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Changes in the Soil Microbial Community Structure and Driving Factors during Post-Fire Recovery of the *Larix gmelinii* Rupr. Forest in Northern China

Hang Li [1,2], Yang Shu [3,4,*], Jiangsheng Wei [1,2,4,*], Pengwu Zhao [3,4], Mei Zhou [3,4] and Wenjie Jia [3]

1 College of Grassland, Resources and Environment, Inner Mongolia Agricultural University, Hohhot 010011, China; 2020202040011@imau.edu.cn
2 Inner Mongolia Key Laboratory of Soil Quality and Nutrient Resource, Hohhot 010011, China
3 Forestry College, Inner Mongolia Agricultural University, Hohhot 010011, China; zhaopengwu@imau.edu.cn (P.Z.); zhoumei@imau.edu.cn (M.Z.); 13722098520@163.com (W.J.)
4 National Orientation Observation and Research Station of Saihanwula Forest Ecosystem in Inner Mongolia, Chifeng 024000, China
* Correspondence: shuyang2018@imau.edu.cn (Y.S.); weijiangsheng1969@163.com (J.W.)

Abstract: Fire is crucial for shaping northern forest ecosystems and can affect soil microbial community structure. However, there are few studies on the long-term effects of forest fire disturbance on soil microbial community diversity. In this study, we employed high-throughput sequencing of 16S rRNA and ITS1 to assess variations in the abundance of bacterial and fungal communities in dominant populations at 1, 6, and 11 years post-fire. Furthermore, a comprehensive analysis was conducted to examine the relationship between soil microenvironmental changes and soil microbial communities after fire disturbances, considering soil physicochemical properties, including bulk density, moisture content, pH, organic carbon, total nitrogen, ammonium nitrogen, nitrate nitrogen, available potassium, and available phosphorus. We found that fire significantly increased soil pH, NO_3^--N, AP, and AK contents, in which the content of NO_3^--N basically recovered to the pre-fire level at 11 years after fire. The soil SOC and TN contents decreased significantly 1 year after the fire. However, compared to the unfired site, the SOC content essentially recovered 11 years after the fire, while TN content was still significantly higher 11 years after fire. Furthermore, fire changed the diversity and richness of soil microbial communities to some extent. PCoA and NMDS analyses suggested that the bacterial community structures in soil samples from different burned areas with different recovery periods exhibited similarity. However, notable differences were observed in the fungal community structures between the 1-year and 6-year post-fire study sites when compared to the unburned control site. Bacterial communities predominantly comprised Proteobacteria, Actinobacteria, and Acidobacteria, while fungal communities were mainly dominated by Ascomycota and Basidiomycota. RDA confirmed the significant roles of SOC, TN, and NO_3^--N in affecting the diversity of soil microbial communities. Therefore, our study not only enhances our understanding of the long-term effects of forest fire disturbances on soil properties and soil microbial community structure, but also provides insights for further utilizing and controlling carbon and nitrogen content to regulate soil microbial activity and accelerate the recovery process of burned areas.

Keywords: forest fire; soil properties; microbial community; high-throughput sequencing

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1. Introduction

Wildfire disturbance is a common natural phenomenon that has significant implications for boreal forest ecosystems. It can alter soil microbial communities and microbially mediated biogeochemical processes, thereby impeding vegetation regeneration in the affected area [1,2]. Incomplete data have suggested that wildfires affect approximately 1% of global forests annually, and their frequency, duration, extent, and intensity are projected to increase in response to ongoing global warming [3,4]. Due to the complicated

climatic conditions, forest fires display abruptness and unpredictability, rendering accurate forecasting and prompt containment efforts challenging. Hence, gaining insights into the ecological recovery process of burned areas has become a focal point in global change and environmental impact research.

Soil microbial involvement is critical for ecosystem energy flow and material cycling, with 80%–90% conversions of soil processes relying on their activity. Consequently, the recovery of soil ecology after a wildfire is profoundly affected by the impact of fire on soil microorganisms [5,6]. Forest fires have both direct and indirect effects on soil microorganisms. High temperatures from wildfires significantly affect the activity of soil microorganisms [7]. Soil microorganisms exhibit heightened temperature sensitivity, with bacteria having a lethal temperature threshold 40 °C higher than that of fungi, under both wet and dry conditions [8]. However, wildfires can reach temperatures ranging from 50 °C to 1500 °C, causing extensive microorganism mortality, reducing their abundance and activity, and hindering a rapid recovery to pre-fire levels [9,10]. Mabuhay [11] observed a significant reduction in the microbial biomass carbon abundance and diversity in the first year following wildfires in Japanese red pine forests. Similarly, Dooley [12] reported a mean decrease of 33.2% in bacterial abundance and 47.6% in fungal abundance owing to fire. Indirectly, fires alter soil texture factors, increase bulk density, and decrease permeability [13–15]. This directly affects aerobic microorganism activity in the soil. Most soil bacteria prefer the pH range of 5.5 to 6.5. However, fire disturbance significantly raises soil pH by introducing alkaline ions from burned plant ash. This creates a favorable habitat for soil microorganisms and enhances their activity [16–18]. Changes in post-fire soil moisture and nutrient levels also affect microbial activity. Holden [19] found that decreased soil moisture and organic matter were key factors limiting post-fire soil microbial decomposition. Consequently, investigating the long-term effects of fire disturbance on soil microbial community diversity can obtain a better understanding of post-fire soil ecological recovery.

The Daxing'anling Forest Region, a crucial component of northern forest ecosystems, demonstrates heightened sensitivity to global climate change and ranks among the regions that are most frequently affected by wildfires [20]. *Larix gmelinii*, a crucial tree species in northern forests, exhibits wide distribution in this area. It is not only the most commonly planted tree species but also pivotal for post-fire vegetation succession [21]. The area of this study was situated within the Daxing'anling Forest Region of Inner Mongolia, China. Between 1990 and 2019, 1311 forest fires occurred with a burnt area of 4.018×10^5 ha. On average, there were 45.2 fire incidents annually, contributing to an average burnt area of 1.385×10^4 ha hectares per year. Therefore, it can be imperative to investigate the recovery characteristics of soil microbial communities in Daxing'anling region forests after wildfires. This study utilized high-throughput sequencing of the 16S rDNA and ITS1 genes to analyze changes in bacterial and fungal community composition and the underlying factors in severely burned soils at different recovery periods (1, 6, and 11 years after fire) within *Larix gmelinii* Rupr. forests. The objective was to elucidate the relationship between soil microbial community structure and function and post-disturbance recovery time, thereby providing valuable insights into the restoration process of soil structure and function, and offering targeted recommendations for ecosystem restoration.

