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Response of Soil Physicochemical Properties and Microbial Community Composition in *Larix olgensis* Plantations to Disturbance by a Large Outbreak of Bark Beetle

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Abstract: Forests are affected by a wide range of disturbances globally, resulting in the decline or death of large areas of them. There is a lack of comparative studies on how soil properties change in forests that die under the influence of disturbances, especially considering different levels of disturbance. For this study, we took *Larix olgensis*—a major plantation forest species in northeast China—as the research object, one in which a large outbreak of bark beetle led to large-scale forest death, and set up fixed sample plots characterized by different disturbance intensities. We investigated the responses of soil physicochemical properties and microbial community compositions to different disturbance intensities through the determination of soil nutrient indices and high-throughput sequencing. The results show that there were significant differences ($p < 0.05$) in the effects of different disturbance intensities on soil physicochemical properties, where the soil moisture content, total nitrogen, total carbon, and total phosphorus in the control group were significantly higher than those in the disturbed groups. The soil pH was highest under low-intensity disturbance and the soil total potassium content was highest under high-intensity disturbance. At different disturbance intensities, the highest soil moisture content was found in the high-intensity group. *Proteobacteria*, *Actinobacteria*, *Verrucomicrobia*, *Acidobacteria*, *Candidatus*_Rokubacteria, *Chloroflexi*, *Gemmatimonadetes*, and *Thaumarchaeota* were the dominant populations with higher abundances; meanwhile, the relative abundance of *Bacteroidetes*, *Tenericutes*, and a tentatively unclassified fungus differed significantly ($p < 0.05$) across disturbance intensities. Among the dominant microbial populations, *Acidobacteria* showed a significant negative correlation with soil pH and a significant positive correlation with total potassium content, *Thaumarchaeota* showed significant positive correlations with soil moisture content and total nitrogen content, and Firmicutes and *Gemmatimonadetes* showed significant negative correlations with total carbon content in the soil. Furthermore, soil total nitrogen content was the key factor driving changes in microbial communities. The results of this study provide a scientific basis for the study of the long-term effects of tree mortality caused by insect pests on soil microbial communities and their response mechanisms, which is of great theoretical value for the establishment of scientific and effective methods for woodland restoration.

Keywords: tree mortality; high-throughput sequencing; forest ecosystem health; ecological impact of pest outbreaks

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1. Introduction

Earth's forests play a critical role in the global carbon cycle, absorbing up to 25% of human-generated CO_2 emissions annually [1,2]. However, forests are currently being impacted by a wide range of disturbances globally, including changing wildfire regimes, biotic disturbances, and climate change [3–5], which have multiple impacts on biodiversity, ecosystem service function, and the carbon cycle [6]. For example, climate change-induced bark beetle outbreaks in countries such as Canada and the United States have led to widespread tree mortality [7,8]. Upon disturbance, the total amount of dead branches and fallen logs on the forest floor increases, resulting in greater carbon inputs to the soil, thus affecting the nutrient and microbial composition of the soil [9]. Tree mortality is a fundamental process of forest succession, directly affecting changes in forest structure, species composition, nutrient cycling, and biomass accumulation rate [10–12]. Tree mortality is normally low in forests, and the results of changes in mortality are often not obvious. In recent years, global warming has resulted in higher temperatures and more frequent insect infestations [13–15]. The frequency and scale of tree mortality has increased, leading to the destruction of evergreen forests in many areas globally [16]. For this reason, it is becoming increasingly important to clarify how ecosystem components respond when forest stands are disturbed.

Coniferous forests in the northern hemisphere are more vulnerable to disturbances. After being disturbed, forest stands experience population decline, increased risk of forest fires, and increased forest mortality [17–19]. In recent years, many scholars have begun to pay more attention to the effects of disturbance factors on forest stands, conducting a series of relevant studies. In his research report, Williams [20] highlighted that warming-induced aridification has led to forest die-off. European scholars [21] have studied the relationship between soil nutrients and bark beetle outbreaks in spruce forests and concluded that increased litter increases cation and soil carbon concentrations in the forest floor. Soil carbon concentrations could explain changes in other soil component concentrations. Morehouse et al. [22] and Griffin et al. [23] have suggested that insect infestations resulting in the death of large numbers of conifer species increase the total amount of dead branches and fallen logs which, in turn, affects soil nutrient inputs. In a long-term monitoring study, Sandén et al. [24] found that soil carbon stocks did not change over for up to 8 years after insect infestations had resulted in the death of birch forests. Trahan et al. [25] and Marschner et al. [26] have concluded that insect infestations causing widespread tree mortality can lead to a sharp decline in soil nutrient content in the short-term. Recent research on the effects of tree mortality on soil nutrients has focused on how nutrients change after death, but there remains a lack of research on the differences and changes in soil nutrients between different stages of tree mortality. Soil micro-organisms are an important part of the ecosystem, and play an important role in the decomposition of soil organic matter and the transformation and cycling of soil nutrients [27,28]. Their community characteristics are sensitive to changes in external environmental factors, and can thus reflect the changes in soil quality in a timely manner [29]. Bacteria play an important role in soil carbon, nitrogen, and phosphorus cycles [30], and Wang et al. [31] have concluded that changes in nitrogen content lead to a decrease in soil fungal abundance when poplar is used as a study subject. Through their study, Mayer et al. [32] found that the number of dead standing trees retained after tree mortality significantly affected the structure and composition of soil fungal communities. Fernández [33] has concluded that soil pH is one of the most influential factors determining the growth and composition of soil bacterial communities. Yao et al. [34] have concluded that adaptive responses in genes and proteins that respond to the process of elemental P changes in soil play a regulatory role in soil phosphorus effectiveness. Li et al. [35] found that tree diversity significantly increases microbial biomass and decreases microbial diversity. While microbial biomass–plant–microbial community–soil interactions can determine aboveground plant abundance and diversity [36], the relationship between their interactions is very complex, posing a challenging problem that has not yet been fully resolved and which requires more exploratory research [37].

Larix olgensis A. Henry is a tree species native to the northeast of China, and is also the main tree species for afforestation and forest regeneration in the region, having rich timber reserves (existing plantation forests cover an area of 2,038,100 hectares). Larch bark beetle (*Ips subelongatus* Motschulsky) is the main pest of China's *L. olgensis*, and can harm both healthy and weak standing trees. Since the 1880s, a large area of *L. olgensis* plantation forests have been infested by bark beetle, with the mortality rate of trees having increased significantly [38]. In this study, we investigate the surface soil physicochemical properties and microbial community structural changes in a typical *L. olgensis* plantation in northeastern China using high-throughput sequencing. We explore the correlation between soil properties and microbial communities, assessing their responses to significant bark beetle disturbances under the background of environmental changes. This research aims to provide a scientific basis by which to understand the long-term impacts of bark beetle outbreaks on soil and micro-organisms, allowing for the development of effective post-infestation forest restoration methods and informing deadwood management strategies.

2. Materials and Methods

2.1. Overview of the Study Area and Sample Plots

The experimental sample site was located in Mengjiagang forest (130°32′42″–130°52′36″ E, 46°20′16″–46°30′50″), Jiamusi city, Heilongjiang province, which is characterized by an east Asian continental monsoon climate, with an average annual temperature of 2.7 °C, average annual precipitation of 550 mm, average annual sunshine hours of 1955 h, and an average annual frost-free period of 120 d. The site is located in the western foothills of Wanda mountain, with an average elevation of 250 m. The soil in the *L. olgensis* plantation cultivation area is mostly dark brown loam, and it is a plantation base with larch as the main business. At present, it is a secondary deciduous broad-leaved mixed forest dominated by *Betula dahurica*, *Populus davidiana*, *Betula platyphylla* and *Pinus koraiensis*, with *Pinus sylvestris* var. *mogolica*- and *Larix olgensis*-dominated planted conifer forests [39].

