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A long-term effect of *Larix* monocultures on soil physicochemical properties and microbes in northeast China

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ABSTRACT

Forest monocultures are generally considered to alter the microbial community, reduce soil fertility, and presumably reduce forest productivity compared to mixed species in plantations or mixed species in natural stands. As the mechanisms of alteration of soils by monocultures have not been elucidated very well, we compared indicators of stand productivity and soils in three forest types all growing on the same soils that developed under the natural forests of the Maoershan Forest Farm in northeastern China. The three forest types were a planted monoculture of *Larix* plantation but where other species were allowed to ingrow (LP), a mixed species natural stand (NS), and a mixed species stand created by planting *Larix* seedlings into a natural stand (LP_NS). We measured the vegetation response as aboveground biomass (AGB), species richness and diversity, the soil response as soil physicochemical properties, and microbial community response as phospholipid fatty acids (PLFAs). The objectives were to determine (1) if the productivity of the *Larix* plantation was reduced relative to the natural stand, and if so, (2) which soil or microbial community factors were related to the declining productivity, and (3) how vegetation characteristics, soil physicochemical properties, and microbial community interacted. The three forest types appeared similar in aboveground biomass and litter biomass. As expected, 83% of 178.3 Mg ha^{-1} overstory AGB in LP was conifers and 87% of 134.7 Mg ha^{-1} AGB in NS was broadleaved species, and LP_NS with 176.9 Mg ha^{-1} had similar conifer AGB (53%) and broadleaved AGB (47%). Species richness for understory was greater in LP ($N0 = 34$, $P < 0.05$) than in NS ($N0 = 22$) and in LP_NS ($N0 = 19$); but species diversity was not different among the three forest types ($2.1 \leq H' \leq 2.7$, $P > 0.09$). LP showed significant reductions ($P < 0.006$) in measures of soil fertility as lower soil N and P concentrations, lower soil pH, and higher soil bulk density than NS and LP_NS. Microbial PLFA did not differ among the three stands ($P > 0.11$) except for the ratios of G+/G-, sat/mono, and cy/prec PLFAs ($P < 0.03$). Our findings indicate that the plantation of *Larix* where ingrowth of other species occurred did not show declines in overstory productivity. However, AGB and litter biomass shifts from broadleaved species to conifers in LP relative to NS and LP_NS caused some soil changes that may relate to long-term productivity losses.

1. Introduction

Forest plantations play an important role in providing forest products and other ecosystem services to our society though they may come with costs. Among about 278 million hectares of plantations world-wide, monocultures are the dominant practice, especially in commercial plantations. These single-species plantations, relative to mixed-species plantations or natural forests (i.e., forests allowed to develop species mixes without major human intervention), may be associated with the loss of soil fertility and subsequent forest productivity, changes in the hydrological cycle, increased insect and disease infestations, reductions of above- and below-ground biological diversity, and decreased resistance to climate change [1].

Most of the evidence of negative effects from monocultures on productivity has been associated with detrimental alterations to soil physicochemical properties and to microbial communities. A number of studies in China found that monocultures of *Cunninghamia lanceolata*, *Eucalyptus* spp., *Populus* spp., *Pinus massoniana*, or *Larix* species reduced soil fertility and presumably forest productivity to various degrees when compared to the natural forest or to mixed species [2]. There are studies that have shown evidence of declines in productivity, such as Evans [3] who reported declining soil fertility and productivity in a

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second rotation *Picea asperata* plantation in Europe during the 19th century. Others have found that productivity of mixed species plantations was considerably higher than monocultures [4], especially while mixed with a nitrogen-fixing species. Mixed species plantings by mimicking the natural forests have become more popular in plantations [1]. However, the mechanisms of the effects of species composition on forest productivity directly or indirectly through soil processes have not received much attention [5]. A better understanding of the mechanisms of monocultures on soil degradation is critical for effectively restoring forests and sustainably managing them for maximizing ecosystem services.

It is generally accepted that forest species can influence soil physicochemical properties by various mechanisms. It has been shown that coniferous stands have lower soil pH, lower soil organic carbon, lower total soil nitrogen (N) and lower soil phosphorus (P), and higher soil C/N ratios versus broadleaved forests or mixed species stands [2,6,7]. These soil effects are most likely caused by the differences of litter quantity measured by higher litter input and faster decomposition rate in mixed-species forests than in monocultures. For example, mixed-species plantations of *C. lanceolata* with *Tsoongiodendron odorum* or *Alnus japonica* produced 30% more litter biomass than monocultures of *C. lanceolata* alone [8,9]. Chen et al. [9] reported that annual litter fall was 6.5 Mg hm^{-2} in a mixed plantation of *L. olgensis* with *Betula platyphylla*, an increase of 41% over *L. olgensis* monoculture alone. The decomposition rate of leaf litter has been shown to be reduced in monocultures compared to mixed-species plantations or natural stands, particularly when the shift is from broadleaf to conifer needles. Yang [2] reported that in a subtropical region in China, the leaf litter of evergreen broadleaved species required 3–4 years for 95% to decompose compared to 6 years for needles of *C. lanceolata* needles. In the temperate zone, the needles of *Pinus* spp. required 8–17 years and *Larix* 9–12 years for 95% decomposition. In contrast, 80% of the leaf litter of *B. platyphyllas* and *Quercus mongolica* mixture decompose within one year. Soil C and N concentrations, mineralization rates, microbial biomass, and the enzymatic activities were lower in the *Larix* plantations than in the adjacent secondary forests [7]. Fu et al. [10] found that understory vegetation reduced soil acidity in monocultures of *C. lanceolata*. Higher soil bulk density was also found in the pine plantation versus the natural mixed-species conifer or oak stands [11].

Vegetation composition can also affect the structural and functional diversity of microbes in forest soils [6,12]. Studying microbial communities 27 years after restoration, Fu et al. [10] found positive relationships between understory aboveground biomass and the functional traits of the soil microbial communities. In the temperate forest zone, mixed species of conifer with broadleaf species were found to have a higher α-diversity and distinctive bacterial communities when compared with coniferous forests [6]. They interpreted this was due to soil texture and pH. Yang et al. [7] suggests that differences in organic matter quality between *Larix* monoculture and secondary natural forests were caused by different soil microbial processes.

The microbial community can also be influenced by soil chemistry and environmental factors, which in turn affect forest vegetation. Brant et al. [13] found that root C inputs affected microbial composition at the Detritus Input and Removal Treatment (DIRT) experiment. Högberg et al. [14] found that soil C/N ratios and pH levels contributed the most to soil microbial community composition on a 34-year-old, N-loading experiment in a boreal forest. In a comprehensive review, Llado et al. [15] examined environmental effects on microbial communities and found that regardless of local and continental scales, higher soil pH was consistently associated with more abundant microbes. By analyzing 126 forest soil samples across North America, Zhou et al. [16] showed a strong positive relationship between mean annual temperature and microbial diversity.

Microbes also affect plant diversity and ecosystem productivity. In their comprehensive review, van der Heijden et al. [17] summarized the soil microbial effect on various ecosystem processes from various studies. Not surprisingly, it appears that aboveground vegetation, roots, soil physicochemical properties, and microbial communities are all interrelated. The main challenge is to understand the mechanisms by which these factors or processes interact with one another.

In this study, we measured overstory tree growth, overstory and understory species richness and diversity, soil physicochemical properties, and microbial community structure in three adjacent forests consisting of monoculture of *Larix gmelinii*, natural vegetation mixed broadleaf species and conifers, and an intermediate monoculture/natural forest where *Larix* seedlings were interplanted into a natural stand. We sought to determine (1) variation in long-term productivity among the three forest types, and if different, (2) which soil or microbial community factors were related to the differences, and (3) how vegetation characteristics, soil physicochemical properties, and microbial communities interacted. We hypothesized that tree productivity and soil fertility of the *Larix* monoculture would be reduced versus the other two forests, and this reduction would be due to a change in the microbial community structure through the detrimental effects of soil property alterations. *Larix gmelinii* was chosen as it is one of most important coniferous species planted as monocultures in northeastern China, having been used on more than half of the total afforested plantations in this region.