2. Materials and Methods

2.1. Overview of the Study Area

The study site was located in Genhe City, Inner Mongolia Autonomous Region, China (121°41′19″ E, 50°52′49″ N). This region exhibited a distinct cold temperate continental climate, with an annual precipitation range of 450–550 mm. It was characterized by significant diurnal temperature fluctuations, prolonged winters, and short summers. The freezing period spanned approximately nine months, resulting in an average annual temperature of −5.3 °C, making it the coldest region in China. The prevalent soil type in this area was dominated by freeze–thaw soils. The dominant arboreal vegetation included *Larix gmelinii* and *Betula platyphylla*, while the shrub layer comprised species such as

Vaccinium vitis-idaea, *Rosa davurica*, and *Rhododendron dauricum*. The herbaceous community consisted of *Carex* spp., *Epilobium angustifolium*, and *Galium aparine* [22].

2.2. Placement of Sampling Points

According to the fire suppression records maintained by Genhe Forest Industry Co., Ltd. in Inner Mongolia, the space–time substitution method was employed to select experimental zones from the burnt areas from 2019, 2014, and 2009. The distance between the different recovery years of the burned areas ranged from approximately 12 to 15 km. The burned areas had respective fire sizes of 120.15 ha, 161.93 ha, and 216.50 ha. The fires were all caused by lightning strikes and lasted for 3–5 days, and the fire intensity at the sampling sites was all heavy fire. To ensure the accuracy of the experiment, sample plots were set in areas with similar geomorphic conditions in the different recovery years of the burned areas. The severely burned areas were determined based on major characteristics such as a proportion of damaged standing trees $\geq$ 70%, consumption of the surface organic layer $\geq$ 80%, and tree scorch height $\geq$ 7 m [18]. For each recovery period, three sample plots were established within the burnt areas; the distance between each sampling site ranges from 100 to 150 m, all measuring 20 m $\times$ 20 m, totaling nine sample plots. Adjacent forest stands with identical vegetation types to the 2019 burnt area but without fire disturbances were selected as control areas. These control sample plots were designed to match the burnt areas, totaling three sample plots. Specific site details are shown in Figure 1.

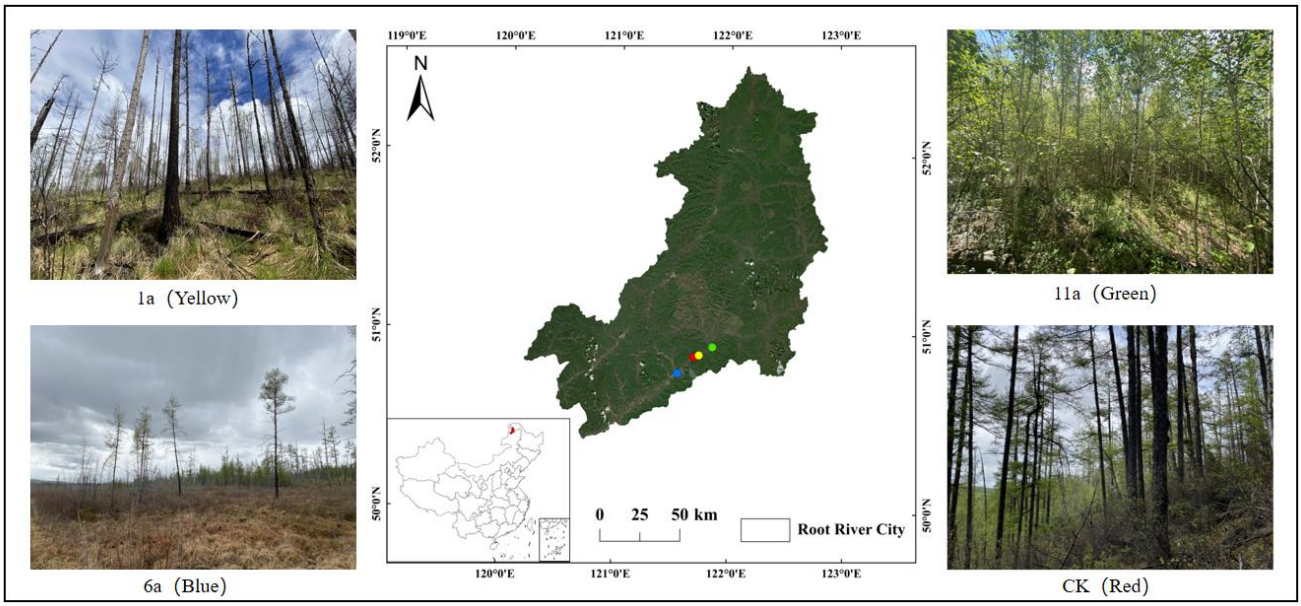

Figure 1. Location of the study area and pictures of fire samples from different restoration years. CK, unfired sample site; 1a, fire sample site 1 year after fire; 6a, fire sample site 6 years after fire; 11a, fire sample site 11 years after fire.