This *L. olgensis* plantation experienced a major outbreak of bark beetles in 2020, which caused extensive tree mortality. For this study, we selected a *L. olgensis* plantation with different levels of larch bark beetle disturbance in Mengjiagang forest farm, including control (no bark beetle disturbance), low-intensity disturbance, medium-intensity disturbance, and high-intensity disturbance. Different disturbance intensities were designed according to tree mortality rates: control (0%–5% tree mortality), low intensity (10%–30% tree mortality), medium intensity (40%–60% tree mortality), and high intensity (70%–90% tree mortality); see Figure 1 and Table 1.

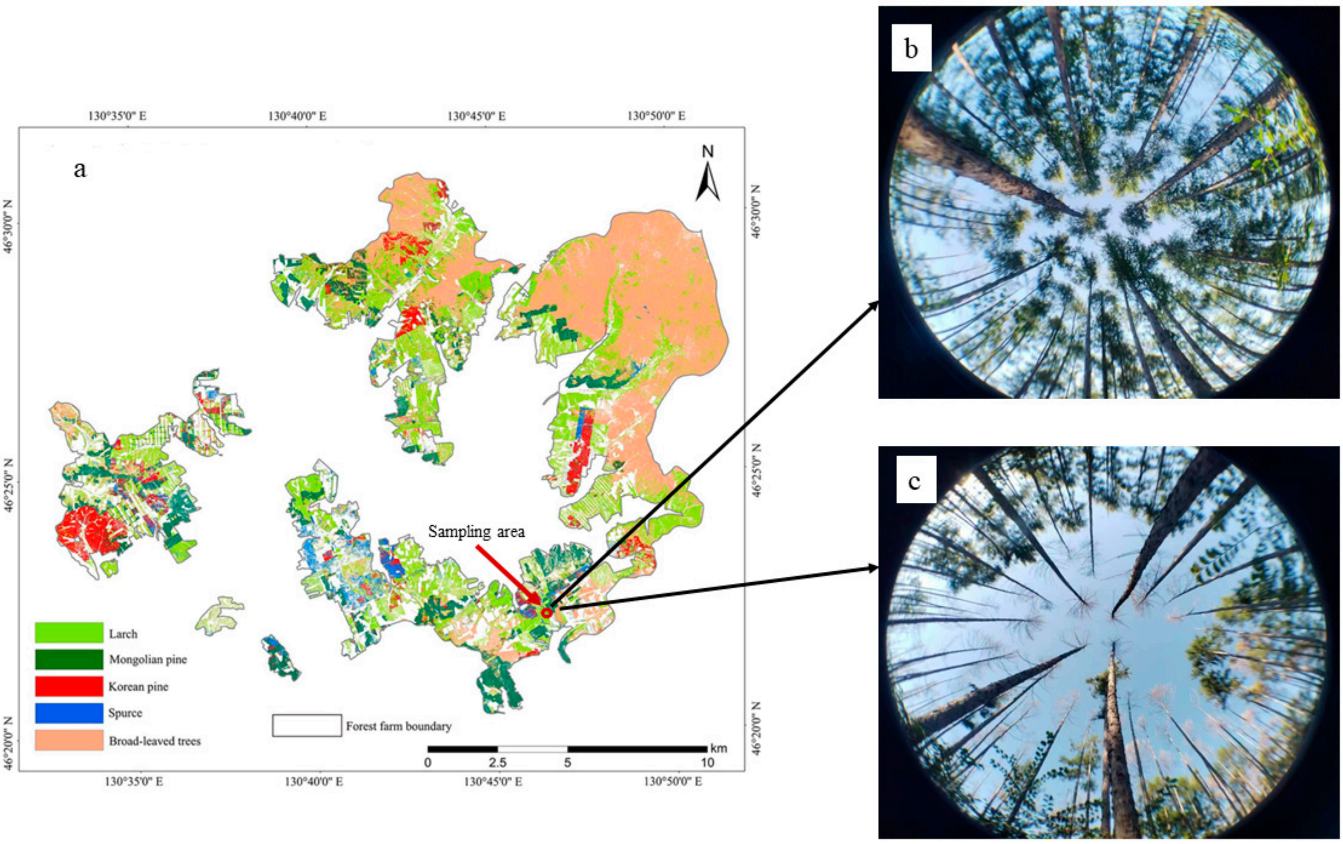

Figure 1. Study area. (**a**): location of the study area; (**b**,**c**): study plots.

Table 1. Basic characteristics of the stands in the study plots. CK: control group; LI: low intensity; MI: moderate intensity: HI: high intensity.

Damage Intensity	Planting Time	Stand Initial Density (Trees·ha^{-1})	Stand Density (Trees·ha^{-1})	Canopy Coverage (%)	Average DBH (cm)	Stand Volume (m^3·ha^{-1})
CK	1988	4440	2016	88	16.21	262
LI			1714	74	16.42	219
MI			1353	58	17.15	189
HI			1263	47	16.92	173

2.2. Sample Collection and Analysis

In October 2021, we selected 3 sample plots for each of the 4 different mortality intensities separately, for a total of 12 plots. Soil samples from 0–10 cm were taken at each sample site using a 5 cm diameter soil auger in an "S" pattern, and 5 soil samples from each sample site were mixed well and repeated 3 times, with withered material and humus in the topsoil layer being removed before sampling. Fresh soil samples were placed in an insulated box and brought back to the laboratory, plant roots and stones in the soil samples were removed and sieved through a 2 mm sieve. The soil was divided into three parts, one of which was placed in a refrigerator at 4 °C for storage and used for the measurement of soil moisture content, and another of which was air-dried, pulverized, and used for the later determination of soil pH, total carbon, total nitrogen, total phosphorus, and total potassium content. The other copy was taken in a centrifuge tube and stored in a −80 °C refrigerator for subsequent sequencing of microbial macro-genomes [40].

Soil pH was determined potentiometrically. Soil organic carbon content was determined through combustion using the solid module of the multiC/N 3000 analyzer (Anailtik Jena AG, Jena, Germany), soil total nitrogen content was determined using the liquid module of the multiN/C3000 analyzer, soil total phosphorus content was determined using the acidic solubilization-molybdenum antimony colorimetric method, and soil total potassium content was determined using the alkali fusion-flame photometer method.

Total DNA was extracted from the microbial communities of soil samples according to the instructions of the E.Z.N.A.®soil DNA kit (Omega Bio-tek, Norcross, GA, USA), and DNA concentration and purity were determined through electrophoresis on 1% agarose gels. PCR amplification of the corresponding regions was carried out with universal primers. The V3 to V4 regions of the bacterial r RNA gene were amplified using the universal primers 338F (5′-ACTCCTACGGGAGGCAGCA-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′), and the endogenous transcribed spacer region of the fungal r RNA gene was amplified using the universal primers ITS1F (CTTGGTCATTTAGAGGAAGTAA) and ITS2R (GCTGCGTTCTTCATCGATGC), The databases used are SwissProt, the Protein Information Resource (PIR), the Protein Research Foundation (PRF), and the Protein Data Bank (PDB). The PCR products were mixed and the products were detected through electrophoresis on 2% agarose gels for identification. Product purification was performed using a kit to recover the products. The library was constructed using a NEXTflexTM Rapid DNA-Seq Kit (Bioo Scientific, Austin, TX, USA), and the qualified PCR products were subjected to Illumina High-Throughput Sequencing by Shanghai Meiji BioPharmaceuticals Technology Co. (Shanghai, China). Quality control of the raw sequencing data was performed to remove the sequencing junction sequences, low-quality bases, N (N stands for uncertain base information) bases, and short sequences that seriously affect the quality of the subsequent analysis, in order to obtain high-quality control data (clean data). Then, the obtained short fragment sequences were assembled for the subsequent analysis.

