Effects of Alder on Soil Amino Sugar Accumulation in Liaodong Larch Plantations: Postprint

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Date: 2018-05-10T00:00:00+00:00

Abstract

Microbial residues are an important component of the stable soil carbon pool; however, their response to nitrogen-fixing tree species remains unclear. Using Japanese larch (Larix kaempferi) monoculture plantations and larch-alder mixed forests in the mountainous region of eastern Liaoning as research subjects, and employing amino sugars as biomarkers of microbial residues, we investigated the effects of alder introduction on amino sugar accumulation in rhizosphere and bulk soils of larch plantations. The results showed that the contents of all amino sugar monomers in both rhizosphere and bulk soils of larch in mixed forests were significantly higher than those in monoculture forests, indicating that alder introduction is beneficial for the accumulation of soil microbial residues. Specifically, the contents of glucosamine in rhizosphere and bulk soils of larch in mixed forests were 99.5% ($P<0.01$) and 154% ($P<0.01$) higher than those in monoculture forests, respectively; muramic acid contents were 66.1% ($P<0.01$) and 132.3% ($P<0.01$) higher, respectively. Alder introduction did not significantly affect the glucosamine/muramic acid ratio but significantly increased the contribution of amino sugars to soil organic carbon. Redundancy analysis indicated that total nitrogen was the primary factor driving changes in soil amino sugars. This demonstrates that alder introduction significantly enhances the microbially-sequestered soil carbon pool and organic carbon stability, which is of great significance for the sustainable management of larch plantations.

Full Text

Effects of N-Fixing Tree Species (*Alnus sibirica*) on Amino Sugars in the Soils of a *Larix kaempferi* Plantation in Eastern Liaoning Province, China

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Abstract: Microbial residues constitute an important component of stable soil carbon (C) pools, yet their response to N-fixing tree species remains unclear. This study investigated the effects of alder (*Alnus sibirica*) on microbial residues (as indicated by amino sugars) in rhizosphere and non-rhizosphere soils of a larch (*Larix kaempferi*) plantation in the mountainous region of eastern Liaoning Province, China. The contents of individual amino sugars in both rhizosphere and non-rhizosphere soils of larch were significantly higher in the mixed plantation than in the pure plantation, indicating that alder introduction benefits microbial residue accumulation. Specifically, glucosamine (GluN) contents were 99.5% ($P<0.01$) and 154% ($P<0.01$) higher in the rhizosphere and non-rhizosphere soils of larch in the mixed plantation compared to the pure plantation, respectively, while muramic acid (MurN) contents were 66.1% ($P<0.01$) and 132.3% ($P<0.01$) higher. Alder had no significant effect on the GluN/MurN ratio but significantly enriched the amino sugar proportion of soil organic C. Redundancy analysis revealed that total nitrogen was the dominant factor driving changes in amino sugars. These results demonstrate that alder introduction significantly enhances the soil C pool immobilized by microorganisms and the stability of soil organic C, which has important implications for the sustainable management of larch plantations.

Keywords: *Larix kaempferi*; rhizosphere; amino sugars; N-fixing tree species

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1. Study Area Overview

The experimental site was located at the Forest Management Research Institute Experimental Forest Farm in Caokou Town, Qingyuan Manchu Autonomous County, Benxi City, Liaoning Province (123°34 53 –125°45 42 E, 40°48 50 – 41°33 50 N). The region has a temperate continental monsoon climate with an average annual temperature of 6.1°C and annual precipitation of 926.3 mm. The growing season lasts 124–144 days. The vegetation belongs to the Changbai flora region. The existing forest consists primarily of natural secondary forest, with Japanese larch (*Larix kaempferi*) being the main plantation species. Soil type is predominantly dark brown forest soil with a silt loam texture. Understory shrubs include honeysuckle (*Lonicera altamanni*), Manchurian hazel (*Corylus mandshurica*), Korean pine (*Pinus koraiensis*), and winged euonymus (*Euonymus alatus*). Herbaceous plants are mainly fern (*Pteridium spp.*), Artemisia (*Artemisia spp.*), and *Filipendula palmata*.

2. Plot Setup and Sample Collection

In July 2015, we conducted a comprehensive survey of stand conditions and selected pure Japanese larch plantations and larch-alder strip-mixed plantations with similar site conditions and management measures. The mixed plantation had a larch to alder ratio of 7:3. Plot area was 20 m × 30 m. In the pure plantation, we selected three standard larch trees; in the mixed plantation, we selected three standard trees of each species. Rhizosphere soil samples were collected using the shaking method: fine roots were excavated and soil adhering to root surfaces was collected as rhizosphere soil. Non-rhizosphere soil samples (0–10 cm) were collected at different positions around each standard tree. All soil samples were brought back to the laboratory, where visible plant residues were removed for determination of soil chemical properties and amino sugars.

