



# Article Soil Microbial and Organic Carbon Legacies of Pre-Existing Plants Drive Pioneer Tree Growth during Subalpine Forest Succession

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**Abstract:** Fast-growing pioneer tree species play a crucial role in triggering late successional development in forests. Experimental evidence of the soil legacy effects of pre-existing plants on pioneer tree performance is lacking. We explored the legacy effects of soils conditioned by early successional herbs (*Poa poophagorum* Bor and *Potentilla fragarioides* L.) and mid-successional shrubs (*Rhododendron fortunei* Lindl. and *Enkianthus quinqueflorus* Lour.) on late-successional ectomycorrhizal (ECM) pioneer tree (*Betula platyphylla* Sukaczev) seedling growth. The soils were analyzed for soil nutrient status and fungal and bacterial compositions using ITS and 16S rRNA gene sequencing. *B. platyphylla* seedlings produced higher biomass in soils conditioned by shrubs. Soil organic carbon (SOC) and bacterial and fungal legacies most impacted pioneer tree seedling growth. Additionally, the partial least squares path model revealed that soil nutrients, especially SOC, indirectly affected seedling biomass by their direct effects on the bacterial and fungal communities. The changes in bacterial community composition had a stronger effect on seedling biomass than those of fungi in enhancing nutrient availability. Our study integrates soil microbial and nutrient legacies to explain the potential mechanisms of pioneer tree regeneration.

Keywords: secondary forest succession; herb; shrub; pioneer tree; soil legacy

# 1. Introduction

The loss of natural forest is an unprecedented current challenge, and the remaining forests are fragmented and degraded [1–3]. Plant communities change from grassland and shrubland to forest during secondary forest succession [4]. A primary determinant of the recovery of forest community structure and composition is the successful establishment of pioneer tree seedlings, which can provide a template for further community assembly [5]. Pioneer tree species are generally light-demanding species [6], which can regenerate quickly, colonize large regions prosperously [7], and create canopy cover suitable for the establishment of mature forest trees [8]. Therefore, understanding the mechanisms regulating pioneer tree recruitment is of increasing importance for forest restoration [6,9].

The environmental conditions during forest succession change and influence the growth of trees over time [10]. Most studies on pioneer tree seedling establishment have focused on the microclimate (gap size, light, humidity, and temperature) [11,12], seed bank dynamics (seed dispersion and seeding predation) [13,14], and interspecific interactions (nutrient, water, light, and space competition) [15,16]. In addition, some reviews have suggested that durable beneficial legacy in soil supports the rapid colonization of pioneer species in secondary succession [17,18]. The allelochemical, microbial, and relevant effects



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**Copyright:** © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). can persist even if the initial plants are no longer present; therefore, such plants can have legacy effects on subsequent plant recruitment, reproduction, and growth, producing a selective advantage of one plant over another [19,20]. In forest ecosystems, the seedlings and saplings of tree species occur not only on bare soil but also in shrub and herb patches [21]. Thus, the legacies of pre-existing plants might also persist in the soils with pioneer tree colonization [22]. It is known that the abiotic tolerances and competitive ability of plants can be modified by soil legacies from pre-existing plants [20,23], which may potentially mediate pioneer tree establishment. However, experimental evidence for soil legacy effects on pioneer tree establishment is lacking.

Soil microbes have direct effects on plants via fungal mutualists (ectomycorrhizal, arbuscular, etc.), pathogens, or plant-associated bacteria. The beneficial interactions between plants and fungal mutualists could improve plant performance by increasing plant resistance to pathogens or stress and by enhancing nutrient acquisition [24]. Ectomycorrhizal (ECM) association is a major contributor to ecosystem functioning and a powerful regulator of forest productivity and composition [25]. Soil pathogens can decrease plant performance by causing a range of damping-off diseases [26]. Saprotrophic microbes are mainly involved in litter decomposition and carbon (C) cycling and influence plant growth by altering nutrient availability [27]. Some bacteria can promote the growth of plants by producing stimulative substances or providing protection against pathogens [28]. However, microbial activities can induce indirect effects on plants by changing the rates of nutrient acquisition and resource partitioning [29]. C and nitrogen (N) are the two most important soil nutrients in terrestrial ecosystems [30]. Both bacterial and fungal communities have been shown to be generally limited by soil C [31]. The availability of soil nutrients, including N and phosphorous (P), is an essential element that limits plant productivity and growth [32,33]. In addition, plants and microbes differ in terms of their threshold value for nutrient availability limitation [34], indicating that soil legacy effects may vary when nutrient availability changes [35]. However, the relative effects of the pre-existing fungi, bacteria, and nutrient availability left by plants in the soils on future pioneer tree seedling growth are still unclear.