2.3. Data Processing and Analysis

The data were organized using the software Excel 2016. The coefficient of variation (CV = (SD/Mean) × 100%) was calculated, which indicates the degree of fluctuation of soil physicochemical properties with the intensity of disturbances. The data on soil physicochemical properties under different mortality intensity conditions were analyzed through one-way ANOVA using the software SPSS v23, combined with the least significant difference method to test the differences between the comparison parameters. The significance level was set at $\alpha = 0.05$. α diversity was calculated and displayed using the QIIME2 and R4.0.4 software. The diversity of micro-organisms in the environment was analyzed in terms of the richness of microbial communities, reflected by the Alpha diversity of a single sample (i.e., the species richness ACE index can reflect community richness). The test of variability in the relative abundance of microbial phyla between different sample groups was performed using the Kruskal–Wallis test. The community bar plot analysis was based on the corresponding taxonomic data table and mapped using the R software. The correlation heatmap is based on the correlation coefficients between environmental factors and selected species, and R software was used to visually display the obtained numerical matrix as a Heatmap. According to the permutational multivariate analysis of variance (PERMANOVA) method, the total variance was decomposed using a half-metric or metric distance matrix to analyze the degree of explanation of the sample variance by the different environmental factors, with a larger R^2 indicating that the environmental factor indicator explains a higher degree of variance.

3. Results

3.1. Changes in Physical and Chemical Properties of Soil with Different Levels of Disturbance

Changes in soil physicochemical properties under different disturbance conditions are shown in Figure 2. Under different disturbance intensities, soil moisture content fluctuated with disturbance intensity (CV = 26%). The soil moisture content in the control group was significantly higher than that in the disturbed stands, the lowest soil moisture content was observed for the medium-intensity disturbance group, there were higher values for the low- and high-intensity disturbance groups, and no significant difference was found in soil moisture content between low- and high-intensity disturbance groups. Soil pH fluctuated least with disturbance intensity (CV = 3%) and was significantly higher in the low-intensity group than in the control group. There was no significant difference in pH between the medium- and high-intensity groups, when compared with the control group. Soil total carbon content showed a decreasing trend and large fluctuation (CV = 28%) with increasing disturbance intensity; the total carbon content in the control group was significantly higher than that for the disturbed stands. The total carbon content in the high-intensity group was the lowest, significantly differing from those of all other stands. Soil total nitrogen content presented the same trend as soil water content, showing a trend of decreasing and then increasing, and fluctuated the most with the change in disturbance intensity (CV = 31%). The soil total nitrogen content at different levels of disturbance was significantly lower than that for the control group. Compared with the control soil, soil total phosphorus content was significantly lower in the low-, medium-, and high-intensity groups, while there was no significant difference in total phosphorus content between the disturbed stands. The soil total potassium content fluctuated little with the intensity of disturbance (CV = 9%) and showed a tendency to increase with increasing disturbance level. The soil total potassium content was significantly higher in the high-intensity group than in the other stands, while that in the control group was significantly lower than in the other stands.

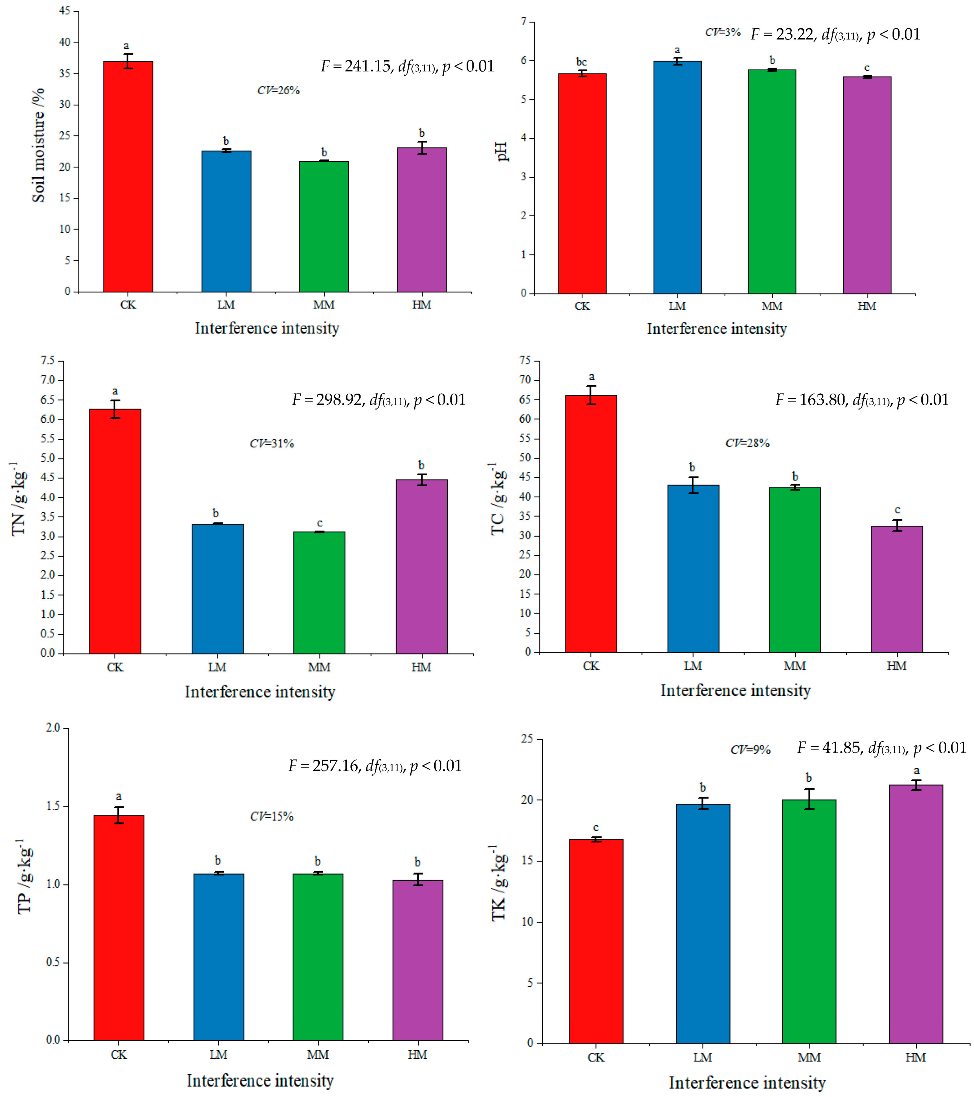

Figure 2. Effects of different levels of disturbance on soil physicochemical properties. TN: total nitrogen; TC: total carbon; TP: total phosphorus; TK: total potassium. Lowercase letters indicate significant differences in the effects of different levels of disturbance on soil physicochemical properties. The figure shows the means ± SD.

3.2. Characterization of Soil Microbial Community Structure at Different Levels of Disturbance

According to the results of high-throughput sequencing, as shown in Figure 3, different disturbance levels increased the biodiversity of the soil microbial community (CK = 319.3, LM = 325.5, MM = 332.5, HM = 329.3); however, these results were not significant ($p > 0.05$). In soils with different levels of disturbance, *Proteobacteria*, *Actinobacteria*, *Verrucomicrobia*, *Acidobacteria*, *Candidatus*_Rokubacteria, *Chloroflexi*, *Gemmatimonadetes*, and *Thaumarchaeota* were the dominant populations, with high abundance (Figure 4). The microbial population with the highest relative abundance was *Proteobacteria* (0.35, 0.33, 0.35, 0.34), while the second-most dominant population was *Actinobacteria* (0.33, 0.31, 0.33, 0.35). The medium-intensity group was most similar to the control in terms of soil dominant population composition and relative abundance size. In the low-intensity group, *Proteobacteria* and *Actinobacteria* had the lowest relative abundances. The difference in relative abundance between *Proteobacteria* and *Actinobacteria* was 0.02 for the control, low-intensity, and medium-intensity groups. The difference in relative abundance between *Proteobacteria* and *Actinobacteria* in the high-intensity group was 0.01, which was closer than in the other groups. *Proteobacteria* was the microbial population with the highest relative abundance.