3. Analytical Methods

Soil organic carbon (SOC) and total nitrogen (TN) were measured using an elemental analyzer (Heraeus Elementar Vario EL, Hanau, Germany). Available nitrogen was determined by the alkali-hydrolysis diffusion method. Total phosphorus (TP) was measured by concentrated sulfuric acid digestion followed by molybdenum-antimony colorimetry. Available phosphorus was extracted using sodium bicarbonate. Microbial community structure was analyzed using phospholipid fatty acid (PLFA) analysis, where bacterial communities were indicated by 15:0iso, 15:0anteiso, 16:00, 16:0iso, 17:0iso, 17:0anteiso, 16:1 7c, 17:00, 17:0cyclo, 18:1 7c, and 19:0cyclo, and fungal communities by 18:1 9c, 18:2 6c, and 16:1 5c.

Soil amino sugar content was determined by gas chromatography of aldononitrile acetate derivatives. The procedure was as follows: 0.3 mg of air-dried soil was placed in a hydrolysis bottle with 100 L of N-methylglucosamine, oscillated, and filtered. The filtrate was evaporated to dryness using a rotary evaporator, redissolved in distilled water, centrifuged (3000 r/min), and the supernatant was lyophilized. The residue was dissolved in methanol, centrifuged again (3000 r/min), and dried. Derivatives were dissolved in ethyl acetate and transferred to chromatography vials for GC analysis (GC-7890B, Agilent, USA) using an HP-5 capillary column (30 m × 0.25 mm × 0.25 m).

4. Calculation of Amino Sugar Content in Soil

The amino sugar content was calculated using the formula:
$\text{Content} = (mi \times Ax) / (Ai \times Rf)$
where mi is the mass of added inositol, Ax and Ai are the peak areas of amino sugar and inositol in the sample, respectively, and Rf is the relative correction factor for each amino sugar.

Statistical analysis was performed using SPSS 16.0 for ANOVA and multiple comparisons (LSD). Origin 8.5 was used for graphing, and Canoco 4.5 for redundancy analysis (RDA) of relationships between amino sugars and environmental factors.

1. Soil Properties

Rhizosphere soil had higher organic carbon, available nitrogen, and microbial biomass than non-rhizosphere soil. However, rhizosphere effects had no significant influence on total phosphorus and fungal/bacterial ratios. Alder introduction significantly affected soil organic carbon, total nitrogen, available nitrogen, and microbial biomass. In the mixed plantation, larch rhizosphere soil organic carbon, total nitrogen, and microbial biomass were 74.1%, 93.4%, and 52.4% higher than in the pure plantation, respectively, while non-rhizosphere values were 45.6%, 125.2%, and 58.2% higher. Stand type had no significant effect on soil total phosphorus or fungal/bacterial ratios. The pH value in the pure plantation was significantly lower than that of larch in the mixed plantation, but other soil properties showed no significant differences between the two.

2. Amino Sugar Content

Under different treatments, rhizosphere glucosamine, muramic acid, and galactosamine ranged from 1099.5-2193.3, 80.6-141.7, and 361.1-920.4 mg/kg, respectively, while non-rhizosphere values ranged from 488.9-1320.9, 20.1-65.2, and 218.6-585.2 mg/kg. Alder introduction significantly promoted amino sugar monomer contents in both rhizosphere and non-rhizosphere soils. Compared with the pure plantation, glucosamine in larch rhizosphere and non-rhizosphere soils of the mixed plantation was 99.5% ($P<0.01$) and 154% ($P<0.01$) higher, respectively. Muramic acid showed the same trend, being 66.1% ($P<0.01$) and 132.3% ($P<0.01$) higher in rhizosphere and non-rhizosphere soils of the mixed plantation. No significant differences in amino sugar monomer contents were observed between larch and alder in the mixed plantation.

3. Effects of Abiotic Factors on Amino Sugars

The GluN/MurN ratio in rhizosphere soil ranged from 17.8-21.7, and in non-rhizosphere soil from 13.7-16.4. Alder introduction had no significant effect on this ratio. However, the contribution of amino sugars to soil organic carbon was significantly higher in both rhizosphere and non-rhizosphere soils of the mixed plantation compared to the pure plantation. Redundancy analysis identified the degree of influence of abiotic factors on soil amino sugars. The first and second ordination axes explained 99.7% of amino sugar variation, with soil amino sugars showing positive correlations with soil organic carbon, total nitrogen, and available nitrogen. Total nitrogen was the most important factor affecting amino sugars.