A natural succession sequence of grassland–shrubland–secondary birch forest was formed due to damage to the original tree species in the southwestern subalpine forests [36]. Betula platyphylla is a representative ECM birch species, and it is the foundation tree species in this area [37]. Birches are widely distributed pioneer tree species in Asia, Europe, and America [38]. Recently, there has been increasing interest in reforestation with birch in post-agricultural lands [39]. Our goals were to determine whether early successional herbs and mid-successional shrubs contributed to legacy effects affecting B. platyphylla seedling growth. Here, we generated species-specific soil legacies by individually growing herbs (early successional species) or shrubs (mid-successional species) in a common starting soil. Then, we planted pioneer tree seedlings (B. platyphylla) in all soils to explore the pre-existing legacy effect affecting their performance. We characterized the bacterial and fungal compositions and nutrient availability and determined their relationships with pioneer tree seedling biomass. We hypothesized that i) the soil legacy effects on pioneer tree seedling performance could differ between herb and shrub soils because the soil microbial communities and nutrient availability depend on plant taxonomy and identity; ii) the fungal community will overwhelmingly promote *B. platyphylla* growth by increasing the resistance and nutrient acquisition associated with ECM fungi.

#### 2. Materials and Methods

## 2.1. Soil and Seed Collection

The sampled sites were located in subalpine forests in Miyaluo, Lixian County, Sichuan, China (31°42′–31°51′ N, 102°41′–102°44′E). The altitude of the study area is 2200–5500 m above sea level, the mean annual temperature is 4.8–6.9 °C, and the mean annual precipitation is 700–800 mm. *B. platyphylla* appears frequently as a primary pioneer species in pure and mixed stands with *Picea asperata* and *Abies fabri* [37]. We used the typical pioneer

tree *B. platyphylla* as the target species in the test phase of our experiment. Two earlysuccessional herbs (*Poa poophagorum* and *Potentilla fragarioides*) and two mid-successional shrubs (*Rhododendron fortunei* and *Enkianthus quinqueflorus*) were selected as the pre-existing plants. In this region, the original coniferous tree species, such as *P. asperata* and *A. fabri*, were logged. A natural succession sequence of "grassland–shrubland–secondary birch forest" formed successively [36,40]. All species are typical in secondary succession after land abandonment in subalpine forests.

Common starting soil includes 90% sterilized bare soil and 10% specific inoculum collected from each species (bare soil and specific inoculum were collected from the sites with identical altitude and slope aspect) [41]. In June 2019, bare soil was collected from a bare area (unvegetated site) in this region, mixed 7:3 (v/v) with silica sand and sterilized by 25 kGy gamma irradiation [41]. To minimize the influence of different plant species' overlapping roots, the specific soil inoculum was collected from monodominant plots of each species [42]. Soil inoculum was randomly collected (0–20 cm depth) from the rooting zones of each studied species (herbs, <0.2 m from the base of the stem; shrubs, <0.5 m from the base of the stem; pioneer tree, <1 m from the base of the stem) at five points (separated by >6 m) [43,44], homogenized, and then divided into two subsamples. One subsample was used for inoculating soil in the conditioning phase, and one was to analyze microbial communities. All soils were sieved using a mesh (<5 mm) to remove large stones and roots before experiments. Mature seeds of all species were obtained at their natural dispersal time from July to October in 2018 and 2019. Seeds of all species were collected from more than 20 plants. All seeds were surface-sterilized (70% ethanol, 2.625% NaOCl, ethanol, distilled water for 1 min, 3 min, 1 min, and 1 min, respectively) and sown on sterile sand and germinated in a cabinet under a regime of 16 h/21 °C (light):8 h/16 °C (dark).