2.3. Sample Collection and Measurement

Sample collection was conducted in July 2020. Three soil profiles were excavated in each sample plot following an "S-shaped" pattern. A total of 36 soil samples were collected. Prior to soil sampling, the surface litter layer was cleared using an iron shovel to avoid external contamination of the samples. The soil profiles were excavated vertically with an iron shovel, and a hoe was used to remove deeply buried vegetation roots, while keeping the profiles as close to vertical as possible. Before soil collection, the litter layer was carefully removed using a shovel to prevent external contamination. The ring knife was first pressed into the soil surface layer (0–10 cm) using the shovel. Subsequently, the sample was removed, sealed in a self-sealed bag, and numbered. This numbered sample will be used for the determination of soil bulk density (BD). For other sampling of soil

physicochemical properties and soil microbial communities, soil samples (not less than 200 g) were collected from the 0–10 cm soil layer using a soil spatula, and then the soil samples were placed in sterile, self-sealing bags. The three soil profiles were combined into sterile self-contained bags, and the collected soil samples were transported to the laboratory for assays and analyses in insulated containers with ice packs. Upon arrival at the laboratory, each bag of soil samples was divided into three portions. One portion was utilized to determine soil pH, soil organic carbon (SOC), soil total nitrogen (TN), soil fast-acting phosphorus (AP), and soil potassium (AK). Another portion was stored in a refrigerator at −4 °C for the assessment of soil moisture content (SMC), soil ammonium nitrogen (NH_4^+-N), and soil nitrate (NO_3^--N). The final portion was stored at −80 °C for subsequent analysis of soil microbial community diversity.

2.3.1. Determination of Soil Physical and Chemical Properties

The methods employed in this study included the determination of soil bulk density using the Ring Knife Method, measuring soil moisture content through the drying differential method, assessing soil acidity by means of a glass electrode with a 2.5:1 soil–water ratio, quantifying soil organic carbon through potassium dichromate oxidation, analyzing soil total nitrogen utilizing the Kai-type nitrogen determination method, and employing intermittent element analyzers (CleverChem 380, Hamburg, Germany) to measure soil ammonium nitrogen and nitrate nitrogen levels. Furthermore, soil quick-acting phosphorus content was determined via sodium bicarbonate extraction followed by molybdenum antimony colorimetry, and soil quick-acting potassium content was assessed using NH_4OAc immersion-flame photometry, in accordance with established protocols for rigorous scientific analysis [23].

2.3.2. Determination of Soil Microbial Community Diversity

DNA Extraction and PCR Amplification

Total DNA of the microbial communities was extracted following the guidelines provided by the E.Z.N.A.® soil DNA kit (Omega Bio-tek, Norcross, GA, USA). The quality of DNA extraction was assessed using 1% agarose gel electrophoresis, and DNA concentration and purity were determined using a NanoDrop2000. For bacterial samples, PCR amplification of the V3–V4 variable region of the 16S rRNA gene was conducted using the primers 338F (5′-ACTCCTACGGGAGGCAGCAG-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′). For fungal samples, PCR amplification of the ITS region of the rRNA gene was carried out using the primers ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′). Each procedure was repeated three times for each sample.

High-Throughput Sequencing

PCR products from the same sample were combined and recovered using a 2% agarose gel. Subsequently, the recovered products were purified using an AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Ltd., Union City, CA, USA). A 2% agarose gel electrophoresis was conducted, and the recovered products were quantified using a Quantus™ Fluorometer (Promega, Madison, WI, USA). The library was prepared using the NEXTFLEX Rapid DNASeq Kit, and sequencing was performed using the MiSeq PE300 platform (Illumina, San Diego, CA, USA). The original data were uploaded to the NCBI SRA (Sequence Read Archive) database.

2.4. Statistical Analysis

The SPSS software (IBM, USA) was utilized to calculate the mean and standard deviation for each physical and chemical factor, as well as to perform a significance analysis. For soil microbial community diversity analysis, we utilized the Meiji BioCloud Platform (https://cloud.majorbio.com) (Accessed on 23 March 2023) and followed the following steps. Mothur software (http://www.mothur.org/wiki/Calculators) (Accessed on 23 March 2023) was employed to calculate alpha diversity index. Principal coordinate

analysis (PCoA) based on the Bray–Curtis distance algorithm at the OTU level, along with non-metric multidimensional scale analysis (NMDS), was applied to assess the similarity of microbial community structures between samples. The impacts of soil physical and chemical indices on the structure of the soil microbial community were investigated using distance-based redundancy analysis (db-RDA). Data processing and visualization were performed using Excel 2019 and Origin 2023 software (Origin Laboratories Ltd., Northampton City, MA, USA).

3. Results

3.1. Effects of Fire Disturbance on Physical and Chemical Properties

As shown in Table 1, fire disturbance significantly affected the contents of soil pH, SOC, TN, NO_3^--N, AP, and AK ($p < 0.05$). Compared to the control (CK), the contents of soil pH, NO_3^--N, AP, and AK were significantly increased one year after the fire, with a decrease in NO_3^--N, AP, and AK content at 6 years after the fire, followed by an increase at 11 years after the fire. Additionally, pH content was increased at 6 years after the fire and then decreased at 11 years after the fire. The contents of soil SOC and TN were decreased significantly 1 year after the fire, with SOC content decreasing 6 years after the fire and essentially returning to pre-fire levels 11 years after the fire. TN content was also gradually increased with the increase in post-fire recovery time. However, fire did not have a significant effect on BD, SMC, and NH_4^+-N ($p > 0.05$).

Table 1. Effects of fire disturbance on soil physicochemical properties.

Physical and Chemical Properties	CK	1a	6a	11a
BD ($g \cdot cm^{-3}$)	0.80 ± 0.02^{a}	0.95 ± 0.05^{a}	0.89 ± 0.02^{a}	0.85 ± 0.03^{a}
SMC (%)	41.27 ± 1.30^{a}	35.49 ± 1.87^{a}	38.20 ± 0.99^{a}	40.87 ± 0.48^{a}
pH	4.92 ± 0.18^{c}	5.56 ± 0.25^{ab}	5.71 ± 0.17^{a}	5.28 ± 0.15^{b}
SOC ($g \cdot kg^{-1}$)	128.95 ± 13.84^{a}	94.47 ± 6.78^{b}	78.98 ± 8.57^{c}	116.92 ± 14.70^{a}
TN ($g \cdot kg^{-1}$)	2.16 ± 0.33^{b}	1.17 ± 0.39^{c}	1.54 ± 0.28^{c}	3.04 ± 0.57^{a}
NH_4^+-N ($mg \cdot kg^{-1}$)	61.54 ± 6.11^{a}	52.29 ± 4.71^{a}	55.02 ± 3.04^{a}	64.08 ± 3.12^{a}
NO_3^--N ($mg \cdot kg^{-1}$)	1.32 ± 0.57^{b}	2.20 ± 0.15^{a}	1.16 ± 0.18^{c}	1.27 ± 0.30^{bc}
AP ($mg \cdot kg^{-1}$)	22.72 ± 1.76^{b}	29.02 ± 2.20^{a}	24.38 ± 2.75^{b}	30.41 ± 5.54^{a}
AK ($mg \cdot kg^{-1}$)	239.42 ± 50.94^{b}	270.47 ± 93.54^{ab}	206.75 ± 14.12^{c}	276.96 ± 6.96^{a}

