)] [[PubMed](#)]
69. Xu, J.; Klopper, J.W.; Huang, P.; McInroy, J.A.; Hu, C.H. Isolation and characterization of N₂-fixing bacteria from giant reed and switchgrass for plant growth promotion and nutrient uptake. *J. Basic Microbiol.* **2018**, *58*, 459–471. [[CrossRef](#)]
70. Peng, L.; Shan, X.; Yang, Y.; Wang, Y.; Druzhinina, I.S.; Pan, X.; Jin, W.; He, X.; Wang, X.; Zhang, X.; et al. Facultative symbiosis with a saprotrophic soil fungus promotes potassium uptake in American sweetgum trees. *Plant Cell Environ.* **2021**, *44*, 2793–2809. [[CrossRef](#)]
71. Walter Osorio, N.; Habte, M. Soil Phosphate Desorption Induced by a Phosphate-Solubilizing Fungus. *Commun. Soil Sci. Plant Anal.* **2014**, *45*, 451–460. [[CrossRef](#)]
72. Zhang, H.; Wu, X.; Li, G.; Qin, P. Interactions between arbuscular mycorrhizal fungi and phosphate-solubilizing fungus (*Mortierella* sp.) and their effects on *Kosteletzkya virginica* growth and enzyme activities of rhizosphere and bulk soils at different salinities. *Biol. Fertil. Soils* **2011**, *47*, 543–554. [[CrossRef](#)]
73. Pinzari, F.; Reverberi, M.; Piñar, G.; Maggi, O.; Persiani, A.M. Metabolic profiling of *Minimedusa polyspora* (Hotson) Weresub & P.M. LeClair, a cellulolytic fungus isolated from Mediterranean maquis, in southern Italy. *Plant Biosyst.* **2014**, *148*, 333–341. [[CrossRef](#)]
74. Guest, R.K.; Smith, D.W. A potential new role for fungi in a wastewater MBR biological nitrogen reduction system. *J. Environ. Eng. Sci.* **2002**, *1*, 433–437. [[CrossRef](#)]
75. Yu, Z.; Gao, Q.; Guo, X.; Peng, J.; Qi, Q.; Chen, X.; Gao, M.; Mo, C.; Feng, Z.; Wong, M.H.; et al. Phylogenetic Conservation of Soil Microbial Responses to Elevated Tropospheric Ozone and Nitrogen Fertilization. *Msystems* **2023**, *8*, e00721-22. [[CrossRef](#)] [[PubMed](#)]

Disclaimer/Publisher’s Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.