## 2.2. Greenhouse Experiment

#### 2.2.1. Conditioning Phase

The major purpose of the conditioning phase was to obtain soils with specific soil communities of five species (two early-successional herbs, two mid-successional shrubs, and one target late-successional tree) (Figure 1, upper panel). In the conditioning phase, 25 pots (15  $\times$  22 cm) were filled with 3.5 kg of common starting soil for each species. For shrubs and pioneer trees, saplings of comparable size were monocultured in each replicate soil for 5 and 8 months, respectively. For herbs, 20 one-week-old seedlings were transplanted into each pot for 3 months. Dead saplings and seedlings were replaced during the first week of the experiment. All pots were arranged in a completely randomized design in the greenhouse under a regime of 16 h/21 °C: 8 h/16 °C (light:dark) and 72% relative humidity. All pots were watered with demineralized water twice a week. At the end of the conditioning phase, shoots were clipped at the soil surface. The main roots were removed, and the fine roots of each conditioning species were cut into fragments ( $\leq 2$  cm) and left in the soil so as to preserve the microbial communities and mined nutrients that had developed in the rhizosphere. Soils conditioned by each plant species were homogenized and pooled [45], and then divided into three subsamples. One subsample was used for determining nutrient concentration, one was stored at -20 °C in order to analyze microbial communities, and one was used in the following test phase.



**Figure 1.** Graphical illustration of the experimental design. In the conditioning phase, five different plant species, PF: *Potentilla fragarioides* L., PP: *Poa poophagorum* Bor, RF: *Rhododendron fortune* Lindl., EQ: *Enkianthus quinqueflorus* Lour., BP: *Betula platyphylla* Sukaczev, were monocultured in soils created by 90% sterilized bare soil and 10% specific inoculum. In the test phase, *Betula platyphylla* was monocultured in preconditioned soils.

## 2.2.2. Test Phase

In the test phase, the soils conditioned by different plant species were transferred to 1 L pots ( $7.5 \times 9.8$  cm) (Figure 1, lower panel). We had 50 1 L pots in the test phase (5 conditioned soil  $\times$  10 replicates = 50 pots). The recently germinated seedlings (at the second leaf stage) of *B. platyphylla* were replanted as single individuals in each pot (home vs. away) and grown for 5 months in a glasshouse. The seedlings that died were replaced during the first week of the experiment. The growth conditions and watering regime were consistent with those described for the conditioning phase. After 5 months of growth, all shoots were harvested, and roots were gently washed from the soil (the old root fragments in the conditioning phase were removed using 0.15 mm sieves). All shoots and roots were dried at 70 °C for 48 h, and the dry mass was weighed.

#### 2.3. Soil DNA Extraction and Sequence Analyses

Soil DNA was extracted from 0.5 g soil samples using a PowerSoil DNA extraction kit (MoBio, Carlsbad, CA, USA) according to the manufacturer's instructions. The concentration and purity of the extracted DNA were measured using a NanoDrop spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). The V4 region of the 16S rRNA for bacteria and the first internal transcribed spacer (ITS1) region of the rRNA operon for fungi were amplified using the general primers 515F/806R [46] and ITS1F/ITS2 [47], respectively. The PCR volume was 50  $\mu$ L, containing 25  $\mu$ L 2 × Taq Master Mix, 20  $\mu$ L ddH<sub>2</sub>O, 1  $\mu$ L each primer, and 3  $\mu$ L genomic DNA. The PCR amplification was conducted as follows: 5 min at 94 °C for initialization; 30 cycles of denaturation at 94 °C for 30 s, 30 s annealing at 52 °C, and 30 s extension at 72 °C; followed by 10 min final elongation at 72 °C. The PCR products from all samples were extracted and purified by 1% agarose gel electrophoresis and the E.Z.N.A. Gel Extraction Kit (Omega, Norcross, GA, USA). Sequencing was conducted on an Illumina NovaSeq 6000 Sequencing System (Guangdong Magigene Biotechnology Co., Ltd., Guangzhou, China). Trimming and filtering of raw sequencing data were performed

with fastp [48]. Low-quality reads with lengths less than 200 bp and quality scores less than 20 were removed. Chimera sequences and singletons (with only one read) were removed during the procedure. Each sample was subsampled to the same number of reads as the lowest soil sample (31,468 and 50,251 reads for fungi and bacteria, respectively). Taxonomic annotation for fungi and bacteria was performed based on the UNITE [49] and SILVA [50] databases, respectively. The raw sequences have been uploaded to the NCBI Sequence Read Archive (SRA) database under accession number PRJNA843373.