Note: Different letters indicate significant differences between recovery years ($p < 0.05$), and the same letters indicate no significant differences between recovery years ($p > 0.05$). CK, control plot without fire disturbance; 1a, 1 year after fire disturbance; 6a, 6 years after fire disturbance; 11a, 11 years after fire disturbance. BD, bulk density; SMC, soil moisture content; pH, soil acidity/alkalinity; SOC, soil organic carbon; TN, total nitrogen; NH_4^+-N, ammonium nitrogen; NO_3^--N, nitrate nitrogen; AP, available phosphorus; AK, available potassium.

3.2. Effects of Fire Disturbance on Soil Microbial Community Diversity

3.2.1. Soil Microbes in Fire Sites of Different Recovery Years: α-Diversity

As shown in Table 2, the richness (Ace index, Chao index) of soil bacterial and fungal communities was not significantly affected by fire ($p > 0.05$). However, there was a significant difference ($p < 0.05$) in the Shannon index of soil bacterial communities between one year and eleven years after the fire, but the index of fungal communities was not significantly affected by fire ($p > 0.05$).

Table 2. Effects of fire disturbance on soil bacterial diversity.

Microbiological	Diversity Index	CK	1a	6a	11a
Bacterial	Ace	1720.05 ± 146.27 [a]	1647.79 ± 233.87 [a]	1432.25 ± 201.02 [a]	1671.9 ± 221.32 [a]
	Chao	1707.1 ± 168.46 [a]	1676.35 ± 237.73 [a]	1430.34 ± 194.77 [a]	1664.05 ± 236.49 [a]
	Shannon	5.25 ± 0.22 [ab]	5.1 ± 0.19 [b]	5.23 ± 0.22 [ab]	5.43 ± 0.26 [a]
Fungal	Ace	619.80 ± 159.07 [a]	474.62 ± 203.76 [a]	507.80 ± 150.4 [a]	566.06 ± 229.86 [a]
	Chao	553.29 ± 142.72 [a]	453.62 ± 213.88 [a]	463.41 ± 117.79 [a]	497.76 ± 183.51 [a]
	Shannon	3.05 ± 0.24 [a]	2.09 ± 0.81 [a]	2.50 ± 1.13 [a]	2.93 ± 0.45 [a]

Note: Different letters indicate significant differences between recovery years ($p < 0.05$), and the same letters indicate no significant differences between recovery years ($p > 0.05$). CK, control plot without fire disturbance; 1a, 1 year after fire disturbance; 6a, 6 years after fire disturbance; 11a, 11 years after fire disturbance.

3.2.2. Soil Microbes in Fire Sites of Different Recovery Years: β-Diversity

To further compare the changes in soil bacterial and fungal community structures across burned areas with varying recovery periods, the Bray–Curtis algorithm was employed for PCoA and NMDS analysis (Figure 2). The proximity of the study sites (Figure 2a) revealed a similarity in the soil bacterial community structures among different recovery periods. At 1 year and 6 years post-fire, these sites were distinct from the unburned site, whereas the site at 11 years post-fire was closer to the unburned site (Figure 2b). This observation indicated significant differences in the soil fungal community structure between the study sites at 1 year and 6 years post-fire when compared to the unburned site. Conversely, the soil fungal community structure at the site 11 years post-fire resembled that at the unburned site.

