



The influence of tree species on soil properties and microbial communities following afforestation of abandoned land in northeast China



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ABSTRACT

The effect of tree species on microbial communities, secondary metabolites (phenolic acids) and soil chemical properties need to be further studied. Four tree species (poplar, Korean pine, larch and Mongolian pine) and abandoned land were selected, and the impact on soil microbial communities, soil chemical properties and phenolic acids were studied in northeastern China. This study found that soil organic carbon (SOC), total nitrogen (TN), and total phosphorus (TP) were higher in all plantations compared to the abandoned land and were significantly higher in the poplar plantation. The complex phenol content was significantly higher in forest soils compared to the abandoned land, and the complex phenol and water-soluble phenol contents showed the opposite trend. All plantations except Mongolian pine had improved soil microbial biomass to varying degrees compared with the abandoned land. Fungal biomass was changed by afforestation to a greater extent than by bacteria. Principal component analysis (PCA) showed that the tree species significantly influenced the soil microbial composition and structure. Redundancy analysis (RDA) indicated that soil C, N and the N/P ratio were significantly associated with PLFA markers. Pearson correlation analysis showed that SOC and TN were positively associated with fungi, while soil phenolic acids did not show any significant correlation with soil microorganisms. The soil chemical properties and microbial communities were improved by the conversion of abandoned land to forest. The poplar plantation had improved soil nutrient and fungal biomass contents compared to the three conifer plantations.

1. Introduction

Coal-mining subsidence has become a bottleneck and difficult problem to restrict the sustainable development of land use in our country. Afforestation is one of the important measures for ecological restoration of coal mining subsidence area. Nevertheless, it is still true that we know little about the effects of various tree species on maximising soil function (e.g. enhancing mineral nutrient storage and promoting nutrient cycling), and particularly on the changes in soil microbial communities in coal-mining areas. This fundamental information would be beneficial to make reasonable administrative decisions to maximise both above and below-ground diversity and to promote sustainable landscape functioning.

Interactions between aboveground and belowground components of terrestrial ecosystems are receiving much attention for their importance in driving ecosystem processes that govern productivity and carbon sequestration [1]. Tree species can influence soil properties through many mechanisms including leaching of dissolved organic material and

nutrients from the forest floor, permeation by roots that may alter soil physical structure, addition of organic matter in the form of root litter, and exudation of ions and organic compounds [2]. Most literature indicates that afforestation can significantly influence soil carbon (C) and nitrogen (N) content as well as ecological stoichiometry [3,4]. Specifically, compared with conifers (e.g. black spruce), broadleaf trees (e.g. trembling aspen) have higher growth rates, litter and root turnover, and higher N, P levels in leaves and roots [5], which indirectly increase the content of available nutrients in the soil. The tree litter and roots can release phenolic acids into the soil; the amount and type of phenolic acids differ greatly between tree species [6].

Accompanied by changes in soil chemistry, soil microbial biomass and community structure could be expected to shift following the establishment of plantation [7]. Afforestation cause a sharp increase in microbial biomass [8]. Afforestation typically promotes the development of fungal communities [9], whereas bacteria appear to be less sensitive to land use changes [10]. In addition, the fungal and bacterial biomass have been shown to increase after afforestation [11]. Hence,

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soil microbial communities might serve as a primary indicator of ecosystems recovery.

Soil microorganisms are an important part of the ecosystem and play vital roles in tree litter decomposition, nutrient mineralization and sustainable utilization of the forest ecosystem [12,13]. Some studies have demonstrated that species identity has a stronger effect than species diversity [11,14]. Tree species not only directly affect the soil microbial communities but also indirectly influence these microbial communities through changes in the soil physical and chemical properties, such as the pH; total C, N, and P contents; and phenolic acid content (e.g. inhibiting or stimulating microflora) [14–16].

Poplar, Korean pine, larch and Mongolian pine are all native afforestation tree species in Northeast China. We selected adjacent abandoned land and the plantations described above to eliminate the influence of site condition and forest age as variables. The aim of this study was to evaluate the effect four species of juvenile plantations on soil chemical characteristics, microflora, and phenolics. We hypothesized that 1) poplar will improve soil nutrient conditions more than conifer species; 2) fungal biomass will increase faster than bacterial biomass; 3) soil nutrients are more influencing factor on microbial community than phenolic acids content in soil.

2. Materials and methods

2.1. Study area and soil sampling

The study was conducted at an ecological restoration area (131°16′–131°42′E, 46°21′–46°37′N) located in Baoshan district, Shuangyashan city, Heilongjiang province, P.R. China. The region belongs to a temperate monsoon climate with a mean annual temperature of 3.4 °C (22 °C maximum in July and –18 °C minimum in January). The mean annual precipitation is 620 mm (most in summer). The mean annual solar period is 2500 h, and the soil is mostly Hap-Boric Luvisol. Poplar (*P. simonii* Carr. × *P. nigra* L.), Korean pine (*Pinus koraiensis* Sieb. et Zucc.), larch (*Larix gmelinii* (Rupr.) Kuzen.) and Mongolian pine (*Pinus sylvestris* L. var. *mongolica* Litv.) were planted on the abandoned land of former mining waste during 2005. The plantations that were the same age were adjacent with abandoned land and in a similar site condition with an average slope of 4°. Three plots (20 m × 30 m) were made in each of the four plantations during July 2015. The abandoned land, which had not been farmed for almost ten years, was used as a control group. Five soil samples were randomly collected at 0–20 cm depths from each plot using a soil augur and were transported in an ice box to the laboratory. Soil samples were sieved through 2-mm mesh to remove plant remains and then homogenized. Soil samples were divided into two parts. One part was lyophilized with an Ultra-low Freeze Dryer (FD-1A-50 Boyikang Experiment Instruments Co., Beijing) for 24 h and stored at –40 °C for PLFA measurement. The other part was air-dried to measure soil physicochemical properties and phenolic acid content.