## 2.4. Soil Properties

Soil inorganic N (ammonium (NH<sub>4</sub><sup>+</sup>-N), nitrate (NO<sub>3</sub><sup>-</sup>-N)) was extracted using 2 M KCL (1:5, w/v) to quantify the concentration on a calorimeter with a microplate reader (Varioskan LUX, Thermo Scientific, America) [51,52]. Soil organic carbon (SOC) was determined by the potassium dichromate oxidation method [52]. Soil available phosphorus (AP) was extracted using 0.5 M NaHCO<sub>3</sub> (1:5, w/v) solution (pH = 8.5) and analyzed calorimetrically [52].

# 2.5. Statistical Analysis

We conducted one-way ANOVAs to analyze the effects of herbs and shrubs on soil chemical properties in the conditioning phase and biomass response in the test phase. We used plant species as explanatory variables, and pairwise differences between them were further evaluated using Tukey HSD tests.  $Log_{10}$  transform was used when necessary for homogeneity of variance and normality. A PERMANOVA model was used to investigate the effects of different successional plants on soil microbial communities using Bray-Curtis distances in the "vegan" package. Patterns of similarity among background (inoculum) and conditioning soils were visualized based on nonmetric multidimensional scaling (NMDS) ordination using the metaMDS function in the "vegan" package. Principal coordinates analysis (PCoA) based on Bray–Curtis distances was conducted to analyze the bacterial and fungal communities. We used the scores of the first and second PCoA axes as a variable to represent overall variation in bacterial and fungal community composition [53]. The Spearman correlation analyses between bacterial and fungal phyla and biomass are shown in a heatmap. The fungal putative functional guild was determined using the FUNGuild database [54]. The best subset model via stepwise multiple regression was conducted to assess the effects of biotic and abiotic soil legacies on pioneer tree biomass. Partial least squares path modeling (PLS-SM) was subsequently applied to assess the direct and indirect effects of soil nutrient availability and fungal and bacterial communities on seedling biomass. The model was conducted in the R package "plspm" using the "innerplot" function [55]. All statistical analyses were conducted in R v.4.0.4.

## 3. Results

#### 3.1. Soil Physicochemical Properties and Microbial Community Composition

At the end of the soil conditioning stage, AP,  $NH_4^+$ -N,  $NO_3^-$ -N, and SOC were significantly affected by the conditioning species (p < 0.05) (Table 1). Soil nutrient availability generally decreased at the end of the conditioning phase, except for the inorganic nitrogen in *P. fragarioides* (PF) soil and the organic carbon in *E. quinqueflorus* (EQ) and *R. fortunei* (RF) soils. The lowest organic C was observed in soil conditioned by *B. platyphylla* (Table 1). The soils planted with grasses had the lowest  $NH_4^+$ -N. Soil conditioned by *P. fragarioides* had a higher  $NO_3^-$ -N content but a lower  $NH_4^+$ -N content than the sterile bare soil. Except for RF and PF, other species did not significantly affect available P (Table 1).