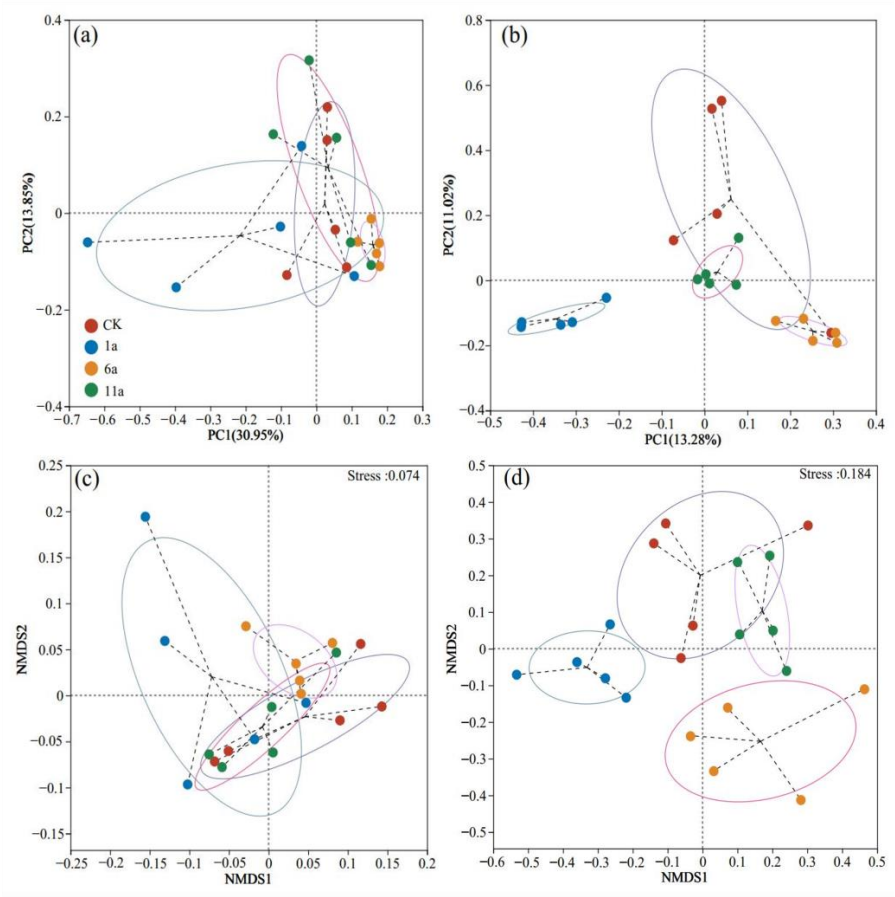

Figure 2. PCoA and NMDS analysis of bacterial (**a**,**c**) and fungal communities (**b**,**d**). CK, Control; 1a, 1 year after the fire; 6a, 6 years after the fire; 11a, 11 years after the fire.Each circle represents a different group. Dash line represents the line between the same points.

3.2.3. Effects of Fire Disturbance on Soil Microbial Community Composition

As depicted in Figure 3a,b, Venn diagrams offered a valuable tool for quantifying shared and unique operational taxonomic units (OTUs) within a sample. They provided a visual representation of OTU composition similarities and dissimilarities across various environmental samples. Between the CK site and the soil bacteria/fungi at 1-year, 6-year, and 11-year post-fire sites, we identified 1371 shared OTUs and 179 unique OTUs. At the CK site, there were 64 unique OTUs for soil bacteria and 304 for soil fungi. In the 1-year post-fire site, these numbers increased to 403 and 613, respectively. At the 6-year post-fire site, there were 37 unique OTUs in soil bacteria and 240 in soil fungi, and the 11-year post-fire site exhibited 40 unique OTUs in soil bacteria and 287 in soil fungi. This pattern indicated that short-term fire disturbance increased the number of OTUs in both soil bacteria and fungi, with a subsequent decline and subsequent rise as the recovery period was extended.

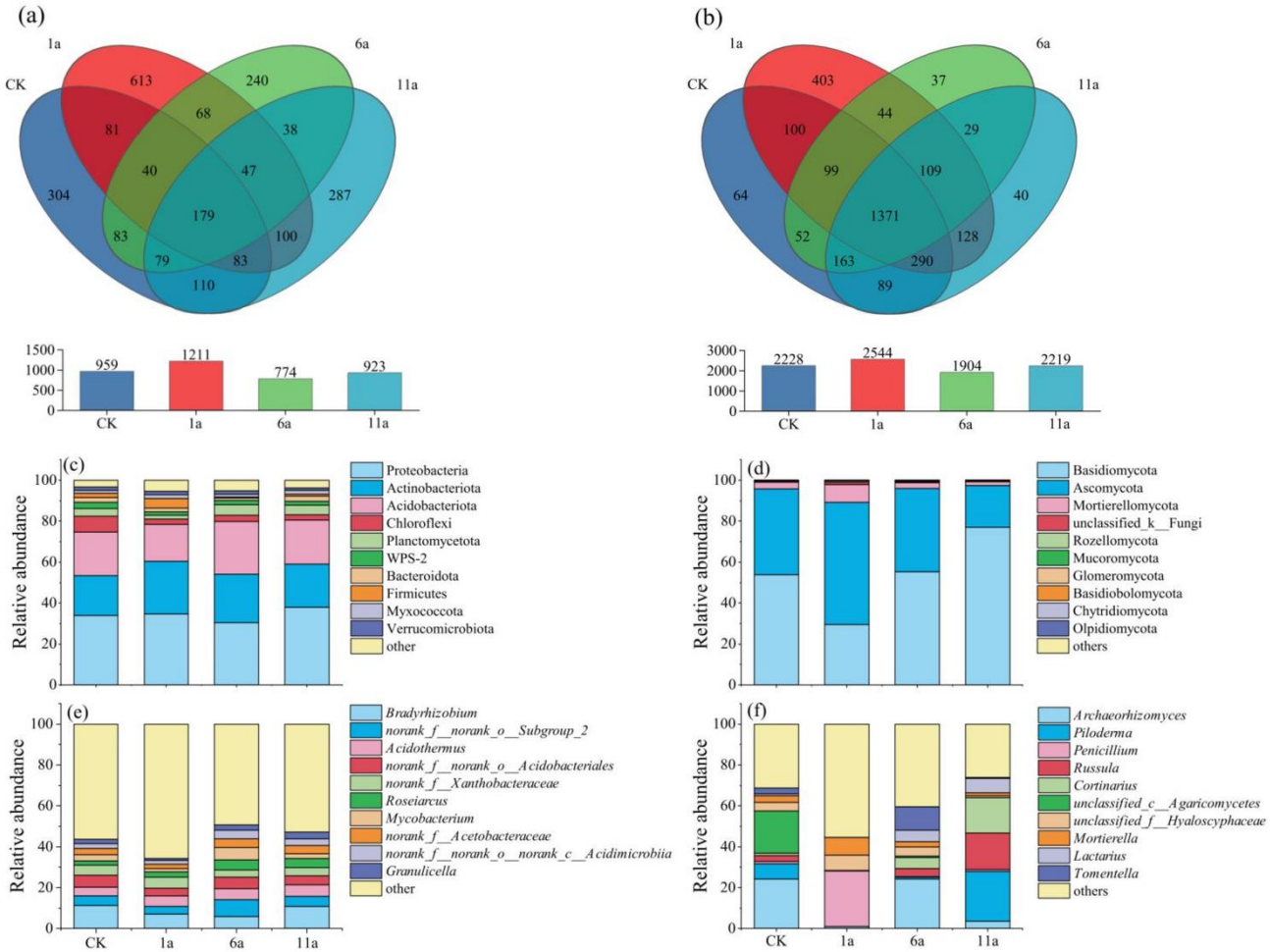

Figure 3. Venn diagram of the soil bacterial community (**a**) and the fungal community (**b**). Composition of soil bacterial communities at the phylum level (**c**) and genus level (**e**). Composition of soil fungal communities at the phylum level (**d**) and genus level (**f**). CK, Control; 1a, 1 year post-fire sample site; 6a, 6 years post-fire sample site; 11a, 11 years post-fire sample site.