2. Materials and methods

2.1. Study site and soil sampling

The study was conducted at the Laoshan Experimental Station on the Maoershan Forest Farm (Lat. 45°22′ N, Long. 127°30′ E, Elev. 300 m) in the Changbai Mountains, China. The typical natural forest in this region was a late-seral, temperate, mixed-coniferous-broadleaved forest. *Pinus koraiensis* is the dominant species that is mixed with multiple broadleaf species. The primary forest was clear-cut early in the last century and was naturally regenerated as a secondary forest. The broadleaved species are also a main component of the secondary forest and include *Betula platyphylla*, *Fraxinus mandshurica*, *Juglans mandshurica*, *Populus davidiana*, *Quercus mongolica*, *Phellodendron amurense*, and *Tilia amurensis*. Other evergreen coniferous species include *Pinus sylvestris* var. *mongolica*, and the deciduous conifer *Larix gmelinii*. Understory shrubs include *Acanthopanax senticosus*, *Corylus heterophylla*, *Lonicera* spp., *Rhamnus parvifolia*, *Syringa reticulata*, etc. A herbaceous layer is composed of *Aegopodium alpestre*, *Cardamine leucantha*, *Athyrium filix-femina*, and *Carex* spp.

The climate is temperate-continental-monsoon with mean annual precipitation 649 mm, annual mean temperature 2.7 °C, and a frost-free period of 120–140 days, based on climatic data available at Maoershan township, about 5-km air distance west of the study site. The soil is mostly Hap-Boric Luvisols according to Chinese taxonomy with granite bedrock as the parent material.

At the time of this study, three forest stands had already been established adjacent to one another and within the same site conditions with a south-facing slope of 18%. The secondary forest had been clear-cut 50 years ago and a major silvicultural experiment was implemented to study the effects of regeneration methods and species mixtures on stand dynamics and forest productivity. Treatments were a conversion of the natural forest to three types of forest. The *Larix* monoculture plantation (LP) was planted at a 2.5 × 2.5 m spacing after eradicating most woody plants grown on the post-harvest site during site preparation in 1967; a natural stand (NS) was allowed to establish itself from natural regeneration without management activities; and an interplanted stand (LP_NS) was created by planting *Larix* seedlings at a 5.0 × 5.0 m spacing on the post-harvest ground without any treatment in 1967. Since that, no other management activities have been applied (Guojiang Li, pers. comm.). Age differences were less than 5 years among overstory trees either planted or naturally regenerated. Competing vegetation was not controlled in any of the stands and

vegetation included conifers, broadleaved tree species, shrubs, and herbaceous species. Each stand was about 50 m wide and > 400 m long parallel to the mountain contour lines.

2.2. Soil measures

We randomly established three 20 m × 20 m plots in each of the three forest stands in May 2014. We collected soils from 0 to 5 cm, 5–10 cm, and 10–20 cm depths from three soil profiles per plot. Three samples from each depth were then composited into a single sample. The soils were placed in a dry-iced cooler in the field and transported to the laboratory for further processing. At the same sampling area, a second set of soil cores with sampler diameter of 3.36 cm were collected and not composited for measuring both soil bulk density, soil moisture content, and fine root biomass. The forest floor litter material in 1 m^2 subplots were collected at each plot center, and these were sorted to separate coniferous needles and broad leaved species.

2.3. Aboveground vegetation measures

In each plot, all overstory trees with dbh ≥ 4 cm were measured for diameter at breast height (1.37 m, dbh) and understory vegetation structure was measured in five 1-m^2 subplots. We tallied each plant within these plots or subplots; the species name was identified based on Flora of China (http://frps.eflora.cn/). Several variables that characterized these plots were derived. (1) Stand density, overstory composition and stand biomass using allometric equations developed at the very site [18] were calculated based on overstory tree measurements. (2) Species richness (N0), was estimated as the number of species at each plot, and the Shannon-Wiener Index (H') based on overstory and understory species inventory [19] was calculated as:

$$H' = -\sum\left(\frac{n_i}{N} \times ln\frac{n_i}{N}\right) \quad [1]$$

Where n_i is number of individuals of the *i*th species, *N* is total number of individuals for the treatment plot, and *ln* is the natural log of the number. The range of H′ is 0.0 and 5.0, representing a diverse and equally distributed community (5.0) and a community with just one species (0.0), respectively.

2.4. Soil physicochemical properties

The composited soil samples were sieved (2-mm mesh) to remove plant residues and rock fragments, homogenized and split into two batches. One batch was air-dried for measuring soil chemical properties, and the other batch was immediately kept in −80 °C freezer for phospholipid fatty acid (PLFA) analysis.

For the air-dried soil, we measured pH using a pH meter (MT-5000, Shanghai), total C and N were measured with combustion methods using a Macro elemental analyzer (vario MACRO, Elementar Co., Germany), and total P was measured colorimetrically (UV, spectrophotometer) after wet digestion with $HClO_4$–H_2SO_4.

On the set of soils that were not composited, we calculated soil water content (SWC) and bulk density (BD) of unsieved soils by weighing soils of known sample volume following drying at 105 °C to constant weight. Then, both SWC (% loss per dry weight) and bulk density (g dry weight cm^{-3}) were calculated. Fine root biomass was measured by hand sorting, washing, drying, and weighing the fine roots (< 2 mm) from the soil cores.

The total stocks of C, N, and P in each soil profile (0–5 cm, 5–10 cm, and 10–20 cm) was calculated as the product of mean BD, soil depth, and concentration of element.

2.5. Soil PLFA for microbial communities

PLFAs were extracted from 2 g of freeze-dried soil and analyzed using standard protocols [20]. These extracted soil lipids were placed in 25 ml glass tubes and added a mixture of 2 ml chloroform, 4 ml methanol and 1.6 ml phosphate buffer. PLFAs were quantified on a PerkinElmer AutoSystem XL gas chromatograph (GC) and calculated by means of an internal standard (the fatty acid 19:0). Fatty acids were designated X:YωZ, where X indicates the number of C atoms, followed by the number and position (ω) of double bonds. Prefixes 'i' and 'a' indicate branching at the second or third carbon atom, respectively, from the ω end. The prefix 'cy' indicates cyclopropyl group and the prefixes 't' and 'c' indicate trans and cis stereoisomers, respectively.

Concentrations of each PLFA were quantified based on the relative retention time of standard substances with 19 acid methyl ester and mass spectrogram compared with the database. The sum of the PLFAs 12:0, 14:0, i15:0, a15:0, 15:0, 16:0, i17:0, cy17:0, 17:0, 10me18:0, 18:1 ω 9, 18:0, cy19:0, 19:0, 20:0 and 22:0 was used for the total bacterial biomass. Among them, the sum of PLFAs i15:0, 10me18:0 and i17:0 were used as indicators for Gram-positive (G+) bacteria PLFAs, cy17:0, cy19:0, 16:1ω9c, 16:1ω7, and 18:1 ω 9 for Gram-negative (G−) bacterial PLFAs, 18:1ω9c and 18:1ω9 for fungal PLFAs, and 10-Me branch saturated fatty acid for actinomycetes. We calculated the ratio of saturated to mono-unsaturated fatty acids (sat/mono) as (12:0, 14:0, 15:0, 16:0, 17:0, 18:0, 21:0, 22:0)/(16:1 ω 9c, 18:1 ω 9c, 18:1 ω 10c) and the ratio of cyclopropyl fatty acids and mono-enoic precursors (cy/prec) as cy17:0, cy19:0/16:1 ω 7. Both ratios were used as indicators of nutritional stress, while cyclopropyl fatty acids were suggested to be indicators of environmental stress [21]. The i17:0 and i15:0 stands for iso- (i) branching PLFAs and a-15:0 is for anthe-iso- (a) branching PLFA.