	AP (mg kg <sup>-1</sup> )	$NO_3^N$ (mg kg <sup>-1</sup> )	$ m NH_4^+- m N$ (mg kg $^{-1}$ )	SOC (g kg <sup>-1</sup> )
PP	$48.19\pm1.5bc$	$4.04\pm0.25~\mathrm{e}$	$1.06\pm0.29~\mathrm{c}$	$18.58\pm0.46~\mathrm{c}$
PF	$62.5\pm8.13~\mathrm{ab}$	$30.29\pm0.52~\mathrm{a}$	$3.95\pm0.32\mathrm{b}$	$22.73\pm0.36\mathrm{bc}$
RF	$82.58\pm0.92~\mathrm{a}$	$8.13\pm0.12~\mathrm{c}$	$3.4\pm0.26\mathrm{bc}$	$26.87\pm3.0~\mathrm{ab}$
EQ	$56.97\pm1.7~\mathrm{bc}$	$15.7\pm0.39~\mathrm{b}$	$5.3\pm0.05~\mathrm{b}$	$30.12\pm1.48~\mathrm{a}$
BP	$47.19\pm3.28\mathrm{bc}$	$6.7\pm0.14~{ m cd}$	$3.97\pm0.68\mathrm{b}$	$18.11\pm0.83~\mathrm{c}$
Str.	$37.13\pm8.16~\mathrm{c}$	$6.26\pm0.18~\mathrm{d}$	$21.04\pm0.89~\mathrm{a}$	$26.11\pm0.47~\mathrm{ab}$

**Table 1.** Soil physiochemical properties (mean  $\pm$  SE) and statistical results at the end of the conditioning phase and in the sterile soil.

AP, soil available phosphorus;  $NO_3^-$ -N, soil nitrate nitrogen;  $NH_4^+$ -N, soil ammonium nitrogen; SOC, soil organic carbon. PP, PF, RF, EQ, and BP refer to the soil conditions of *Poa poophagorum*, *Potentilla fragarioides*, *Rhododendron fortunei*, *Enkianthus quinqueflorus*, and *Betula platyphylla*, respectively. Str. was the sterile bare soil. Different lowercase letters within each column refer to significant differences at p < 0.05 between different treatments.

The fungal communities were dominated by Ascomycota, Basidiomycota, Mortierellomycota, Chytridiomycota, and Rozellomycota at the phylum level (Figure 2). Similarly, the dominant phyla of bacteria in soils conditioned by herbs were Proteobacteria, Actinobacteria, and Acidobacteria (Figure 2). The dominant fungal genera of fungi were Solicoccozyma, Mortierella, and Coniochaeta, while the dominant bacterial genera were Pseudarthrobacter, Massilia, and Candidatus Udaeobacter (Figure S1). PERMANOVA tests suggested that the species were significantly different in all background (inoculum) and conditioning soils (Figure 3). The first PCoA axis based on Bray–Curtis distances of bacterial communities explained 37.6% of the total variation, whereas the second axis explained 22.2% of the total variation (Figure S3a). In fungal communities, the first axis explained 32.8%, and the second axis explained 27.3%, of the total variation (Figure S3b). For fungi, the potential functional profiles were predicted using the FUNGuild database, including three main types of trophic modes: symbiotroph (ectomycorrhizal, endophyte, epiphyte), pathotroph (fungal parasite, insect parasite, plant pathogen), and saprotroph (wood/dung/plant saprotroph). No significant relationship was observed between the predicted fungal functional guilds and seedling biomass (Figure S2).



**Figure 2.** Relative abundance of (**a**) bacterial and (**b**) fungal compositions at the phylum level. PP, PF, RF, EQ, and BP refer to the background (inoculum) soils of *Poa poophagorum, Potentilla fragarioides, Rhododendron fortunei, Enkianthus quinqueflorus,* and *Betula platyphylla,* respectively. CPP, CPF, CRF, CEQ, and CBP refer to the soil conditioned by the above species.



**Figure 3.** (a) Bacterial (stress = 0.081) and (b) fungal (stress = 0.079) communities of background (inoculum) and conditioned soils. NMDS using Bray–Curtis distance of background (inoculum) soils and conditioning plants (colors: blue depicts conditioned soils, red depicts background soils) and differential successional stages (shapes: circles represent herbs, triangles represent shrubs, and crosses represent trees). PERMANOVA results, including R<sup>2</sup> and significance *p* for the model, are given in the figure.

## 3.2. Linking Soil Biotic and Abiotic Legacies with Pioneer Tree Seedling Performance

The seedling biomass of *B. platyphylla* significantly responded to the conditioning plant guild (herbs vs. shrubs). Specifically, we found significantly lower total biomass in the soil conditioned by herbs than in the soil conditioned by conspecifics (Figure 4). The seedlings growing in *E. uinqueflorus* (EQ) soils exhibited a higher seedling biomass than those growing in conspecific soil and produced significantly higher biomass than those growing in herb soils (Figure 4).