An analysis of the changes in the soil microbial composition at various post-fire recovery stages revealed the identification of 25 phyla, 58 orders, 136 phyla, 214 families, and 342 genera of bacteria. In addition, 11 phyla, 37 orders, 80 phyla, 150 families, and 225 genera of fungi were identified. Sequences that could not be categorized at known taxonomic levels were grouped as "others". At the phylum level, Proteobacteria (33.95% in

CK, 34.71% in 1a, 30.43% in 6a, and 37.88% in 11a), Actinobacteria (19.44% in CK, 25.78% in 1a, 23.62% in 6a, and 21.23% in 11a), and Acidobacteria (21.41% in CK, 18.02% in 1a, 25.88% in 6a, and 21.51% in 11a) were more abundant in soil bacterial communities across burned areas with different recovery periods (Figure 3c). In contrast, Basidiomycota (50.85% in CK, 29.04% in 1a, 52.68% in 6a, and 76.87% in 11a) and Ascomycota (44.82% in CK, 59.85% in 1a, 43.46% in 6a, and 20.55% in 11a) showed higher abundances in soil fungal communities (Figure 3d). At the genus level, the most abundant bacterial genera were *Bradyrhizobium*, *norank_f__norank_o__Subgroup_2*, *Acidothermus*, *norank_f__norank_o__Acidobacteriales*, and *norank_f__Xanthobacteraceae* (Figure 3e). In addition, the predominant fungal genera in terms of abundance were *Archaeorhizomyces*, *Piloderma*, *Penicillium*, *Russula*, and *Cortinarius* (Figure 3f).

3.3. Analysis of Driving Factors of Soil Microbial Community in Fire Fields

The results of the RDA concerning the bacterial community and soil physicochemical factors (Figure 4a) revealed that the eigenvalues for the first and second axes were 0.2913 and 0.1270, respectively, which collectively explained 41.83% of the total variance. This suggested that the soil physicochemical properties effectively elucidated the relationship with the soil bacterial community. Notably, longer arrows indicated a more substantial impact, and SOC, TN, and NO_3^--N were significant factors affecting the diversity of soil bacterial communities across various post-fire recovery years. These factors exhibited explanatory percentages of NO_3^--N: 69.21%, $p = 0.01$; SOC: 49.29%, $p = 0.003$; and TN: 41.50%, $p = 0.015$ (Table 3). SOC and TN were positively correlated with soil bacterial communities in both the CK plot and the 11 years post-fire plot, whereas NO_3^--N was positively correlated with soil bacterial communities, specifically in the 1 year post-fire plot. Similarly, the RDA results of the fungal community and soil physicochemical factors (Figure 4b) showed eigenvalues of 0.7223 and 0.0371 for the first and second axes, respectively, explaining a total variance of 75.94%. This demonstrated the explanatory power of soil physicochemical properties concerning the soil fungal community relationship. Longer arrows indicated a more substantial influence, and TN emerged as a significant factor affecting the diversity of soil fungal communities across different post-fire recovery years, with an explanatory percentage of 47.47% ($p = 0.003$) (Table 3). Notably, TN was positively correlated with soil fungal communities, specifically in the 11 years post-fire plot.

Table 3. Significance of soil physicochemical properties and microbial community structure.

Microbiological	Physical and Chemical Properties	R^2	*p*
Bacterial	BD	0.1033	0.410
	SMC	0.0094	0.934
	pH	0.2335	0.101
	SOC	0.4929	0.003 **
	TN	0.4150	0.015 *
	NH_4^+N	0.2858	0.054
	NO_3^-N	0.6921	0.001 ***
	AP	0.0220	0.838
	AK	0.0875	0.463
Fungal	BD	0.1582	0.231
	SMC	0.2046	0.138
	pH	0.1295	0.292
	SOC	0.1591	0.198
	TN	0.4747	0.003 **
	NH_4^+N	0.0234	0.802
	NO_3^-N	0.1778	0.182
	AP	0.0794	0.518
	AK	0.1476	0.260

Note: Statistical significance is indicated as *, **, and *** at the levels of 0.05, 0.01, and 0.001, respectively. BD, bulk density; SMC, soil moisture content; pH, soil acidity/alkalinity; SOC, soil organic carbon; TN, total nitrogen; NH_4^+-N, ammonium nitrogen; NO_3^--N, nitrate nitrogen; AP, available phosphorus; AK, available potassium.