2.6. Statistical analysis

We analyzed all aboveground variables (overstory and understory species richness, Shannon-Wieners diversity index H', aboveground biomass (AGB) and litter biomass of conifer and broadleaved species) based on a completely randomized design using SAS PROC MIXED (SAS Institute Inc., NC, USA), in which forest types were regarded as the fixed effect and plot as a random effect. For physicochemical variables (C, N, and P concentrations, total C, N, P stocks of the top 20-cm soil depth, bulk density, pH, fine root biomass, and C/N), total microbial PLFAs, and their functional group percentages, depth was regarded as a category term and included as a fixed effect to the model. The full model was:

$$y_{ijk} = \mu + \alpha_i + \gamma_j + \alpha\gamma_{ij} + \varepsilon_{ijk} \quad [2]$$

Where y_{ijk} is the dependent variable measured for the *i*th forest type and the *j*th depth, and *k*th replicated plot, μ is the overall mean, α_i is the fixed effect of the *i*th forest type (I = 1, 2, and 3), γ_j is the fixed effect of the *j*th depth (j = 1, 2, and 3), and ε_{ijk} is an experimental error, $\varepsilon_{ijk} \sim iid\ N(0, \sigma_e^2)$.

Normality and homoscedasticity assumptions were examined using residuals for each variable and data transformation with either natural log or square-root transformation as needed. We used the Tukey-Kramer test to compare differences among forest types or depths by controlling for the overall $\alpha = 0.05$. Principal component analysis (PCA) for each individual PLFAs was conducted with Canoco 5 for each forest type to elucidate major variation patterns among depths and between forest types. Redundancy analysis (RDA) was also performed to determine the canonical correlations among physicochemical properties, microbial community composition, vegetation biomass, and species richness and diversity.

3. Results

3.1. Overstory trees and understory vegetation

Aboveground biomass (AGB) of conifer trees differed significantly among forest types ($F = 6.87$, $P < 0.03$). AGB for all trees (i.e. conifer

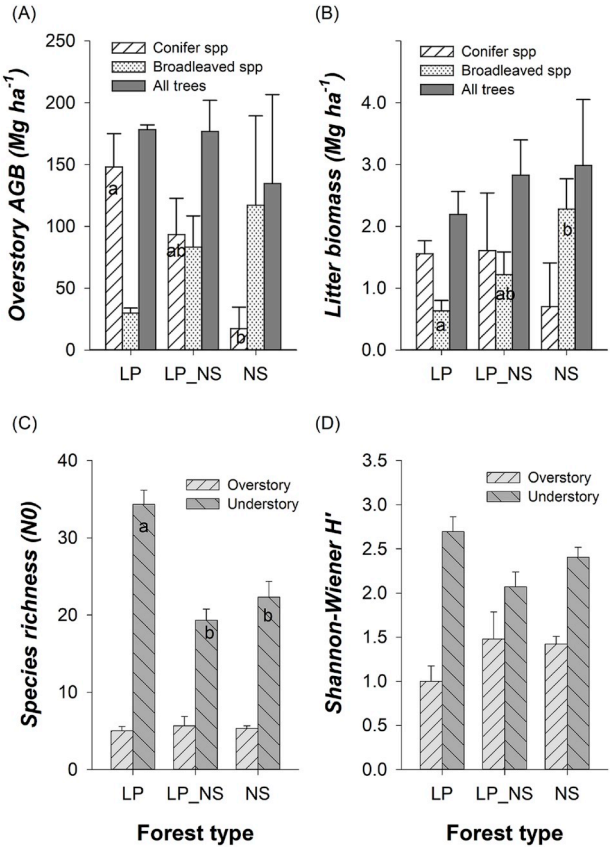

Fig. 1. Stand aboveground biomass (A) and litter biomass (B) of conifers, broadleaved trees, and all trees, and species richness (C) and diversity index (D) for both overstory trees and understory vegetation for three forest types in northeastern China. LP is *Larix* plantation; NS is natural stand; and LP_NS is mixed stand created by planting *Larix* seedlings in a natural stand. Significant differences among forest types for each variable are showed by different letters.

and broadleaved species combined) was not different among forest types, ($F = 0.40$, $P > 0.68$). As expected, LP showed significantly more conifer AGB (148.1 Mg ha^{-1}) than NS (17.4 Mg ha^{-1}) because *Larix* seedlings were planted in LP. Conifer AGB was 93.5 Mg ha^{-1} in LP_NS (Fig. 1A). The trend of conifer AGB among forest types corresponded to conifer density with 750 trees ha^{-1} (1000 overstory trees) in LP, 67 trees ha^{-1} (750 overstory trees) in NS, and 342 trees ha^{-1} (742 overstory trees) in LP_NS, respectively. AGB for broadleaved species in NS (117.4 Mg ha^{-1}) was more than 83.4 Mg ha^{-1} in LP_NS and 30.2 Mg ha^{-1} in LP, but these differences were not statistically significant ($F = 1.00$, $P > 0.42$). However, a difference in broadleaved litter biomass was statistically significant between LP (0.64 Mg ha^{-1}) and NS (2.28 Mg ha^{-1}) (Fig. 1B); neither total litter nor coniferous litter biomass differed significantly among forest types.

Natural regeneration of many vegetation species occurred simultaneously with planted *Larix* seedlings in the plantation (LP). This resulted in the species richness of the monoculture plantation overstory as almost identical to the other two stands with 5 or 6 species occurring in each of the forest stand types (Fig. 1C). However, understory richness differed significantly with more species in LP ($N0 = 35$) than in the other two forest types ($N0 \approx 20$). The extra understory species in LP was not due to invasive species (http://frps.eflora.cn/). Shannon-Wiener plant diversity index (H′) was similar among forest types although understory vegetation (shrubs and herbaceous species) showed much higher H' (2.0–2.7) than overstory trees (1.0–1.5) (Fig. 1D).

3.2. Soil physicochemical properties

LP had significantly lower concentrations of soil C, N, and P than the other two stands over the entire 20-cm soil profile (Table 1). Because BD was highest in LP, total C and N stocks over the entire 20-cm soil profile were nearly the same as the other stands (Table S1), although total P stock in LP was statistically lower than LP_NS. LP and LP_NS soils had lower pH compared to NS. Fine root biomass was also lower in LP than in other stands (Table 1). C/N ratios were similar among the three forest stands (Table S1).

Higher values for concentrations of C and N, total C, N, and P stocks, and C/N were observed and found to be statistically significant in the uppermost soils. Note that higher stocks of C, N, and P at 10–20 cm in some forest types were due to a greater soil volume than sampled at 0–5 cm and 5–10 cm depths (Table 1 and S1). There was no difference in either soil bulk density or pH among depths.

3.3. Soil microbial community

Total PLFAs, bacterial, fungal, actinomycetes, G+ and G- PLFAs, and the ratio of bacterial and fungal PLFAs were not statistically significant different among forest types (Table 2 and S2). However, LP soils showed a trend of slightly higher PLFAs than other forest types

Table 1
Means (± standard errors) of soil physicochemical properties and fine root biomass at three depths from three forest types at Laoshan experimental station. Probabilities for testing fixed effect of forest type, soil depth, and their interactions are indicated.

Forest Type	Depth (cm)	C (%)	N (%)	P (mg Kg^{-1})	BD (g cm^{-3})	pH	Fine roots (g m^{-3})
***Larix* plantation**	0–5	6.0 ± 0.02	0.7 ± 0.08	0.3 ± 0.06	1.07 ± 0.10	5.5 ± 0.11	0.45 ± 0.18
	5–10	4.7 ± 0.23	0.5 ± 0.02	0.3 ± 0.02	1.27 ± 0.04	5.4 ± 0.12	0.31 ± 0.16
	10–20	3.1 ± 0.13	0.4 ± 0.07	0.2 ± 0.04	1.28 ± 0.13	5.5 ± 0.03	0.07 ± 0.03
	Mean	***4.6 ± 0.44***a	***0.6 ± 0.05***a	***0.3 ± 0.02a***	***1.21 ± 0.06***a	***5.5 ± 0.05***a	***0.27 ± 0.09***a
***Larix*-Natural stand**	0–5	9.6 ± 1.19	0.9 ± 0.08	1.3 ± 0.26	0.93 ± 0.13	5.5 ± 0.08	0.71 ± 0.19
	5–10	5.8 ± 0.47	0.7 ± 0.09	0.6 ± 0.12	0.86 ± 0.02	5.5 ± 0.04	0.68 ± 0.08
	10–20	4.0 ± 0.12	0.5 ± 0.03	0.5 ± 0.10	0.94 ± 0.06	5.4 ± 0.06	0.31 ± 0.09
	Mean	***6.5 ± 0.91***b	***0.7 ± 0.07***b	***0.8 ± 0.15b***	***0.91 ± 0.05***b	***5.4 ± 0.04***a	***0.57 ± 0.09***b
Natural stand	0–5	9.7 ± 0.81	1.0 ± 0.15	0.4 ± 0.03	1.01 ± 0.06	6.1 ± 0.09	0.72 ± 0.12
	5–10	5.9 ± 0.32	0.7 ± 0.06	0.6 ± 0.22	1.02 ± 0.12	5.8 ± 0.09	0.32 ± 0.04
	10–20	4.1 ± 0.28	0.6 ± 0.03	0.6 ± 0.18	0.90 ± 0.06	5.6 ± 0.22	0.53 ± 0.27
	Mean	***6.6 ± 0.86***b	***0.8 ± 0.08***b	***0.5 ± 0.09 ab***	***0.98 ± 0.05***b	***5.9 ± 0.10***b	***0.53 ± 0.10*** ab
Pr > F	**Forest type**	**< 0.001**	**0.006**	**0.001**	**0.001**	**< 0.001**	0.059
	Soil depth	**< 0.001**	**< 0.001**	0.185	0.754	0.182	0.054
	FT*SD	0.378	0.702	**0.030**	0.278	0.188	0.416