The relative abundance of the bacterial phylum *Armatimonadetes* ( $\mathbf{r} = 0.78$ , *p* < 0.001) was significantly and positively correlated with the seedling biomass (Figure 5a). The relative abundances of the bacterial phylum *Verrucomicrobia* (Figure 5a, Spearman:  $\mathbf{r} = -0.51$ , *p* = 0.05) and the fungal phylum *Blastocladiomycota* (Figure 5b, Spearman:  $\mathbf{r} = -0.7$ , *p* = 0.004) were negatively correlated with seedling biomass. The best subset model via stepwise multiple regression showed that NO<sub>3</sub><sup>-</sup>-N, SOC, bacterial community, and fungal community significantly explained the seedling biomass (Table 2). The SOC contents (27.8%), fungal community composition (29.7%), and bacterial community composition (34.8%) were the dominant determinants of the variation in seedling biomass (Table 2). Partial least squares path modeling indicated that the coefficients quantifying the directions and strengths between total biomass and fungi and bacteria were -0.52 and 0.97, respectively (Figure 6). *B. platyphylla* biomass was more significantly determined by soil bacterial communities than by fungal communities (Figure 6). In addition, the indirect effect of SOC (coefficient: 0.95) on seedling biomass via bacterial and fungal communities was stronger than that of NH<sup>4</sup><sub>4</sub>-N (0.75), NO<sub>3</sub><sup>-</sup>-N (0.34), and AP (0.59).



**Figure 4.** The total biomass (g) of *Betula platyphylla* in soil conditioned by herbs (PP, PF), shrubs (EQ, RF), and conspecifics (BP). PP, *Poa poophagorum*; PF, *Potentilla fragarioides*; RF, *Rhododendron fortunei*; EQ, *Enkianthus quinqueflorus*; BP, *Betula platyphylla*. Values shown in the figure are mean values  $\pm$  SE (n = 10). Different lowercase letters refer to significant differences at p < 0.05.



**Figure 5.** Spearman correlation analysis between (**a**) bacterial phyla, (**b**) fungal phyla, soil nutrients, and seedling biomass. AP, soil available phosphorus;  $NO_3^-$ , soil nitrate nitrogen;  $NH_4^+$ , soil ammonium nitrogen; SOC, soil organic carbon; Biomass, total seedling biomass. Asterisks represent significant effects (\*, p < 0.05; \*\*, p < 0.01).

Biomass	Independent Variables	Contribution of the Individual (%)	p Value	Adj. R2 of Full Model
Biomass	NO <sub>3</sub> -N	5.6	0.02	
	SŎC	27.8	< 0.001	
	Fungal communities (PC1)	2.3	0.09	0.92
	Fungal communities (PC2)	29.7	< 0.001	(p < 0.001)
	Bacterial communities (PC1)	27.3	< 0.001	
	Bacterial communities (PC2)	7.5	0.009	

**Table 2.** Results of the stepwise multiple regression using the best subset model for the effect of different soil nutrient contents and biotic (bacteria and fungi) factors on the biomass.

PC1 and PC2 of the bacterial and fungal communities were used to measure the composition variations of the microbial communities based on Bray–Curtis distance. Bold values indicate significance at p < 0.05.



Goodness of fit=0.53

**Figure 6.** Partial least squares path modeling disentangling the direct and indirect pathways of the soil nutrients, fungal communities, and bacterial communities in *Betula platyphylla* biomass. Red and gray arrows indicate negative and positive effects, respectively. Asterisks represent significant effects (\*, p < 0.05; \*\*, p < 0.01; \*\*\*, p < 0.001). Only significant pathways are shown. Numbers on the arrows indicate standardized path coefficients.