The relative abundances of *Bacteroidetes*, *Tenericutes*, and a tentatively unclassified fungus differed significantly ($p < 0.05$) between sample groups at different levels of disturbance. The relative abundances of *Bacteroidetes* and *Tenericutes* increased in soils with different intensities of mortality, compared with control (Figure 5a,b). The relative abundance of *Bacteroidetes* was significantly higher in the low-intensity group than in the control, while the relative abundance of *Tenericutes* was significantly higher in the medium-intensity group than in the control. The different intensities of disturbance resulted in a significant decrease in the relative abundance of all tentatively unclassified fungi (Figure 5c). Most of the negative correlations were found between microbial populations and soil moisture content, total carbon, total nitrogen, and total phosphorus content, while most of the positive correlations were found with pH and total potassium content. *Acidobacteria* showed a significant negative correlation with pH and a significant positive correlation with total potassium content. *Thaumarchaeota* showed significant positive correlations with soil moisture content and total nitrogen content. Firmicutes and *Gemmatimonadetes* showed significant negative correlations with total carbon content (Figure 6). PERMANOVA analysis indicated that soil nitrogen content ($R^2 = 0.15289$, $p = 0.081$) was the key factor driving changes in the microbial community.

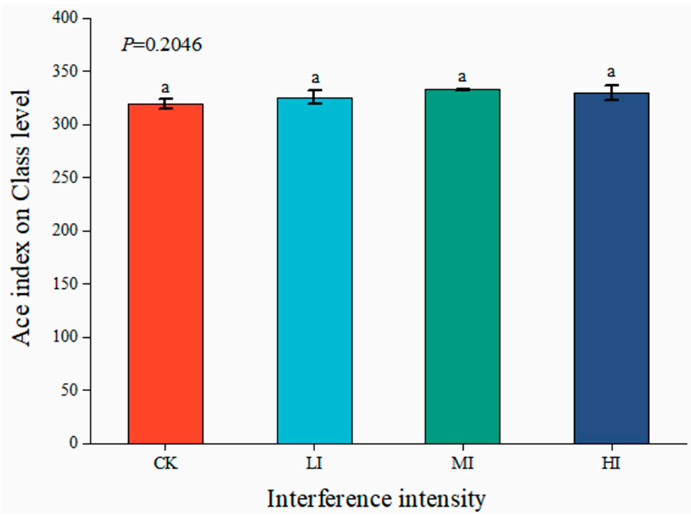

Figure 3. Bar graph of alpha diversity index at different levels of disturbance. Different lowercase letters indicate significant differences between the different sample groups.

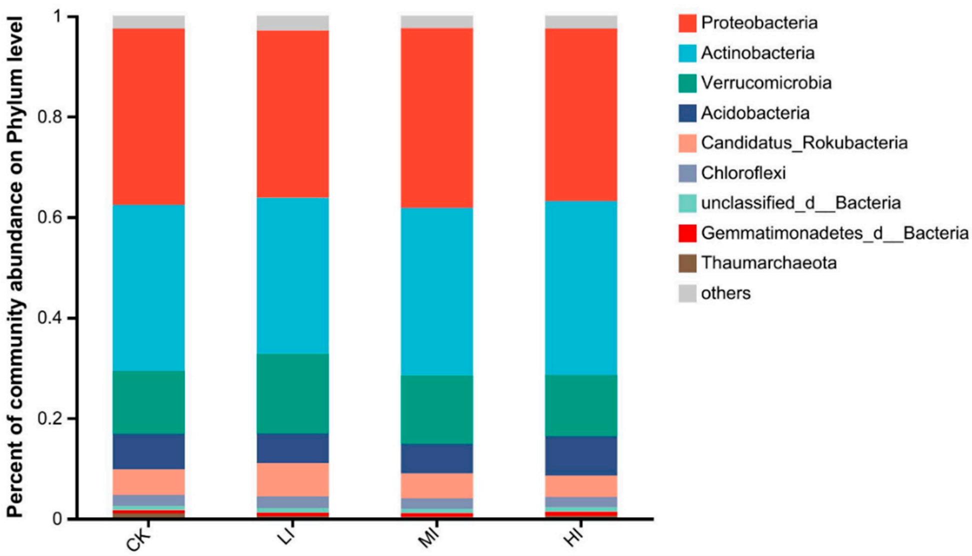

Figure 4. Relative abundance of soil micro-organisms at the phylum level at different levels of disturbance.

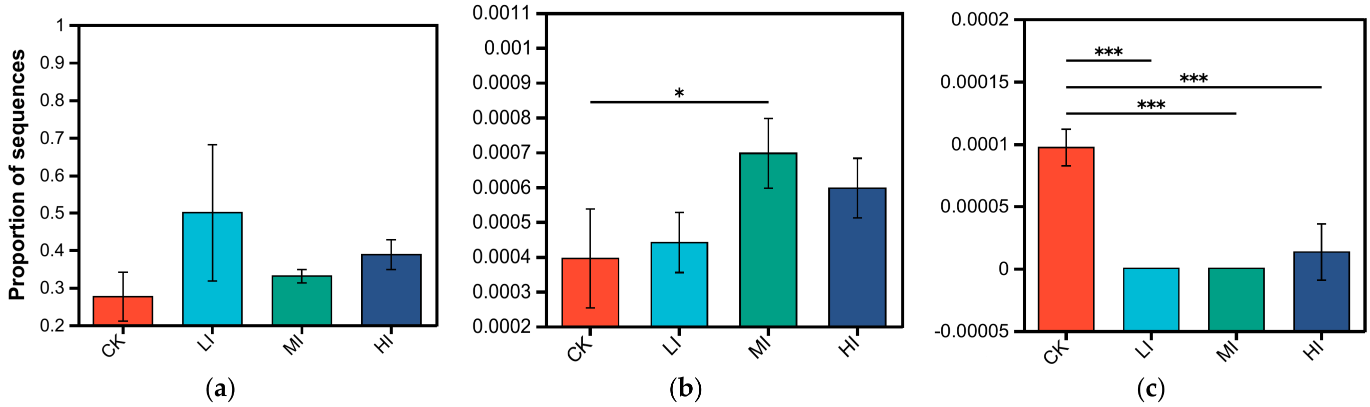

Figure 5. Phyla with significant differences at different levels of disturbance. (**a**) *Bacteroidetes* (low intensity significantly higher than other disturbance intensities); (**b**) *Tenericutes*; and (**c**) unclassified_k__Fungi. *: Significant difference, ***: Very significant difference.