Note: $n = 3$ for depth means; $n = 9$ for forest type means. Different letters within each variable indicate significant difference among forest types at $P < 0.05$.

Table 2
Means (± standard errors) of microbial biomass PLFAs, bacterial and fungal PLFAs, and the ratios of G+/G-, saturated/monounsaturated (Sat/Mono), and cyclopropyl to monoenoic precursors (cy/prec) PLFAs at three depths from three forest types at Laoshan experimental station. Probabilities for testing fixed effect of forest type, soil depth, and their interactions were provided.

Forest Type	Depth (cm)	PLFA (nmol g^{-1})	Bacterial (nmol g^{-1})	Fungal (nmol g^{-1})	G+/G-	Sat/Mono	Cy/Prec
***Larix* plantation**	0–5	361 ± 22.5	315 ± 19.7	30 ± 3.2	1.0 ± 0.03	6.0 ± 0.3	1.1 ± 0.0
	5–10	263 ± 32.4	228 ± 28.2	15 ± 3.4	1.1 ± 0.03	6.8 ± 0.4	1.0 ± 0.1
	10–20	335 ± 63.5	297 ± 55.7	16 ± 5.1	1.4 ± 0.27	8.3 ± 0.2	0.8 ± 0.2
	Mean	***320 ± 26.2***a	***280 ± 23***a	***20 ± 3.2***a	***1.2 ± 0.11***a	***7.0 ± 0.4*** ab	***1.0 ± 0.1***a
***Larix*-Natural stand**	0–5	330 ± 31.3	291 ± 26.8	25 ± 3.2	1.2 ± 0.10	7.1 ± 0.6	1.1 ± 0.1
	5–10	275 ± 26.9	243 ± 23.2	18 ± 2.3	1.0 ± 0.04	8.1 ± 0.7	1.2 ± 0.1
	10–20	147 ± 4.67	129 ± 3.5	8 ± 0.6	1.5 ± 0.08	7.7 ± 0.7	0.8 ± 0.2
	Mean	***251 ± 29.7***a	***221 ± 26***a	***17 ± 2.8***a	***1.2 ± 0.08***a	***7.6 ± 0.4***a	***1.0 ± 0.1***a
Natural stand	0–5	339 ± 36.8	295 ± 32.4	29 ± 6.4	1.0 ± 0.03	6.1 ± 0.4	1.2 ± 0.1
	5–10	227 ± 57.8	201 ± 50.8	16 ± 4.7	0.8 ± 0.07	6.9 ± 0.5	2.4 ± 0.4
	10–20	206 ± 85.1	180 ± 74.5	19 ± 9.4	1.1 ± 0.13	6.2 ± 0.4	1.0 ± 0.2
	Mean	***257 ± 37.7***a	***225 ± 33***a	***21 ± 4.2***a	***0.9 ± 0.07***b	***6.4 ± 0.3***b	***1.5 ± 0.3***b
Pr > F	**Forest type**	0.161	0.167	0.518	**0.004**	**0.021**	**0.027**
	Soil depth	**0.020**	**0.022**	**0.008**	**0.001**	0.052	**0.002**
	FT*SD	0.288	0.256	0.762	0.376	0.117	0.105

Note: Different letters within each variable indicate significant difference among forest types at $P < 0.05$.

except for fungal PLFA. In contrast, G+/G-, Sat/Mono, and Cy/Prec were significantly different among forest types. Total soil microbes and individual microorganism abundance (bacterial, fungal, actinomycetes, G+ and G- PLFAs) were significantly greater at the top soil depth (0–5 cm) than at 5–10 cm and 10–20 cm depths except for LP soil between 0-5 cm and 10–20 cm. There were no significant interactions between forest type and soil depth.

Other subtle soil microbial community differences among forest types and depths were also reflected from individual loading values for the PLFAs in principle component analysis (Fig. 2). The first and second

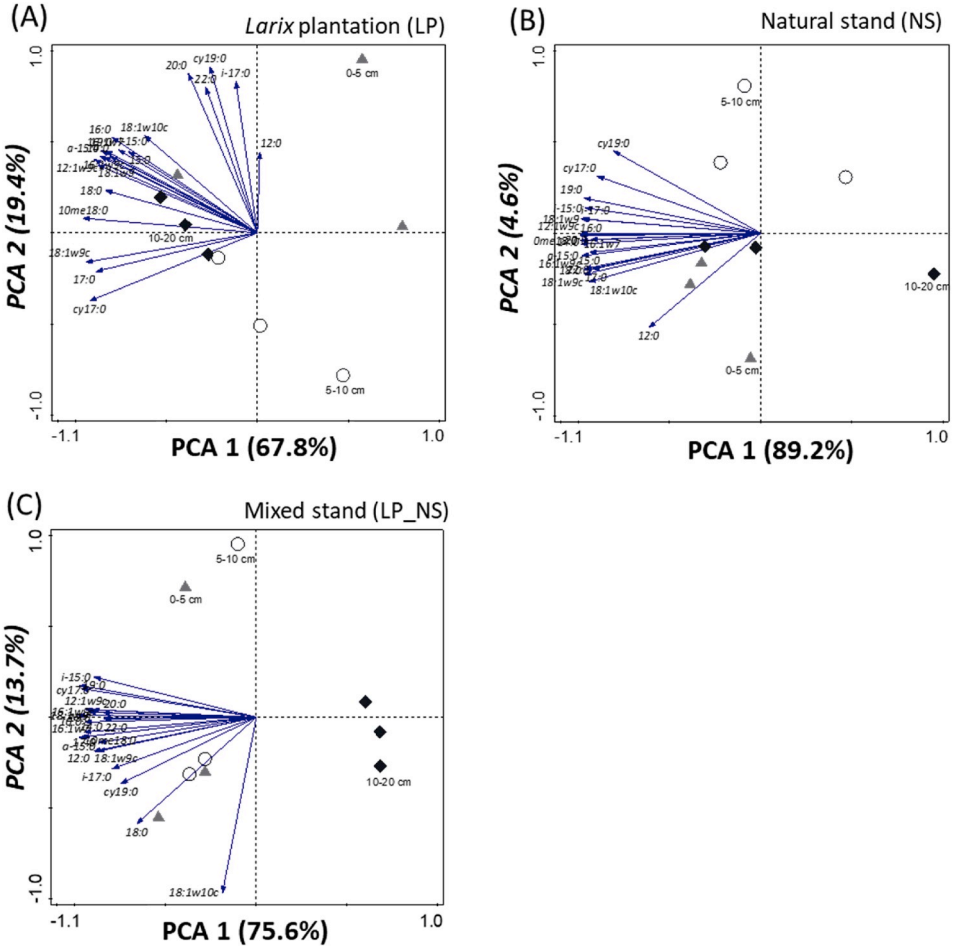

Fig. 2. Principal component analysis (PCA) of the signature lipid biomarkers (phospholipid fatty acids: PLFAs) used for examining soil microbial community at three depths in (A) *Larix* plantation, (B) natural forest stand, and (C) mixed stand grown at Laoshan, northeastern China. The biomarkers are detailed in the methods section.