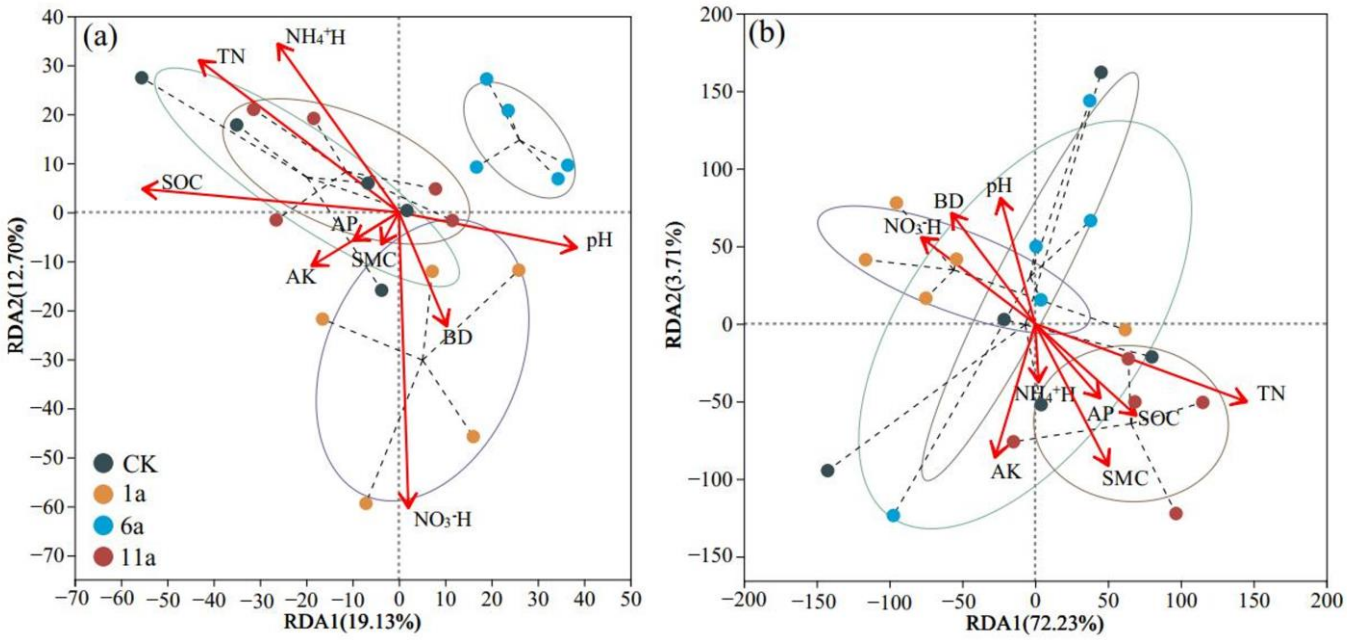

Figure 4. RDA of soil bacterial (**a**) and fungal communities (**b**). CK, control plot without fire disturbance; 1a, 1 year after fire disturbance; 6a, 6 years after fire disturbance; 11a, 11 years after fire disturbance. BD, bulk density; SMC, soil moisture content; pH, soil acidity/alkalinity; SOC, soil organic carbon; TN, total nitrogen; NH_4^+-N, ammonium nitrogen; NO_3^--N, nitrate nitrogen; AP, available phosphorus; AK, available potassium. Each circle represents a different group. Dash line represents the line between the same points. The arrows indicate environmental factors. The length of the arrow line indicates the size of the correlation between the environmental factor and the sample distribution, the longer the line, the greater the correlation, and vice versa, the smaller the correlation. The angle between the line of arrows and the sorting axis and the angle between the lines of arrows indicates the correlation, with acute angles indicating a positive correlation and obtuse angles indicating an inverse correlation. The smaller the angle, the higher the correlation.

4. Discussion

4.1. Effects of Fire on Soil Properties

Wildfire-induced high temperatures on the surface vegetation can be the primary driver of alterations in the soil physicochemical properties [24]. The impact of fire on these properties is significantly driven by the intensity, duration, and frequency of fire events. Gabbasova et al. [25] have confirmed that the slope (13°–15°) had an effect on the soil properties after the fire, which led to extensive soil loss. Although the sample plots of the present study were located on slopes, the slope did not significantly affect soil properties in our study, which might be related to the differences in the study area in terms of soil type, intensity of fire, and the size of the slope. In our study area within the *Larix gmelinii* forest in the Daxing'anling Mountains, the soil exhibited weakly acidic characteristics. After a wildfire, the ash generated from the burnt vegetation contained a high concentration of alkaline ions, including calcium and magnesium. These ions leached into the soil through rainwater, consequently elevating the soil pH levels. This finding was consistent with the previous research conducted by Scharenbroch et al. [26].

Our study revealed a decrease in SOC content after severe burning, which aligned with the findings of Granged et al. [27]. Severe fires, caused by annihilating surface vegetation, generated high temperatures during combustion, causing substantial decomposition of organic carbon in the topsoil. Furthermore, this study indicated that TN and NH_4^+-H levels remained lower than those in the control plots for the first 1 and 6 years after the fire but recovered to pre-fire levels after an 11-year period. This pattern was consistent with the observations of Wang [28]. Temperatures exceeding 500 °C during fires resulted

in the loss of nearly all aboveground nitrogen, and fire temperatures exceeding 800 °C exceeded the volatilization temperature of soil nitrogen [29]. Additionally, reduced vegetation cover and heightened post-fire surface temperature sensitivity led to nitrogen primarily existing as nitrite, which can easily be washed away by rainfall into lakes and seas, causing severe nitrogen loss and exerting long-term impacts on nitrogen recovery in the soil [30]. Wang et al. [31] reported a substantial increase in NO_3^--N levels after burning ($p < 0.05$), a result consistent with our study. The notable augmentation of nitrification enzyme activity following a fire event may be the primary factor contributing to the increase in NO_3^--N levels. After burning, the levels of AP and AK significantly increased. Fernández-García et al. [32] observed higher AP levels in the surface soil of burned areas than unburned sites after high-intensity wildfires. Similarly, Moya et al. noted a significant increase in AP content in the 0–10 cm soil layer of burned forests compared to control plots [33]. This increase was likely attributed to the leaching of nutrients from vegetation and organic matter ash into the soil, leading to an elevation in readily available nutrient content.

4.2. Effects of Fire on Soil Bacterial Communities

The high temperatures generated during a fire can lead to a substantial decrease in soil bacterial populations, consequently reducing the activity of soil bacterial communities. This study observed that the Ace index and Chao index values of soil bacterial communities in burned areas were notably lower than those in unburned control sites (Table 3). Remarkably, even after an 11-year period, the bacterial community abundance in the burned areas failed to recover to pre-fire levels. The fire disturbance caused a temporary reduction in the Shannon index of soil bacteria. However, this effect was relatively short-lived, reaching a level similar to that of the unburned control sites after 6 and 11 years. Notably, the variation in bacterial abundance among the different recovery periods in the burned areas did not yield significant differences ($p > 0.05$). This phenomenon was potentially due to the heat tolerance exhibited by soil bacteria and the recovery observed within the initial year after the fire. Furthermore, the application of PCoA and NMDS (Figure 2a,c) did not reveal discernible disparities in bacterial community structure among distinct recovery periods following the fire. The results from RDA indicated that SOC (49.29%, $p = 0.003$), TN (41.50%, $p = 0.015$), and NO_3^--N (69.21%, $p = 0.01$) possessed substantial explanatory power concerning the diversity of soil bacterial communities across various post-fire recovery periods (Figure 4a). SOC and TN played pivotal roles in governing microbial communities. They were essential carbon and nitrogen sources required for microbial growth and reproduction and exhibited positive correlations with most soil microorganisms [34,35]. Fire disturbances can cause significant losses of soil carbon and nitrogen sources, severely affecting the activity and diversity of soil microorganisms. Additionally, alteration of the soil carbon-to-nitrogen ratio induced by fire may have enduring inhibitory effects on microbial activity [36,37].