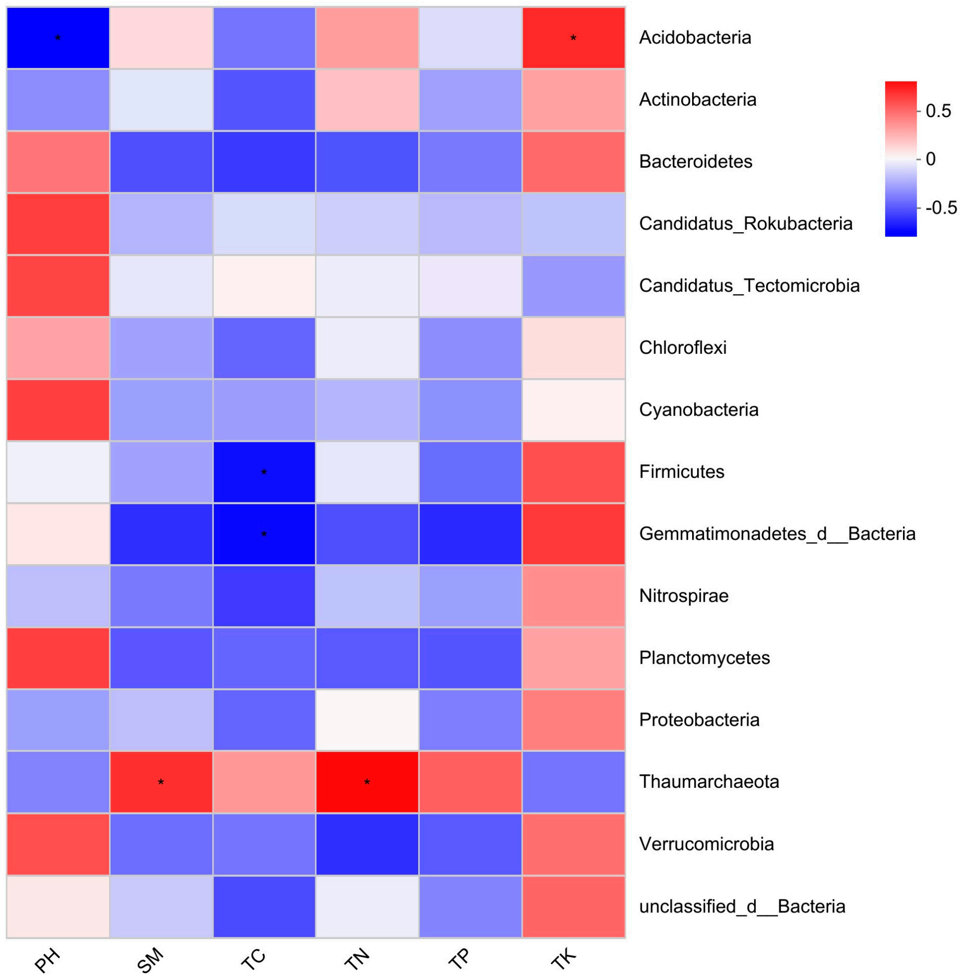

Figure 6. Heatmap analysis of the correlation between soil environmental factors and phyla under different mortality intensities. *: Significant difference.

4. Discussion

4.1. Soil Response to Bark Beetle Disturbance

Global forest ecosystems are currently facing increasing biotic and abiotic environmental stresses, and widespread forest mortality or decline is becoming an escalating global phenomenon [3,41]. In this study, we found that different levels of pest disturbance affected soil physicochemical properties, microbial community composition, and the relationships between these variables in larch plantation forests. Abiotic factors (e.g., fire, wind, logging, and so on) can damage or remove above-ground vegetation in a short period, leading to more significant effects [42,43]; as such, they have received more attention in recent scientific research. Compared with abiotic disturbances, biotic (e.g., insect pests or pathogens) disturbances are a longer process, and the impacts on forest ecosystems need to be validated through further studies [44]. Experimental plots and data on the intensity of different pest disturbances in the natural state are difficult to obtain; therefore, existing research on the disturbance of forest ecosystems with respect to biological factors (pests and pathogens) has focused on the aftermath of the large-scale forest mortality caused by pest disturbances [8,45,46], leading to a lack of studies on the disturbance of forests due to other biological factors and studies on forest disturbance processes in general. In this study, larch forests characterized by different levels of disturbance were selected as research objects, allowing for the further clarification of the soil changes occurring during forest death or decline. Meanwhile, reasonable forest management measures may reduce the impact of disturbance factors on forests [47], providing a theoretical basis for the development of reasonable management measures.

Soil physicochemical properties and the microbial community composition are sensitive to the influence of the external environment and can be a good indicator of changes in soil properties and functions [48,49]. Under similar environments at certain spatial scales, the differences in physicochemical properties and microbial community composition of native soils are not obvious [50]. In this study, an *L. olgensis* plantation with different disturbance intensities in the same area was sampled in its natural state. The differences between soil physicochemical properties and microbial community composition exhibited through the study results further demonstrate the effects of different disturbance intensities on the soil. Although the results of spatially repeated experiments at a large scale may be more representative, the conditions of spatial heterogeneity, environmental factors, and climatic factors lead to the complexity of the factors considered in the research process, and the results of the study are subject to a certain degree of uncertainty [51].

4.2. Relationships between Soil Physical and Chemical Properties at Different Levels of Disturbance

Tree mortality can lead to changes in biodiversity, as well as carbon and nutrient cycling [13]. As the upper soil layers are closely associated with vegetation, they will be more sensitive to external disturbances. Due to tree death, canopy thinning, and increased sunlight hours on the forest floor, the soil moisture content of larch plantation forests in the low-, medium-, and high-intensity disturbance groups was significantly lower than that in the control group and, on the forest floor in the high-intensity disturbance group, a large amount of dead leaves covered the soil surface, resulting in changes in soil and surface temperatures and increased soil moisture [52], leading to a slight increase in soil moisture content in the high-intensity group relative to the low- and medium-intensity groups; however, the difference was not significant. Larch reduced soil pH and cation exchange, such that soil pH in the low- and medium-intensity groups was significantly higher than that in the control group, indicating that pH recovered in the short-term after tree mortality and changes in stand structure, similar to the findings of Farahnak et al. [53]. In contrast, a major outbreak of boll weevil resulted in a higher levels of stand mortality and large amounts of deadwood, leading to increased cation inputs and organic matter, which decreased soil pH [21]. Soil total carbon content decreased significantly in the short term with increasing levels of disturbance, as plant carbon uptake stops when trees die, and soil respiration and carbon content decrease with the loss of root respiration and exudation [45,54], which is basically consistent with the conclusions drawn by Trahan et al. [25], who studied the soil carbon content in subalpine forests after insect damage disturbance. The soil nitrogen content decreased significantly under different levels of disturbance, probably due to the increase in soil microbial activity, the intensification of soil organic nitrogen utilization, the intensification of nitrogen mineralization, and the increase in nitrogen mineralization after large amounts of trees had died. After the death of trees, the forest floor has sufficient light and the surface temperature increases, promoting the biological nitrogen fixation process and enhancing nitrogen fixation. In addition, considering the high number of tree deaths in the high-intensity group, the return of large amounts of deadwood and its decomposition increases the soil nitrogen content, which may be the reason for the observed increase in soil nitrogen content in the high-intensity group. In their study of North American pine forests, Griffin et al. [55] concluded that there was no significant difference in soil nitrogen content under different death intensities, whereas Cigan et al. [52] concluded that input rates of nitrogen and phosphorus increased with increasing intensity of tree mortality in their investigation of nutrient cycling in pine forests in western Canada, which may be related to differences in time of tree mortality, sampling season, tree species, geographic location of the sample site, nutrients in the litterfall, and the role of the root system. Varying levels of disturbance resulted in a significant decrease in soil phosphorus compared with the control group, which could be related to the death of trees after infestation, reduction of root exudates, and changes in microbial loads. Marschner et al. [26] have suggested that this phenomenon is related to a decrease in the rate of phosphorus mineralization, in contrast with reports of an increase in phosphorus content in the upper layers of the soil after beetle disturbance [56]. In this study, soil total potassium content showed an increasing trend with an increase in the disturbance level, and the soil total potassium content in the low-, medium-, and high-intensity groups was significantly higher than that in the control group, which may be attributed to the fact that the decomposition of organic matter by micro-organisms in the soil produces excessive potassium ions, which are difficult to be absorbed into plants, resulting in elevated soil total potassium content.