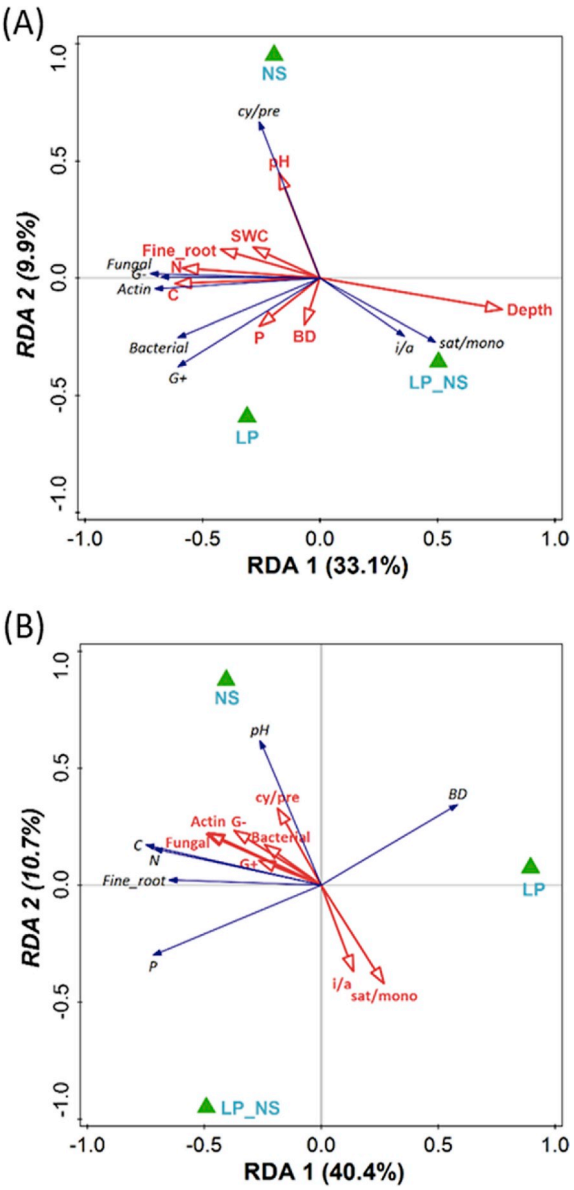

Fig. 3. Ordination plots of the results from the redundancy analysis to identify the relationships between soil physicochemical properties and microbial community composition from three forest types at Laoshan, northeastern China. (A) Microbial PLFAs as responding variables and (B) physicochemical properties as responding variables. cy/pre, the ratios of cyclopropyl to monoenoic precursors PLFAs; sat/mono, the ratios of total saturated to total monounsaturated PLFAs; i/a, the ratios of iso- and anthe-iso- branching PLFAs; BD, bulk density; and SWC, soil water content.

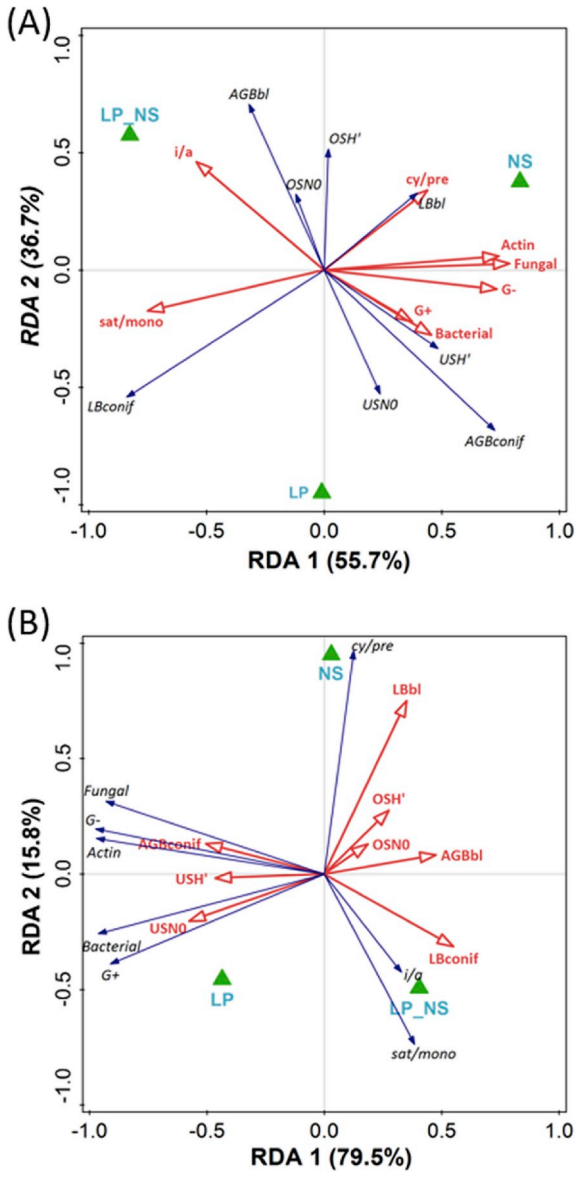

Fig. 4. Ordination plots of the results from the redundancy analysis to identify the relationships between aboveground vegetation variables and microbial community composition from three forest types at Laoshan, northeastern China. (A) Vegetation variables as responding variables and (B) Microbial PLFAs as responding variables. AGBbl, broadleaved species overstory aboveground biomass; AGBconif, conifer overstory aboveground biomass; LBbl, broadleaved litter biomass; LBconif, conifer litter biomass; OSH′, overstory species diversity; OSN0, number of overstory species; USH′, understory species diversity; USN0, number of understory species; cy/pre, the ratios of cyclopropyl to monoenoic precursors PLFAs; sat/mono, the ratios of total saturated to total monounsaturated PLFAs; i/a, the ratios of iso- and anthe-iso- branching PLFAs.

principals (PCA 1 and PCA 2) explained 67.8 and 19.4% of PLFA variation in LP (Fig. 2A), 89.2 and 4.6% in NS (Fig. 2B), 75.6 and 13.7% in LP_NS (Fig. 2C), respectively. It appears that PLFA biomarkers were concentrated in second quadrant in LP, compared to PLFAs between second and third quadrant in NS and LP_NS. Interestingly, microbial activity was higher at 10–20 cm soil depth in LP and at 0–10 cm in LP_NS respectively. There was no clear pattern in NS. In all forest types, PLFA 10Me18:0, one of the biomarkers for both G+ bacteria and actinomycetes and 18:1ω9c for fungi were strongly negatively correlated with the first principal component. In contrast, cy19:0, a biomarker for G- and i-17:0 for G+ were strong-positively correlated with PCA 2 in LP. PLFA 18:1ω10c, one of the monounsaturated fatty acids in LP_NS, had strongly negative loadings on PCA 2.

3.4. Relationships among soil properties, microbial community, and aboveground vegetation

Redundancy analysis (RDA) indicated that physicochemical properties could explain 43.0% of the variance in microbial PLFAs (Fig. 3A), while microbial PLFAs could explain 51.1% of variance in physicochemical properties (Fig. 3B). Both analyses showed that differences were larger along RDA 1 between LP and LP_NS with NS being the middle. Along RDA 2, however, difference between LP and NS was larger in Fig. 3A and so was between LP_NS and NS in Fig. 3B. Apparently, C and N concentrations and fine-root biomass significantly influenced distribution of actinomycetes, fungal, and G-PLFAs. pH was