2.2. Soil physicochemical analysis

Soil organic carbon (SOC) and total nitrogen (TN) were determined by combustion in a Macro Elemental Analyser (vario MACRO, Elementar Co., Germany). Total phosphorus (TP) was measured using a sulphuric acid - soluble perchlorate acid - molybdenum antimony colorimetric method. The pH was determined using a pH meter (MT-5000, Shanghai) in a 1:2.5 soil-water suspension.

2.3. Soil phenolic acids analysis

An ultraviolet spectrophotometer was used to determine the content of total phenol [6]. The content of complex phenols and water-soluble phenols were determined by the Folin reagent colorimetric method [6].

2.4. PLFA analysis

Lipids from soil were extracted using a modified Bligh and Dyer method. The fatty acid methyl esters were separated on a Hewlett-Packard 5890 gas chromatograph fitted with a flame ionization detector and mass spectrometer (GC-MS) [6]. Fatty acid methyl esters (FAMES) were identified by chromatographic retention time compared to a standard mixture of 37 different FAMES ranging from C11 to C24 in length (Sigma corporation, USA).

The PLFAs described as 12:0, 14:0, 15:0, 16:0, 17:0, 18:0, 20:0, i15:0, a15:0, i17:0, 10Me18:0, 16:1 ω 7, 16:1 ω 9c, 18:1 ω 9, cy17:0, and cy19:0 were used to identify bacterial fatty acid [17–19]. Gram-positive bacteria (G^+) were identified by the PLFAs: a15:0, i15:0, i17:0, 10Me18:0 and 18:1 ω 9 [18,20]. Gram-negative bacteria (G^-) were identified by the PLFAs: 16:1 ω 7, 16:1 ω 9c, cy17:0, and cy19:0 [19,21]. The fungi were identified by the PLFAs: 18:1 ω 9c and 18:1 ω 9 [22–24]. Actinomycete fatty acids were identified by the PLFA: 10Me17:0 [22]. The ratio of fungal-to-bacterial PLFAs (the F/B ratio), was calculated using (18:1 ω 9c, 18:1 ω 9)/(12:0, 14:0, 15:0, 16:0, 17:0, 18:0, 20:0, i15:0, a15:0, i17:0, 10Me18:0, 16:1 ω 7, 16:1 ω 9c, 18:1 ω 9, cy17:0, cy19:0) and used as an indicator of the relative abundance of these two microbial groups, as was the same with G^+/G^- . The following two ratios of PLFA relative abundance were also calculated, cyclopropyl/monoenic (Cy/Pre) precursor fatty acids (cy17:0, cy19:0)/(16:1 ω 7, 18:1 ω 7) [14] and total saturated/total monounsaturated (Sat/Mono) fatty acids (12:0, 14:0, 15:0, 16:0, 17:0, 18:0, 20:0)/(16:1 ω 7, 18:1 ω 9c, 18:1 ω 9, 16:1 ω 9c) [25,26].

2.5. Data analysis

Microsoft Excel 2010 and SPSS 19.0 software were used to process the data. Single factor analysis of variance (one-way ANOVA) was used for the differences between treatments (LSD, $\alpha = 0.05$). Pearson correlation analysis was used to determine the relation among soil properties, phenolic acids and the microbial parameters. PCA (principal component analysis) was used for family composition analysis on different sources of soil microbial communities. Redundancy analysis (RDA) was conducted to understand the relationship between soil chemical properties, phenolic acids and soil microbial community. SigmaPlot 12.0 software was used to draw the figures. All data were given as an average \pm standard error.

3. Results

3.1. Soil chemical properties

The conifer tree species had a weak effect on the SOC content. SOC was significantly higher in the poplar plantation than in plantations of other species. Plantation soils (except Mongolian pine) had significant increases in the content of TN compared to the abandoned land. Afforestation (except Korean pine) significantly increased TP content compared to the abandoned land and were 83.70–190.11% higher in forest soils relative to the abandoned land, and TP was highest for poplar and larch plots (Fig. 1a–c). In general, the C/N ratio was significantly higher in poplar than in the abandoned land (Fig. 1d), while the N/P ratio was significantly higher in the abandoned land than in other plantations (except Korean pine) (Fig. 1e). The pH value was significantly higher in the poplar plantation compared to the other plantations and the abandoned land (Fig. 1f).

Total phenol content was highest in larch and Korean pine plantations, and this content increased by 86.5% and 68.9%, respectively, compared to the abandoned land (Fig. 2a). In addition, the complex phenol content was significantly different in the forest soils from that in abandoned land, and this content in larch and poplar plantations increased by 199.8% and 159.6%, respectively, compared to that in abandoned land. Complex phenol and water-soluble phenol content

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