4.3. Changes in Soil Microbial Community Structure at Different Levels of Disturbance

Soil microbial communities regulate many biogeochemical cycling processes and are essential for ecosystem restoration [57]. The present study found that, in soils with different levels of disturbance, *Proteobacteria*, *Actinobacteria*, *Verrucomicrobia*, *Acidobacteria*, *Candidatus*_Rokubacteria, *Chloroflexi*, *Gemmatimonadetes*, and *Thaumarchaeota* were the dominant phyla with high relative abundance, the vast majority of which were bacterial flora, indicating their predominance in the forest soil. The composition of the flora in this study was basically the same at the systematic level, The results for the dominant population composition are similar to those reported by Sun et al. [58] in a study on the soil microbial community; thus, the microbial diversity in the soil was relatively stable. When trees appeared to die under different levels of disturbance due to the bark beetle, significant changes occurred mainly in bacteria, indicating that soil bacteria are highly sensitive to tree mortality. In contrast, Ferrenberg et al. [59] concluded that the soil bacterial community structure did not change when studying the tree mortality associated with bark beetle; the reason for the differing results may be due to the different locations of the study areas and the different number of years of recovery after tree death, suggesting that the response of the soil microbial community structure to changes in tree mortality is related to the host or ecosystem. In this study, it was found that the microbial community composition in the medium-intensity group was the most similar to that of the control group. This may be due to the microbial community in the medium-intensity group having recovered the best in the short-term after the death of the trees. *Actinobacteria* had the highest relative abundance in the high-intensity group, surpassing *Proteobacteria* as the dominant population with the highest abundance. *Actinobacteria* may be the key phylum responsible for the high degree of tree mortality under insect pest disturbance, related to possible changes in soil total nitrogen and phosphorus content. Chen et al. [60], in a global meta-analysis of soil microbial community composition and diversity, found that *Actinobacteria* were largely proportional to soil total nitrogen and phosphorus content. They may also be related to available carbon, as *Proteobacteria* are one of the most dominant bacterial types in the soil and can utilize carbon sources for rapid reproduction [61]. In the present study, it was found that the relative abundances of *Bacteroidetes* and *Tenericutes* in the sample groups with different levels of disturbance were increased relative to the control group, which may indicate these as the pathogens responsible for the death of trees under the influence of bark beetle. It has been suggested that *Bacteroidetes* play a central role in the decomposition of the plant body, being a key microbial group for cellulose and lignin degradation [62]. The increase in the relative abundance of *Bacteroidetes* proves the enhancement of microbial carbon metabolism in this region; regarding *Tenericutes*, there is less literature available evidencing it as a micro-organism present in the intestinal tracts of animals, and the relative abundance of *Tenericutes* was higher under different levels of disturbance than in the control group, possibly due to the soil with a higher level of disturbance containing more beetle feces. Between the disturbance groups and the control group, there was also a significant difference in the relative abundance of a tentatively unclassified fungus, whose relative abundance was opposite that of *Bacteroidetes* and *Tenericutes*, and whose role can also be presumed to be opposite to that of *Bacteroidetes* and *Tenericutes*; it may be that this is a beneficial micro-organism that can increase the resistance of trees to bark beetle and inhibit tree death. *Acidobacterium* spp. are mostly acidophilic bacteria, are better adapted to more acidic environments [2] and, so, are negatively correlated with pH. Some studies have proposed that *Thaumarchaeota* plays an important role in the process of ammonia oxidation [63], as it is a typical ammonia-oxidizing archaea. High C/N inhibits the growth of *Thaumarchaeota*, which may be the reason why *Thaumarchaeota* was positively correlated with soil total nitrogen content. The results of Zhang et al. [64] demonstrate that an increase in nitrogen concentration promotes the growth of the soil bacterial community, and that soil nitrogen and phosphorus content are key factors driving changes in the microbial community, which is consistent with the conclusions of Cui et al. [65]. The structure of soil microbial communities is affected by many factors; however, the driving factors may differ between different ecosystems, which cannot be generalized and need to be further explored.

5. Conclusions

Soil physicochemical properties and microbial community structure were altered in an *L. olgensis* plantation under different levels of bark beetle disturbance. Correlations between the level of disturbance and these variables were analyzed, and soil total nitrogen content emerged as the key factor driving changes in microbial communities. The effects of tree mortality caused by bark beetles on soil physicochemical properties and microbial community structure involve a complex and long-term process, the analysis of which is limited by the sampling size and time. While the effects of the disturbance degree of bark beetles on the soil physicochemical properties and microbial community structure of the *L. olgensis* plantation were studied as short-term effects in this study, the long-term effects and the underlying mechanisms need to be further investigated.

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References

1. Bonan, G.B. Forests and Climate Change: Forcings, Feedbacks, and the Climate Benefits of Forests. *Science* **2008**, *320*, 1444–1449. [CrossRef] [PubMed]
2. Pankratov, T.A.; Ivanova, A.O.; Dedysh, S.N.; Liesack, W. Bacterial populations and environmental factors controlling cellulose degradation in an acidic Sphagnum peat. *Environ. Microbiol.* **2011**, *13*, 1800–1814. [CrossRef] [PubMed]