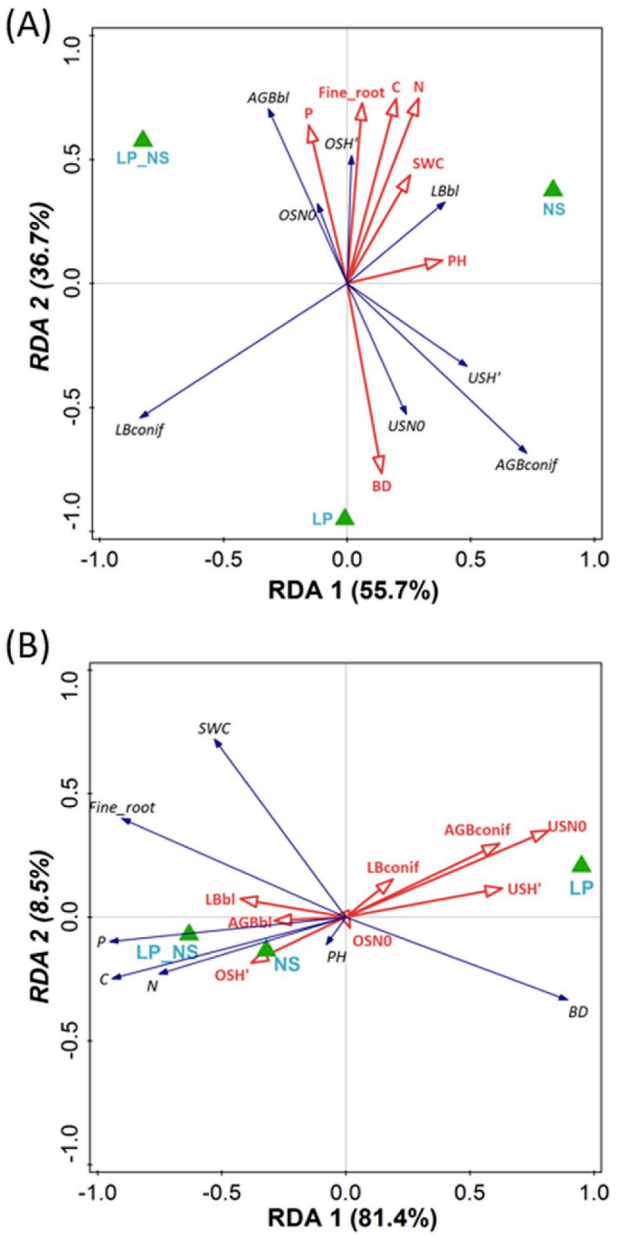

Fig. 5. Ordination plots of the results from the redundancy analysis to identify the relationships between aboveground vegetation variables and soil physicochemical properties from three forest types at Laoshan, northeastern China. (A) Vegetation variables as responding variables and (B) soil physicochemical properties as responding variables. AGBbl, broadleaved species overstory aboveground biomass; AGBconif, conifer overstory aboveground biomass; LBbl, broadleaved litter biomass; LBconif, conifer litter biomass; OSH′, overstory species diversity; OSN0, number of overstory species; USH′, understory species diversity; USN0, number of understory species; BD, soil bulk density; SWC, soil water content.

positively related with cy/pre PLFAs, but negatively associated with i/a and sat/mono PLFAs.

Microbial community provided 92.4% explanation of aboveground vegetation biomass, species richness and H' (Fig. 4A). Fungal, actinomycetes, G-, and sat/mono PLFAs were along the RDA 1. Broadleaved litter biomass (LBbl) was positively related to cy/pre; both were negatively related to conifer litter biomass (LBconif). Bacterial and G + PLFAs were positively related to conifer aboveground biomass (AGBconif), understory H′ and N0; they were negatively related to broadleaved AGB, overstory H' and N0. There were similar relationships with entire microbial community (bacterial, fungal, actinomycetes, G+ and G-) as responding variables (Fig. 4B), with vegetation variables providing 95.3% explanation.

Soil physicochemical properties explained 92.4% variation of aboveground vegetation variables (Fig. 5A). Concentrations of P, C, and N, fine-root biomass, and SWC were positively correlated with broadleaved AGB and litter biomass, overstory H′ and N0, but were negatively related to conifer AGB and litter biomass and understory H' and N0. Whereas, BD showed the opposite relationships. Similar trends were found in Fig. 5B when vegetation variables explained 89.9% of soil properties.

4. Discussion

4.1. Vegetation effect on physicochemical properties

The reduced soil concentrations of elements, roots, and pH on the monoculture LP stand are consistent with literature reports of soil degradation that may in turn cause reduced forest productivity [22]. Similar trends are reported for other plantations relative to natural forests, such as subtropical forests with *Cunninghamia lanceolata* plantations in China [2,10], and perhaps other forests across the world [1].

Soil pH plays a critical role in determining soil microbial community and influencing soil cation exchange, nutrient availability and leaching processes [13–15]. The observed level of reduction of pH in LP is in agreement with trends in previous studies. Yang et al. [7] found that soil pH was about 6.0 in natural secondary forest and 5.4 in the *Larix* plantation in Liaoning Province, which are very similar to our soil pH of 5.5 in the LP and 5.9 in the NS. These values are much higher than what Li et al. [6] found in the Changbai Nature Reserve, where *Larix olgensis* forest had the lowest pH 4.8 compared to spruce-fir (pH 5.0), poplar-birch (pH 5.4), and broadleaf-Korean pine (pH 5.2) mixed forests. The lower absolute pH values are thought to be mainly related to soil parent materials [6].

Compared to observed reductions in soil pH of sub-tropical forests of *Cunninghamia* plantations [10], pH reduction in our *Larix* plantations was less. Fu et al. [10] reported a 0.95 unit reduction in a 27-year-old *Cunninghamia* plantation compared with the native broadleaved forest. Here, we found a 0.4 unit reduction in LP compared to NS at about 50 years. Jackson et al. [23] found soil pH was 0.3 units lower under afforested plantations than adjacent grassland and shrublands, which related to higher Na concentration. However, Liao et al. [24] failed to detect any difference in soil pH between natural forests and plantations in a meta-analysis. Neither did soil Na and Al concentrations vary. In our study, Na and Al concentrations were not measured. But higher biomass, although it is non-significant (Fig. 1A), of both LP and LP_NS than NS may need to be balanced by additional cations [23], which may reduce pH by 0.4 units compared to NS.

Significantly lower C, N, and P concentrations in the surface 20 cm of soil in the LP plantation is consistent with previous studies [6,7,24]. Because LP had a higher soil bulk density than other forest types, total C and N stocks were not significantly different among forest types. NS stocks had 15% more C stock and 8% more N stock than LP. A meta-analysis of 74 studies suggested a 13% reduction of soil C stock when native forest was converted to plantation [25]. However, if nutrient concentrations are considered, LP had 22%, 25%, and 40% lower C, N, and P concentrations, respectively. These numbers are slightly lower than what was found in a meta-analysis with reductions of C and N concentrations being 36.0% and 26.0% in plantations relative to natural forests [24].

Severely compacted soil with extremely high BD can limit root growth. Here, significantly higher (~30%) BD in LP versus other forest types may have been caused by soil compaction during site preparation. Liao et al. [24] found that soil BD in plantations increased by 12.5%. In a review, Daddow and Warrington [26] found that 1.75 Mg m^{-3} for

sandy loams and 1.4 Mg m^{-3} for fine-textured soils limited root growth of ponderosa pine. Although a BD of 1.21 Mg m^{-3} in the LP was not high enough to restrict root growth of ponderosa pine, significantly less fine roots in LP observed here suggests that *Larix* roots may be more sensitive to soil compaction or distributed in deeper soils than 20 cm where we did not sample (Table 1). In addition, because of the small sample sizes with three 3.36 cm dimeter soil cores collected in each plot, the fine-root results in this study must be used with caution, although a general trend of more fine-roots in the mixed-species forests than in the monoculture is consistent with previous studies [27].

4.2. Vegetation effect on microbial communities

Soil microbes are the primary decomposers of soil organic matter and play a key role in soil biogeochemical cycling in forest ecosystems, and can be a sensitive indicator of environmental changes [28]. Vegetation can directly influence microbial communities with the quality and quantity of plant inputs [10] or indirectly by changing soil physicochemical properties. Our results for microbial communities indicated that the *Larix* plantation caused subtle, though non-significant changes in microbial composition and biomass compared to the NS or the LP_NS (Table 2 and S2; Figs. 1 and 2). Our results differ from previous studies, such as Yang et al. [7] who found greater microbial biomass and soil enzyme activities in the secondary forests than in *Larix* plantations. Yang [2] reported that quantities of bacteria, actinomycetes, and fungi declined about 49%, 5%, and 6%, respectively, in the second rotation *Cunninghamia* plantation compared to the first rotation. However, total, bacterial, and fungal PLFAs were not different between broadleaved forest, shrub stand, and 27-year-old *Cunninghamia* plantation with slightly higher biomass in the plantation [10], although the *Pinus massoniana* plantation had significantly lower biomass. Therefore, the effect of plantation establishment on microbial community appears to be related not just to overstory tree species.