# 4. Discussion

In this study, the legacies derived from herbs and shrubs significantly impacted the growth of pioneer tree seedlings. Consistent with hypothesis (i), the pioneer tree seedling biomasses in soils conditioned by herbs and shrubs were significantly different. Tree seedlings achieved more biomass when growing in the shrub (*E. quinqueflorus*) legacy compared to herb and conspecific legacy (Figure 4). The observed facilitative effect of shrub legacy on tree seedlings was consistent with previous observations showing that shrubs (e.g., *Baccharis vernalis*) had apparent facilitative effects on tree establishment [56]. Macek et al. (2018) suggested that shrubs provide situations (such as light, humidity, and temperature) suitable for tree growth. Here, our results suggested that soil nutrient content and bacterial and fungal community compositions changed by conditioning plants dominantly explained seedling biomass (Table 2). The content of  $NH_4^+$ -N being significantly greater in sterilized soil compared to soils that were conditioned by different plant species could be due to a release of nutrients from dead microbes in sterilized soil [57]. All conditioning plant species significantly decreased soil  $NH_4^+$ , resulting from plant absorption [58]. The contents of SOC were the highest in soils conditioned by two shrubs (*R. fortunei* and

*E. quinqueflorus*). The SOC content of sterile bare soil was almost similar to those under *R. fortunei*, possibly because of recalcitrant debris of *R. fortunei* leading to lower C input [59] or higher microbial activity leading to greater C output in R. fortunei soils [60]. The primary sources of organic C entering the soil were root exudates, root debris, and border cells [61]. Significant changes in soil nutrient availability are usually associated with the accumulation of organic matter and increased abundance and activity of soil microbes [62]. B. platyphylla seedlings may largely profit from increased organic carbon in the conditioned soil of shrubs. In addition, the relative abundance of the bacterial phylum Armatimonadetes was positively correlated with the target seedling biomass (Figure 5a). This result is supported by previous studies showing that Armatimonadetes was positively correlated with plant growth [63] by protecting against fungal pathogens [64] and decomposing plant materials and photosynthetic substances [65]. Furthermore, pioneer tree seedlings possessed the highest relative biomass in *E. quinqueflorus* soils with the highest relative abundance of the fungal genus Acremonium (Figure S1). This result was consistent with previous studies showing that the species of Acremonium are not host-specific and could promote the development of different host plants by protecting against root herbivores [66]. The observed negative effects of herb legacy on tree seedlings were consistent with previous observations. Graves et al. (2006) suggested that there are negative effects of herbs on tree seedlings as they competitively inhibited tree seedlings via faster growth and consequent pre-emption of light or nutrients [15,67]. However, the decreased tree seedling growth were eventually ascribed to the changes in soil nutrient content and microbial communities in our study. For instance, the relative abundance of the fungal phylum Blastocladiomycota was negatively correlated with tree seedling biomass (Figure 5b). Some fungal species in *Blastocladiomycota* are considered pathogens of vascular plants and obligate parasites of plants [68,69], which may infect tree seedlings by having a pathogenic relationship with plants and thus inhibit their growth. Fast-growing *B. platyphylla* seedlings were more vulnerable to the pathogens because of a higher investment in advanced fine roots as a strategy to promote nutrient absorption [70,71] rather than the resistance to soil-borne pathogens [72]. Additionally, the contents of SOC were the lowest in soils conditioned by two herbs (P. poophagorum and *P. fragarioides*). This result might be due to higher microbial activity in herbs soils, which result in greater carbon mineralization and soil respiration intensity [60]. However, a higher carbon mineralization might inhibit plant performance due to nutrient starvation as a result of a rapid increment in microbial biomass [73,74]. Collectively, our results agreed with previous studies showing that fast-growing plants produced soil legacies that negatively influenced later plants, whereas slower-growing plants produced a more positive soil legacy effect [75,76]. However, we detected a higher biomass of pioneer tree seedlings when growing in shrub soil for only one plant species. This result indicated that the soil legacy effects of slower-growing shrubs were species-specific, and different soil legacies of pre-existing plant species may differentially influence subsequent plant performance [77].