3. Allen, C.D.; Macalady, A.K.; Chenchouni, H.; Bachelet, D.; McDowell, N.; Vennetier, M.; Kitzberger, T.; Rigling, A.; Breshears, D.D.; Hogg, E.H.; et al. A global overview of drought and heat-induced tree mortality reveals emerging climate change risks for forests. *For. Ecol. Manag.* **2009**, *259*, 660–684. [CrossRef]
4. Jolly, W.M.; Cochrane, M.A.; Freeborn, P.H.; Holden, Z.A.; Brown, T.J.; Williamson, G.J.; Bowman, D.M.J.S. Climate-induced variations in global wildfire danger from 1979 to 2013. *Nat. Commun.* **2015**, *6*, 7537. [CrossRef]
5. Patacca, M.; Lindner, M.; Lucas-Borja, M.E.; Cordonnier, T.; Fidej, G.; Gardiner, B.; Hauf, Y.; Jasinevicius, G.; Labonne, S.; Linkevicius, E.; et al. Significant increase in natural disturbance impacts on European forests since 1950. *Glob. Chang. Biol.* **2022**, *23*, 1359–1376. [CrossRef] [PubMed]
6. Seidl, R.; Thom, D.; Kautz, M.; Martin-Benito, D.; Peltoniemi, M.; Vacchiano, G.; Wild, J.; Ascoli, D.; Petr, M.; Honkaniemi, J.; et al. Forest disturbances under climate change. *Nat. Clim. Chang.* **2017**, *7*, 395–402. [CrossRef] [PubMed]
7. Kurz, W.A.; Dymond, C.C.; Stinson, G.; Rampley, G.J.; Neilson, E.T.; Carroll, A.L.; Ebata, T.; Safranyik, L. Mountain pine beetle and forest carbon feedback to climate change. *Nature* **2008**, *452*, 987–990. [CrossRef]
8. Reed, C.C.; Hood, S.M.; Cluck, D.R.; Smith, S.L. Fuels change quickly after California drought and bark beetle outbreaks with implications for potential fire behavior and emissions. *Fire Ecol.* **2023**, *19*, 16. [CrossRef]
9. Woolley, T.; Shaw, C.D.; Hollingsworth, T.L.; Michelle, C.; Agne; Fitzgerald, S. Beyond red crowns: Complex changes in surface and crown fuels and their interactions 32 years following mountain pine beetle epidemics in south-central Oregon, USA. *Fire Ecol.* **2019**, *15*, 4. [CrossRef]
10. Lutz, J.A.; Halpern, C.B. Tree Mortality during Early Forest Development: A Long-Term Study of Rates, Causes, and Consequences. *Ecol. Monogr.* **2006**, *76*, 257–275. [CrossRef]
11. Zhang, X.Q.; Cao, Q.V.; Duan, A.G.; Zhang, J.G. Modeling tree mortality in relation to climate, initial planting density and competition in chinese fir plantations using a bayesian logistic multilevel method. *Can. J. For. Res.* **2017**, *47*, 1278–1285. [CrossRef]
12. Lugo, A.E.; Scatena, F.N. Background and catastrophic tree mortality in tropical moist, wet, and rain forests. *Biotropica* **1996**, *28*, 585–599. [CrossRef]
13. Michael, C.; Dietze, P.R.; Moorcroft, S. Tree mortality in the eastern and central United States: Patterns and drivers. *Glob. Chang. Biol.* **2011**, *35*, 3312–3326.
14. Collins, B.J.; Rhoades, C.C.; Hubbard, R.M.; Battaglia, M.A. Tree regeneration and future stand development after bark beetle infestation and harvesting in Colorado lodgepole pine stands. *For. Ecol. Manag.* **2011**, *261*, 2168–2175. [CrossRef]
15. Diskin, M.; Rocca, M.E.; Nelson, K.N.; Aoki, C.F.; Romme, W.H. Forest developmental trajectories in mountain pine beetle disturbed forests of Rocky Mountain National Park, Colorado. *Can. J. For. Res.* **2011**, *41*, 782–792. [CrossRef]
16. Brouillard, B.M.; Mikkelson, K.M.; Bokman, C.M.; Berryman, E.M.; Sharp, J.O. Extent of localized tree mortality influences soil biogeochemical response in a beetle-infested coniferous forest. *Soil Biol. Biochem.* **2017**, *114*, 309–318. [CrossRef]
17. Young, D.J.N.; Stevens, J.T.; Earles, J.M.; Moore, J.; Ellis, A.; Jirka, A.L.; Latimer, A.M. Long-term climate and competition explain forest mortality patterns under extreme drought. *Ecol. Lett.* **2017**, *20*, 78–86. [CrossRef]
18. Anderegg, W.R.L.; Chegwidden, O.S.; Badgley, G.; Trugman, A.T.; Cullenward, D.; Abatzoglou, J.T.; Hicke, J.A.; Freeman, J.; Hamman, J.J. Future climate risks from stress, insects and fire across US forests. *Ecol. Lett.* **2022**, *25*, 1510–1520. [CrossRef]
19. Perret, D.L.; Bell, D.M.; Gray, A.N.; Shaw, J.D.; Zald, H.S.J. Range-wide population assessments for subalpine fir indicate widespread disturbance-driven decline. *For. Ecol. Manag.* **2023**, *542*, 121128. [CrossRef]
20. Williams, A.P.; Allen, C.D.; Millar, C.I.; Swetnam, T.W.; Michaelsen, J.; Still, C.J.; Leavitt, S.W. Forest responses to increasing aridity and warmth in the southwestern United States. *Proc. Natl. Acad. Sci. USA* **2010**, *107*, 21289–21294. [CrossRef]
21. Kopácek, J.; Capek, P.; Choma, M.; Cudlín, P.; Kana, J.; Kopácek, M.; Porcal, P.; Santrucková, H.; Tahovská, K.; Turek, J. Long-term changes in soil composition in unmanaged central European mountain spruce forests after decreased acidic deposition and a bark beetle outbreak. *Catena* **2023**, *222*, 106839. [CrossRef]
22. Morehouse, K.; Johns, T.; Kaye, J.; Kaye, A. Carbon and nitrogen cycling immediately following bark beetle outbreaks in southwestern ponderosa pine forests. *For. Ecol. Manag.* **2008**, *255*, 2698–2708. [CrossRef]
23. Griffin, J.M.; Turner, M.G.; Simard, M. Nitrogen cycling following mountain pine beetle disturbance in lodgepole pine forests of Greater Yellowstone. *For. Ecol. Manag.* **2010**, *261*, 1077–1089. [CrossRef]
24. Sandén, H.; Mayer, M.; Stark, S.; Sandén, T.; Nilsson, L.O.; Jepsen, J.U.; Wäli, P.R.; Rewald, B. Moth Outbreaks Reduce Decomposition in Subarctic Forest Soils. *Ecosystems* **2020**, *23*, 151–163. [CrossRef]
25. Trahan, N.A.; Dynes, E.L.; Pugh, E.; Moore, D.J.P.; Monson, R.K. Changes in soil biogeochemistry following disturbance by girdling and mountain pine beetles in subalpine forests. *Oecologia* **2015**, *177*, 981–995. [CrossRef] [PubMed]
26. Marschner, P.; Crowley, D.; Rengel, Z. Rhizosphere interactions between microorganisms and plants govern iron and phosphorus acquisition along the root axis—Model and research methods. *Soil Biol. Biochem.* **2011**, *43*, 883–894. [CrossRef]
27. Zhao, J.; Ni, T.; Li, Y.; Xiong, W.; Ran, W. Responses of bacterial communities in arable soils in a rice-wheat cropping system to different fertilizer regimes and sampling times. *PLoS ONE* **2018**, *9*, e85301. [CrossRef] [PubMed]
28. Ngosong, C.; Jarosch, M.; Raupp, J.; Neumann, E.; Ruess, L. The impact of farming practice on soil microorganisms and arbuscular mycorrhizal fungi: Crop type versus long-term mineral and organic fertilization. *Appl. Soil Ecol.* **2010**, *46*, 134–142. [CrossRef]
29. Shen, J.P.; Zhang, L.M.; Guo, J.F.; Ray, J.L.; He, J.Z. Impact of long-term fertilization practices on the abundance and composition of soil bacterial communities in Northeast China. *Appl. Soil Ecol.* **2010**, *46*, 119–124. [CrossRef]