This study identified the dominant bacteria in the soil bacterial communities across various post-fire recovery periods, revealing Proteobacteria, Actinobacteria, Acidobacteria, Chloroflexi, and Planctomycetota as the prevailing taxa (Figure 3c). This observation aligned with previous research findings [38]. When soil environments are disturbed by external factors, Proteobacteria exhibit rapid responsiveness and ascend to dominance within the bacterial community. Ash resulting from vegetation combustion provides an ample nutrient source for soil microorganisms, thereby promoting the abundance of Proteobacteria and Actinobacteria in the short term, consistent with the findings of Xiang [39]. However, because rainfall washes away nutrients from the soil, it imposes limitations on the growth and reproduction of soil microorganisms. The abundance of Acidobacteria, Chloroflexi, and Planctomycetota was initially lower than those of the unburned control sites during the first year after fire. However, Acidobacteria and Planctomycetota recovered to their pre-fire levels after 6 and 11 years, respectively. Nevertheless, the abundance of Chloroflexi remained lower than that in the unburned control sites, possibly because of

the changes in soil temperature. Severe fires generated high temperatures, resulting in direct mortality of soil microorganisms. Moreover, the complete loss of surface vegetation cover after intense burning exposed the soil to direct sunlight, increasing soil temperatures compared with unburned areas with intact vegetation cover. Therefore, the abundance of Acidobacteria, Chloroflexi, and Planctomycetota in burned soil was lower during the first year after the fire. However, as the recovery progressed, vegetation in the burned area, especially shrubs, was gradually regenerated, reducing direct sunlight exposure. Additionally, the residues left by the fire had a substantial nutrient load. Consequently, Acidobacteria and Planctomycetota recovered to pre-fire levels after 6 years. Research has indicated that Chloroflexi exhibits high sensitivity to temperature and a negative correlation with soil temperature [40]. Hence, the impact of fire on Chloroflexi was more pronounced than on Acidobacteria and Planctomycetota, necessitating an extended duration for recovery.

4.3. Effects of Fire on Soil Fungal Communities

Compared to soil bacterial communities, fungal communities experienced the most significant impact from fire and required a longer recovery period [41]. This study demonstrated that fire significantly reduced the richness (as indicated by the Ace index and Chao indices) and diversity (as measured by the Shannon index) of soil fungal communities. Remarkably, even 11 years after the fire, these communities did not fully recover to their pre-fire levels (Table 3). The utilization of PCoA and NMDS (Figure 2b,d) elucidated notable disparities in the soil fungal community structure between the 1a and 6a fire-affected sites compared to the CK, whereas the fungal community structure of the 1a fire-affected site closely resembled that of the CK site. The RDA results indicated the pivotal role of TN (explaining 47.47%, $p = 0.01$) in elucidating a substantial proportion of the variation in fungal community diversity across distinct post-fire recovery years (Figure 4b). Nitrogen played a fundamental role in the growth and metabolic activity of soil fungi and contributed to several critical biochemical processes. After the fire, the level of TN in the soil was reduced, requiring 11 years to recover to pre-fire levels (Table 2). TN exhibited a positive correlation with the fungal community at the 11a fire-affected site (Figure 4b). Ascomycota and Basidiomycota consistently accounted for higher relative abundance across various post-fire recovery years within the burned areas. Although the relative abundance of these phyla may vary with different recovery periods, their dominance remains unchanged. Basidiomycota and Ascomycota persisted as the principal fungal phyla in areas subjected to varying fire intensities, which was consistent with previous studies on soil fungal community composition in *Larix gmelinii* forests within the Great Xing'an Mountains [42]. Basidiomycota frequently establish mycorrhizal associations with plant roots, enhancing the nutrient uptake and the plant growth in the soil. These fungi exhibit low resistance to environmental disturbances, as fire not only removes aboveground vegetation but also affects belowground plant roots, reducing the relative abundance of Basidiomycota following a fire event [43]. Conversely, Ascomycota, with the capacity to produce extracellular enzymes, excelled at decomposing recalcitrant substances, such as lignin and cellulose. They were notably sensitive to plant residues and debris [44]. After the fire, there was a pronounced increase in dead branches, fallen leaves, and ash from vegetation on the ground surface, increasing the relative abundance of Ascomycota. However, with the increase in recovery years, the dominance of Ascomycota gradually decreased, being replaced by Basidiomycota, which was consistent with the findings of Meng [35].

5. Conclusions

This study investigated the long-term effects of fire on soil properties and microbial communities. Our results showed that fire disturbance significantly affected the contents of soil pH, SOC, TN, NO_3^--N, AP, and AK ($p < 0.05$) in the long term, in which soil pH, TN, AP, and AK contents were increased at after 11 years of fire recovery, but NO_3^--N and SOC contents did not change after 11 years of fire recovery. Fire-induced perturbations also resulted in the substitution of original microbial communities, as more Proteobacteria and

Actinobacteria assumed dominance within bacterial populations, decreasing their relative abundance over time. Conversely, the relative abundance of Ascomycota in fungal communities increased after the fire, but gradually yielded ground to a heightened predominance of Basidiomycota during the recovery phase. The changes in the soil physicochemical environment induced by fire further affect microbial activity. The SOC, TN, and NO_3^--N were the pivotal factors governing the restoration of microbial communities. These findings offered insight into the enduring consequences of fire on soil microbial communities. In the context of fire-affected site restoration, manipulating carbon and nitrogen content to regulate bacterial activity and leveraging the relationships between dominant bacterial and fungal genera and plants can enhance beneficial plant growth, while inhibiting low-value plant growth. Nevertheless, further research is required to examine the intricate relationships between these biological indicators and ecosystem function.

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