Despite the highest disturbance during site preparation in the past, LP plantation showed higher, but non-significant total PLFAs and bacterial, G+, and G- PLFAs. Because these stands were not intensively managed, such as application of fertilizers or herbicides that control competing vegetation, the microbial community might have recovered from the pre-planting disturbances. Using the same PLFA technique, Moore-Kucera and Dick [29] found that composition of the microbial community had recovered back to levels similar to an old-growth Douglas-fir forest after 25 years following clear-cutting.

Fungal to bacterial PLFA ratio is usually used to evaluate the change of fungi and bacteria microbial community biomass in the soil, the relative abundance of the two communities, and the stability of the soil ecosystem [30]. There is generally a low level of nitrogen in the soil substrate, and a lower mineralization rate when the fungi are broken down by fungi eaters in a situation of lower fungi/bacteria ratio. Conversely, the increase in fungal biomass and fungal mycelium as indicated by a high fungi/bacteria ratio means more fungal contact area with soil available nutrients, and thus the soil ecosystem is more stable because of reduced loss of soil nutrient elements [31]. In our study, soil fungal content was higher (Table 2) and bacterial/fungal ratio was lower (Table S2) in NS than in LP, suggesting that the stability of the soil ecosystem is greater in the natural stand.

Cyclopropyl fatty acids were used as indicators of environmental stress [21]. The lower cy/prec suggests less stress levels in both LP and LP_NS than in NS. In contrast, higher sat/mono indicates nutritional stress in bacterial communities, although we have no knowledge how severe the stresses are imposed on the soil microbes, except for lower pH could have caused these stresses.

4.3. Physicochemical properties, microbial community, and vegetation

Changes in soil structure and physical properties not only affect soil nutrient availability, but also influence soil microbial community structure. These coinciding changes occurred with soil depths; for example, decrease of soil C and N concentrations strongly affects the vertical distribution of special microorganisms [32]. Our results showed expected decreasing trends for C and N concentrations with soil depths. However, the trends for microbial composition only showed in the top 5 cm compared to other depths, possibly confounded from the uneven sampling of soil volume. Microbial abundance and biomass were greatest at the top, because the surface soil is where enrichment of fine root biomass, litter accumulation, higher organic matter content and good air-exchange conditions occur, which benefits the growth and reproduction of soil microorganisms.

Soil pH regulates soil biological activities including the microbial community. Generally, microbial biomass and activity declines in a more acidic soil [12,14]. This study showed a significantly lower soil pH in LP and LP_NS than in NS, which was associated with C and N concentrations, positively related to cy/pre and negatively related to i/a and sat/mono. However, pH was not related to microbial biomass (Fig. 3), suggesting that pH was not low enough to cause the direct changes of microbial communities [7].

4.4. Plant diversity and productivity responses

The forest species composition plays a key role in affecting ecosystem function and its sustainable management. Mixed planting of conifer and broadleaf species has been advocated to effectively mitigate a potential soil degradation caused by monoculture practices in plantation forests [2,10,12]. Here, our study demonstrated that although concentrations of soil C and N, and pH were higher and presumably better in NS than in LP, and P was the highest in LP_NS, the aboveground biomass of standing trees was not different among the three forest types (Fig. 1). In fact, overstory AGB was the lowest in the NS (Fig. 1A), suggesting that the LP did not reduce stand overstory tree productivity. We offer several possible explanations: (1) the plantation studied here differs from other plantations where competing vegetation is effectively controlled in the early stage of plantation development. Here, shrubs and other hardwood species freely regenerated from the very beginning of plantation development, so that the plantation ended up being very similar to a natural stand except that LP has ~30% more trees than NS or LP_NS stands. Because understory competition was never controlled, trees in the lower density plots did not grow significantly bigger and therefore, more trees in LP resulted in more biomass than in NS and LP_NS. (2) Total stand productivity differs from the overstory-only measurements because understory vegetation biomass can be greater than that of overstory trees. During the first 20 years understory growth exceeded overstory growth [33], but after that they found that the tree-only stands or plantations grew more biomass due to trees growing over the understory shrubs. Although we only measured overstory biomass in this study, higher AGB in the LP suggests a similar result, because these stands have been with 100% crown closure for many years. LP may reduce its biomass considerably faster after an onset of self-thinning in the future [33]. (3) The non-significant soil total C stock among forest types here may also indicate that site productivity in the LP is similar to the others. Chen et al. [34] reported that decreased soil carbon stocks were restored within 27 years after subtropical forest was converted to *Cunninghamia* plantations. So, it is not surprising that a 50 + year plantation restores soil carbon similar to a secondary-growth stand. (4) Although there was similar AGB among forest types, species composition measured with AGB varied. This species shift from broadleaved species in NS to conifers in LP may cause the soil changes, which may cause a decline in productivity in the future.

The plant species diversity (H') was not different among forest types, and more interestingly, there were more plant species in the LP than in the NS. If it is true that vegetation controls soil microbes, the microbial community should not differ among these stands. Fu et al.

[10] did not find differences on any PLFAs or the fungal/bacterial ratios between shrubland and broadleaf forest. Spake et al. [35] did not find a difference in ectomycorrhizal fungi between a mature plantation and an old-growth forest. Our study showed significant differences in G+/G-, sat/mono, and cy/prec PLFAs among the subject forest types (Table 2), which suggests the different effects of vegetation species on soil microbial community. Conversely, soil microbes may have more influence on aboveground plant diversity and productivity [17].

5. Conclusions

This study demonstrated that the *Larix* plantation with naturally established native species vegetation did not reduce overstory tree productivity (178.3 Mg ha^{-1}) compared to secondary natural stand (134.7 Mg ha^{-1}) or to the mixed planted *Larix* trees within the natural stand (176.9 Mg ha^{-1}), even though LP had significantly lower soil pH and lower soil concentrations of C, N, and P, and higher soil bulk density. As expected, 83% overstory AGB in LP was conifers and 87% of AGB in NS was broadleaved species, and LP_NS had similar conifer AGB (53%) and broadleaved AGB (47%). Understory species diversity was not different among the three forest types ($2.1 \leq H' \leq 2.7$, $P > 0.09$). Microbial PLFA did not differ ($P > 0.11$) except G+/G-, sat/mono, and cy/prec PLFAs ($P < 0.03$). Based on these results, we concluded that the plantation did significantly alter soil physiochemical properties and some microbial communities, but not overall plant diversity and overstory tree productivity. It is the species shifts that may cause some soil changes in LP, which may affect future productivity.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejsobi.2019.103149.

References

[1] C.L.C. Liu, O. Kuchma, K.V. Krutovsky, Mixed-species versus monocultures in plantation forestry: development, benefits, ecosystem services and perspectives for the future, Glob. Ecol. Conserv. 15 (2018) e00419, , https://doi.org/10.1016/j.gecco.2018.e00419.

[2] C.D. Yang, Decline of quantity and quality of soil organic matter is the key factor restricting the growth of plantation in China, Sci. Silvae Sin. 52 (2016) 1–12 (in Chinese with English abstract).

[3] J. Evans, Long-term productivity of forest plantations–status in 1990, Proceedings of the Nineteenth World International Union of Forest Research Organisations Congress, 1 1990, pp. 165–180.

[4] D. Piotto, A meta-analysis comparing tree growth in monocultures and mixed plantations, For. Ecol. Manag. 255 (2008) 781–786, https://doi.org/10.1016/j.foreco.2007.09.065.

[5] R.F. Powers, Long-Term Soil Productivity: genesis of the concept and principles behind the program, Can. J. For. Res. 36 (2006) 519–528, https://doi.org/10.1139/X05-279.

[6] H. Li, D. Ye, X. Wang, M.L. Settles, J. Wang, Z. Hao, L. Zhou, P. Dong, Y. Jiang, Z. Ma, Soil bacterial communities of different natural forest types in Northeast China, Plant Soil 383 (2014) 203–216, https://doi.org/10.1007/s11104-014-2165-y.