30. Nkongolo, K.K.; Narendrula-Kotha, R. Advances in monitoring soil microbial community dynamic and function. *J. Appl. Genet.* **2020**, *61*, 249–263. [CrossRef]
31. Wang, J.X.; Gao, J.; Zhang, H.Q.; Tang, M.; Chen, H. How does tree mortality caused by bark beetle (*Trypophloeus klimeschi*) outbreaks affect changes in soil fungal communities? *Catena* **2022**, *217*, 106516. [CrossRef]
32. Mayer, M.; Rosinger, C.; Gorfer, M.; Berger, H.; Deltedesco, E.; Bässler, C.; Müller, J.; Seifert, L.; Rewald, B.; Godbold, D.L. Surviving trees and deadwood moderate changes in soil fungal communities and associated functioning after natural forest disturbance and salvage logging. *Soil Biol. Biochem.* **2022**, *166*, 108558. [CrossRef]
33. Fernández-Calviño, D.; Rousk, J.; Brookes, P.C.; Bååth, E. Bacterial pH-optima for growth track soil pH, but are higher than expected at low pH. *Soil Biol. Biochem.* **2011**, *43*, 1569–1575. [CrossRef]
34. Yao, Q.M.; Li, Z.; Song, Y.; Wright, S.J.; Guo, X.; Tringe, S.G.; Tfaily, M.M.; Pasa-Tolic, L.; Hazen, T.C.; Turner, B.L.; et al. Community proteogenomics reveals the systemic impact of phosphorus availability on microbial functions in tropical soil. *Nat. Ecol. Evol.* **2018**, *2*, 499–509. [CrossRef] [PubMed]
35. Li, Y.N.; Qian, Z.Y.; Li, D.J. Effects of tree diversity on soil microbial community in a subtropical forest in Southwest China. *Eur. J. Soil Biol.* **2023**, *116*, 103490. [CrossRef]
36. Reinhart, K.O.; Callaway, R.M. Soil biota and invasive plants. *New Phytol.* **2006**, *170*, 445–457. [CrossRef] [PubMed]
37. Ng, E.-L.; Patti, A.F.; Rose, M.T.; Schefe, C.R.; Wilkinson, K.; Smernik, R.J.; Cavagnaro, T.R. Does the chemical nature of soil carbon drive the structure and functioning of soil microbial communities? *Soil Biol. Biochem.* **2014**, *70*, 54–61. [CrossRef]
38. Zheng, W.; Ya, L.; Huimin, W.; Xianjing, M.; Xuewei, L. Ophiostomatoid fungi associated with Ips subelongatus, including eight new species from northeastern China. *IMA Fungus* **2020**, *11*, 3.
39. Yue, Y.; Men, X.; Sun, Z.; Chen, X. Exploring the Role of Stumps in Soil Ecology: A Study of Microsite Organic Carbon and Enzyme Activities in a Larix olgensis Henry Plantation. *Forests* **2023**, *14*, 1027. [CrossRef]
40. Song, U.; Zuo, Z.; Zhao, X.; Qiao, X.; Ya, J.; Li, H.; Yue, X.; Chen, P.; Wang, M.; Medina-Roldán, S.K. Plant functional traits mediate the response magnitude of plant-litter-soil microbial C: N: P stoichiometry to nitrogen addition in a desert steppe. *Cience Total Environ.* **2024**, *915*, 169915. [CrossRef]
41. Jofre, C.; Marta, C.; Miquel, N.; Xavier, P.; Gerardo, S. Widespread crown condition decline, food web disruption, and amplified tree mortality with increased climate change-type drought. *Proc. Natl. Acad. Sci. USA* **2011**, *108*, 1474–1478.
42. Johnson, W.D.; Curtis, S.P. Effects of forest management on soil C and N storage: Meta analysis. *For. Ecol. Manag.* **2001**, *140*, 227–238. [CrossRef]
43. Zhou, D.; Zhao, Q.S.; Liu, S.; Oeding, J. A meta-analysis on the impacts of partial cutting on forest structure and carbon storage. *Biogeosciences* **2013**, *10*, 3691–3703. [CrossRef]
44. Holden, S.R.; Treseder, K.K. A meta-analysis of soil microbial biomass responses to forest disturbances. *Front. Microbiol.* **2013**, *41*, 63. [CrossRef]
45. Moore, D.J.P.; Trahan, N.A.; Wilkes, P.; Quaife, T.; Stephens, B.B.; Elder, K.; Desai, A.R.; Negron, J.; Monson, R.K. Persistent reduced ecosystem respiration after insect disturbance in high elevation forests. *Ecol. Lett.* **2013**, *16*, 731–737. [CrossRef] [PubMed]
46. Sallé, A.; Bouget, C. Victims or perpetrators: Contribution and response of insects to forest diebacks and declines. *Ann. For. Sci.* **2020**, *77*, 104. [CrossRef]
47. Leverkus, A.B.; Buma, B.; Wagenbrenner, J.; Burton, P.J.; Lingua, E.; Marzano, R.; Thorn, S. Tamm review: Does salvage logging mitigate subsequent forest disturbances? *For. Ecol. Manag.* **2021**, *481*, 118721. [CrossRef]
48. Johannes, R.; Erland, B. Growth of saprotrophic fungi and bacteria in soil. *FEMS Microbiol. Ecol.* **2011**, *78*, 17–30.
49. Delgado-Baquerizo, M.; Oliverio, M.A.; Brewer, E.T.; Benavent-González, A.; Eldridge, D.J. A global atlas of the dominant bacteria found in soil. *Science* **2018**, *359*, 320–325. [CrossRef]
50. Yan, J.; Wang, L.; Hu, Y.; Tsang, Y.F.; Zhang, Y. Plant litter composition selects different soil microbial structures and in turn drives different litter decomposition pattern and soil carbon sequestration capability. *Geoderma* **2018**, *203*, 319194. [CrossRef]
51. Xue, R.; Zhao, K.; Yu, X.; Liu, E.; Ye, S.; Xu, J. Deciphering sample size effect on microbial biogeographic patterns and community assembly processes at centimeter scale. *Soil Biol. Biochem.* **2021**, *156*, 108218. [CrossRef]
52. Cigan, P.W.; Karst, J.; Cahill, J.F.; Sywenky, A.N.; Pec, G.J.; Erbilgin, N. Influence of bark beetle outbreaks on nutrient cycling in native pine stands in western Canada. *Plant Soil* **2015**, *390*, 29–47. [CrossRef]
53. Farahnak, M.; Mitsuyasu, K.; Ide, J.; Chiwa, M.; Enoki, T.; Jeong, S.; Otsuki, K.; Shimizu, K.; Kume, A. Soil pH and divalent cations after clear-cutting on a Japanese cypress plantation. *J. For. Res.* **2022**, *27*, 363–370. [CrossRef]
54. Xiong, Y.M.; D'Atri, J.J.; Fu, S.L.; Xia, H.P.; Seastedt, T.R. Rapid soil organic matter loss from forest dieback in a subalpine coniferous ecosystem. *Soil Biol. Biochem.* **2011**, *43*, 2450–2456. [CrossRef]
55. Griffin, J.M.; Simard, M.; Turner, M.G. Salvage harvest effects on advance tree regeneration, soil nitrogen, and fuels following mountain pine beetle outbreak in lodgepole pine. *For. Ecol. Manag.* **2013**, *291*, 228–239. [CrossRef]
56. Kana, J.; Tahovská, K.; Kopácek, J. Response of soil chemistry to forest dieback after bark beetle infestation. *Biogeochemistry* **2013**, *113*, 369–383. [CrossRef]
57. Fierer, N. Embracing the unknown: Disentangling the complexities of the soil microbiome. *Nat. Rev. Microbiol.* **2017**, *15*, 579–590. [CrossRef] [PubMed]

58. Sun, S.; Xing, F.; Zhao, H.; Gao, Y.; Bai, Z.; Dong, Y. Response of bacterial community to simulated nitrogen deposition in soils and a unique relationship between plant species and soil bacteria in the Songnen grassland in Northeastern China. *J. Soil Sci. Plant Nutr.* **2014**, *14*, 565–580. [CrossRef]
59. Ferrenberg, S.; Knelman, J.E.; Jones, J.M.; Beals, S.C.; Bowman, W.D.; Nemergut, D.R. Soil bacterial community structure remains stable over a 5-year chronosequence of insect-induced tree mortality. *Front. Microbiol.* **2014**, *5*, 681. [CrossRef]
60. Chen, C.; Chen, H.Y.H.; Chen, X.L.; Huang, Z.Q. Meta-analysis shows positive effects of plant diversity on microbial biomass and respiration. *Nat. Commun.* **2019**, *10*, 1332. [CrossRef]
61. Janssen, P.H. Identifying the dominant soil bacterial taxa in libraries of 16S rRNA and 16S rRNA genes. *Appl. Environ. Microbiol.* **2006**, *72*, 1719–1728. [CrossRef] [PubMed]
62. Gavande, P.V.; Basak, A.; Sen, S.; Lepcha, K.; Murmu, N.; Rai, V.; Mazumdar, D.; Saha, S.P.; Das, V.; Ghosh, S. Functional characterization of thermotolerant microbial consortium for lignocellulolytic enzymes with central role of Firmicutes in rice straw depolymerization. *Sci. Rep.* **2021**, *11*, 3032. [CrossRef] [PubMed]
63. Pester, M.; Schleper, C.; Wagner, M. The *Thaumarchaeota*: An emerging view of their phylogeny and ecophysiology. *Curr. Opin. Microbiol.* **2011**, *14*, 300–306. [CrossRef] [PubMed]
64. Zhang, C.; Liu, G.B.; Xue, S.; Wang, G.L. Soil bacterial community dynamics reflect changes in plant community and soil properties during the secondary succession of abandoned farmland in the Loess Plateau. *Soil Biol. Biochem.* **2016**, *97*, 40–49. [CrossRef]
65. Cui, J.Y.; Sun, Z.H.; Wang, Z.X.; Gong, L.F. Effects of the Application of Nutrients on Soil Bacterial Community Composition and Diversity in a *Larix olgensis* Plantation, Northeast China. *Sustainability* **2022**, *14*, 16759. [CrossRef]