Inconsistent with hypothesis (ii), our results showed that soil fungal community did not exhibit the overwhelming effects on *B. platyphylla* growth as expected. Instead, our results showed that variation in pioneer tree seedling biomass was driven more significantly by bacterial community composition than by fungal community composition (Figure 6). The different effects of bacterial and fungal communities on pioneer tree seedling growth in our study are partly in line with previous findings which suggested that the soil legacy effects were more strongly reflected in bacterial communities than fungal communities [78]. Bacterial communities can be responsible for direct growth promotion by enhancing nutrient acquisition, preventing the activity or growth of pathogens, and producing phytohormones [79,80]. For example, previous studies indicated that some species in the bacterial phylum *Verrucomicrobia* play important roles in organic carbon degradation and have negative impacts on the other microbial groups [81,82]. A probable explanation is that bacteria are generally considered to be more sensitive than fungi to the availability of C substrates due to their shorter turnover period [83,84]. Most of the easily available organic matter is first trapped and metabolized by bacteria [85,86]. Accelerating soil or-

ganic matter decomposition and available nutrient release is important for promoting plant growth [87,88]. Mycorrhization also plays a very important role in mutualistic interactions by obtaining nutrients and increasing resistance to biotic and abiotic stresses; however, hyphal-growing fungi have a relatively longer generation time [80,89]. Furthermore, fungal communities tend to have more host-specific relationships with plant species [89]. For example, our results showed that the most abundant sequence numbers of ECM were classified into *Sphaerosporella* after the conditioning phase (Table S1). Previous studies have reported that *Sphaerosporella* formed mycorrhizae with *Pinus banksiana*, *Populus*, *Picea*, and *Larix* [90] and may not effectively and specifically benefit *B. platyphylla* seedlings. Thus, our results implied that *B. platyphylla*, an ECM birch species, might be less influenced by pre-existing fungal legacies in this study.

Soil biotic (fungal and bacterial community compositions) and abiotic legacies (especially SOC) synergistically influenced tree seedling growth (Table 2). Soil nutrients (SOC,  $NO_3^-$ ,  $NH_4^+$ , and AP) can indirectly influence plant growth by stimulating the soil microbial community, especially the bacterial community composition [91,92]. The strong association between soil nutrients and microbial communities (both bacteria and fungi) indicated that soil nutrients (especially SOC) were a potential factor influencing pioneer tree seedling recruitment. The interactions between plants and soil microbial communities are modified by variations in soil abiotic properties [93]. Therefore, our results suggested that soil nutrients (SOC,  $NO_3^-$ ,  $NH_4^+$ , and AP) are potential and critical components of soil legacy effects. Furthermore, the results imply that the ability to predict soil legacy effects requires a better understanding of interactions between diverse soil microbes and nutrient availability rather than a focus on singular biotic legacies.

## 5. Conclusions

In conclusion, we found that early- and mid-successional plant species created distinct soil microbial and abiotic legacies, which in turn differentially influenced the subsequent pioneer tree seedlings that established in these soils. Our results suggested that the legacies of shrubs had a facilitative effect on pioneer tree seedling growth and that the bacterial and fungal communities associated with SOC were the crucial explanatory factors. Specifically, the seedling biomass of *B. platyphylla* was positively correlated with the relative abundance of bacterial phylum Armatimonadetes, and negatively correlated with the relative abundance of fungal phylum *Blastocladiomycota*. The changes in bacterial community composition had a stronger effect on seedling biomass than those of fungi. Soil nutrients (especially SOC) were inextricably connected with soil legacy effects on pioneer tree seedling growth. Overall, the results illustrated that soil microbial community composition and soil nutrients together modulated pioneer tree seedling growth. These mechanisms provide evidence for optimizing forest management practices. Further research that focuses on combined experimental studies between plant community legacy effects and individual legacy effects is essential for obtaining a better understanding of the mechanisms that govern pioneer tree species establishment.

**Supplementary Materials:** The following supporting information can be downloaded at: https: //www.mdpi.com/article/10.3390/f13071110/s1, Figure S1: Relative abundance of dominant (a) bacterial and (b) fungal genera; Figure S2: Spearman correlation analysis between the relative abundance of soil fungal functional groups and seedling biomass; Figure S3: Principal coordinate analysis of (a) bacterial and (b) fungal communities based on Bray–Curtis distances; Table S1: Sequence numbers of the dominant genera of ECM under different treatments.

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