[7] K. Yang, W. Shi, J.J. Zhu, The impact of secondary forests conversion into larch plantations on soil chemical and microbiological properties, Plant Soil 368 (2013) 535–546, https://doi.org/10.1007/s11104-012-1535-6.

[8] C.Y. Chen, S.L. Wang, Ecology on Mixed Plantation, Science Press, Beijing, 2004 (in Chinese).

[9] L.X. Chen, X.W. Chen, W.B. Duan, Larch litter and soil fertility, Chin. J. Appl. Ecol. 9 (1998) 581–586, https://doi.org/10.13287/j.1001-9332.1998.0122 (in Chinese).

[10] X. Fu, F. Yang, J. Wang, Y. Di, X. Dai, X.Y. Zhang, H.M. Wang, Understory vegetation leads to changes in soil acidity and in microbial communities 27 years after reforestation, Sci. Total Environ. 502 (2015) 280–286, https://doi.org/10.1016/j.scitotenv.2014.09.018.

[11] K.G. Mattson, J.W. Zhang, Forests in the northern Sierra Nevada of California, USA, store large amounts of carbon in different patterns, Ecosphere 10 (6) (2019) e02778, , https://doi.org/10.1002/ecs2.2778.

[12] E. Hackl, M. Pfeffer, C. Donat, G. Bachmann, S. Zechmeister-Boltenstern, Composition of the microbial communities in the mineral soil under different types of natural forest, Soil Biol. Biochem. 37 (2005) 661–671, https://doi.org/10.1016/j.soilbio.2004.08.023.

[13] J.B. Brant, D.D. Myrold, E.W. Sulzman, Root controls on soil microbial community structure in forest soils, Oecologia 148 (2006) 650–659, https://doi.org/10.1007/s00442-006-0402-7.

[14] M.N. Högberg, P. Högberg, D.D. Myrold, Is microbial community composition in boreal forest soils determined by pH, C-to-N ratio, the trees, or all three? Oecologia 150 (2007) 590–601, https://doi.org/10.1007/s00442-006-0562-5.

[15] S. Llado, R. Lopez-Mondejar, P. Baldrian, Drivers of microbial community structure in forest soils, Appl. Microbiol. Biotechnol. 102 (2018) 4331–4338, https://doi.org/10.1007/s00253-018-8950-4.

[16] J.Z. Zhou, Y. Deng, L.N. Shen, C.Q. Wen, Q.Y. Yan, D.L. Ning, Y.J. Qin, K. Xue, L.Y. Wu, Z.L. He, J.W. Voordeckers, J.D. Van Nostrand, V. Buzzard, S.T. Michaletz, B.J. Enquist, M.D. Weiser, M. Kaspari, R. Waide, Y.F. Yang, J.H. Brown, Temperature mediates continental-scale diversity of microbes in forest soils, Nat. Commun. 7 (2016) 12083, , https://doi.org/10.1038/ncomms12083.

[17] M.G.A. Van Der Heijden, R.D. Bardgett, N.M. Van Straalen, The unseen majority: microbes as drivers of plant diversity and productivity in terrestrial ecosystems, Ecol. Lett. 11 (2008) 296–310, https://doi.org/10.1111/j.1461-0248.2008.01199.x.

[18] C.K. Wang, Biomass allometric equations for 10 co-occurring tree species in Chinese temperate forests, For. Ecol. Manag. 222 (2006) 9–16, https://doi.org/10.1016/j.foreco.2005.10.074.

[19] C.E. Shannon, A mathematical theory of communications, Bell Syst. Tech. J. 27 (1948) 379–423, https://doi.org/10.1002/j.1538-7305.1948.tb01338.x.

[20] E.G. Bligh, W.J. Dyer, A rapid method of total lipid extraction and purification, Can. J. Biochem. Physiol. 37 (1959) 911–917, https://doi.org/10.1139/o59-099.

[21] A. Kaur, A. Chaudhary, A. Kaur, R. Choudhary, R. Kaushik, Phospholipid fatty acid – a bioindicator of environment monitoring and assessment in soil ecosystem, Curr. Sci. 29 (2005) 1103–1112 https://www.jstor.org/stable/24110962.

[22] S. Liu, X. Li, L. Niu, The degradation of soil fertility in pure larch plantations in the northeastern part of China, Ecol. Eng. 10 (1998) 75–86, https://doi.org/10.1016/S0925-8574(97)10024-6.

[23] R.B. Jackson, E.G. Jobbágy, R. Avissar, S.B. Roy, D.J. Barrett, C.W. Cook, K.A. Farley, D.C. le Maitre, B.A. McCarl, B.C. Murray, Trading water for carbon with biological carbon sequestration, Science 310 (2005) 1944–1947, https://doi.org/10.1126/science.1119282.

[24] C.Z. Liao, Y.Q. Luo, C.M. Fang, J.K. Chen, B. Li, The effects of plantation practice on soil properties based on the comparison between natural and planted forests: a meta-analysis, Glob. Ecol. Biogeogr. 21 (2012) 318–327, https://doi.org/10.1111/j.1466-8238.2011.00690.x.

[25] L.B. Guo, R.M. Gifford, Soil carbon stocks and land use change: a meta-analysis, Glob. Chang. Biol. 8 (2002) 345–360, https://doi.org/10.1046/j.1354-1013.2002.00486.x.

[26] R.L. Daddow, G.E. Warrington, Growth-limiting Soil Bulk Densities as Influenced by Soil Texture, Watershed Systems Development Group, USDA Forest Service, Fort Collins, CO, 1983.

[27] B.W. Brassard, H.Y.H. Chen, Y. Bergeron, D. Pare, Differences in fine root productivity between mixed- and single-species stands, Funct. Ecol. 25 (2011) 238–246, https://doi.org/10.1111/j.1365-2435.2010.01769.x.

[28] J.A. Pascual, C. Garcia, T. Hernandez, J.L. Moreno, M. Ros, Soil microbial activity as a biomarker of degradation and remediation processes, Soil Biol. Biochem. 32 (2000) 1877–1883, https://doi.org/10.1016/S0038-0717(00)00161-9.

[29] J. Moore-Kucera, R.P. Dick, PLFA profiling of microbial community structure and seasonal shifts in soils of a Douglas-fir chronosequence, Microb. Ecol. 55 (2008) 500–511, https://doi.org/10.1007/s00248-007-9295-1.

[30] S.A. Boyle, R.R. Yarwood, P.J. Bottomley, D.D. Myrold, Bacterial and fungal contributions to soil nitrogen cycling under Douglas-fir and red alder at two sites in Oregon, Soil Biol. Biochem. 40 (2008) 443–451, https://doi.org/10.1016/j.soilbio.2007.09.007.

[31] F.T. De Vries, E. Hoffland, N. van Eekeren, L. Brussaard, J. Bloem, Fungal/bacterial ratios in grasslands with contrasting nitrogen management, Soil Biol. Biochem. 38 (2006) 2092–2103, https://doi.org/10.1016/j.soilbio.2006.01.008.

[32] N. Fierer, J.P. Schimel, P.A. Holden, Variations in microbial community composition through two soil depth profiles, Soil Biol. Biochem. 35 (2003) 167–176, https://doi.org/10.1016/S0038-0717(02)00251-1.

[33] J.W. Zhang, M.D. Busse, D.H. Young, G.O. Fiddler, J.W. Sherlock, J.D. Tenpas, Aboveground biomass responses to organic matter removal, soil compaction, and competing vegetation control on 20-year mixed conifer plantations in California, For. Ecol. Manag. 401 (2017) 341–353, https://doi.org/10.1016/j.foreco.2017.07.023.

[34] L.C. Chen, H. Wang, X. Yu, W.D. Zhang, X.T. Lu, S.L. Wang, Recovery time of soil carbon pools of conversional Chinese-fir plantations from broadleaved forests in subtropical regions, China, Sci. Total Environ. 587–588 (2017) 296–304, https://doi.org/10.1016/j.scitotenv.2017.02.140.

[35] R. Spake, S. van der Linde, A.C. Newton, L.M. Suz, M.I. Bidartondo, C.P. Doncaster, Similar biodiversity of ectomycorrhizal fungi in set-aside plantations and ancient old-growth broadleaved forests, Biol. Conserv. 194 (2016) 71–79, https://doi.org/10.1016/j.biocon.2015.12.003.