3. Discussion

3.1 Effects of Alder on Soil Properties

Previous studies have found that introducing N-fixing tree species enhances soil carbon and nitrogen sequestration potential, consistent with our results. Alder introduction improved soil nutrient conditions in the larch plantation, likely alleviating nitrogen limitation in this temperate forest ecosystem where nitrogen is a key limiting factor for plant growth. Higher leaf nitrogen content in N-fixing species promotes nutrient release from litter, increases plant net productivity, and enhances soil microbial activity and litter production. We also found no significant differences in soil nutrient content between larch and alder in the mixed plantation, possibly because larch can obtain nutrients either directly through contact with alder roots or indirectly through decomposition of alder litter, ultimately increasing soil carbon and nitrogen retention.

The pH value was significantly lower in the pure plantation than in the mixed plantation larch, a finding consistent with many studies on N-fixing species, indicating that alder introduction intensified soil acidification. This may occur because N-fixing species enhance soil nitrification, producing more H+ and increasing nitrate nitrogen content, which leads to greater leaching of base cations. Our study soil’ s silt loam texture may have further exacerbated base cation loss.

3.2 Effects of Alder on Amino Sugar Content and Ratios

We found that glucosamine and muramic acid contents were significantly higher in rhizosphere than non-rhizosphere soils of the pure plantation, primarily due to higher microbial biomass in rhizosphere soils. This rhizosphere effect on microbial biomass is consistent with previous research. Different tree species have unique microbial residue accumulation characteristics, and N-fixing species introduction may significantly affect soil microbial residues. Our study found that all amino sugar monomer contents were significantly higher in the mixed plantation than in the pure plantation, indicating that alder introduction benefits microbial residue accumulation.

Previous studies have shown that when soil nutrients are deficient, amino sugars can be utilized by microorganisms as carbon or nitrogen sources, while nutrient addition promotes amino sugar accumulation. In our study, alder introduction increased soil carbon and nitrogen sources, thereby increasing microbial biomass, which was the main reason for enhanced amino sugar accumulation. We also found that alder’ s promotion effect on amino sugars in non-rhizosphere soil was greater than in rhizosphere soil, suggesting stronger stimulation of microbial residues in nutrient-poor soils. Li et al. found similar results where amino sugars increased more in low organic matter soils after straw addition, possibly because nitrogen limitation is more severe in non-rhizosphere soils with lower nutrient availability, so alder introduction has greater impact on microbial activity and turnover in these soils.

Our RDA results showed total nitrogen was the most important factor affecting amino sugars, supporting this conclusion. No significant difference in GluN/MurN ratio between pure and mixed plantations indicates that alder introduction had little effect on the relative contributions of fungi and bacteria to larch forest soils, suggesting balanced accumulation of fungal and bacterial residues. Zhang et al. found that high nitrogen deposition (150 kg N hm ^{2} yr ^{1}) decreased the GluN/MurN ratio, while our results suggest that current alder introduction intensity (50 kg N hm ^{2} yr ^{1}) may represent low nitrogen addition levels that do not affect this ratio. Amino sugar transformation and accumulation are influenced by available carbon and mineral nutrients, and their ratios reflect long-term microbial community dynamics. Future studies using isotopic labeling to simultaneously observe temporal changes in amino sugars and microbial community structure would help elucidate mechanisms by which N-fixing species affect soil amino sugar ratios.

As important components of stable soil carbon pools, amino sugars play a significant role in long-term carbon sequestration. The higher contribution of amino sugars to soil organic carbon in the mixed plantation indicates that alder introduction significantly enhanced larch plantation soil organic carbon stability. Compared with the pure plantation, the mixed plantation increased both soil organic carbon and amino sugar content. However, the increase in non-rhizosphere soil organic carbon was significantly greater than the increase in amino sugars, suggesting that plant-derived organic carbon diluted amino sugar concentration in non-rhizosphere soil organic carbon. This dilution effect did not occur in rhizosphere soil, possibly because amino sugars, as components of signaling substances produced by rhizobia in N-fixing plants and necessary for establishing plant-rhizobia symbiosis, were transferred to larch rhizosphere through belowground processes.

4. Conclusion

Alder introduction promotes the accumulation of all amino sugar monomers in larch soils, with greater promotion of glucosamine and muramic acid in non-rhizosphere than rhizosphere soils. Alder introduction does not affect the relative contributions of fungal and bacterial residues but significantly enhances amino sugar contribution to soil organic carbon. This study suggests that alder introduction improves soil nutrient status, particularly nitrogen content, which is an important reason for promoting microbial residue accumulation in mixed plantations. When afforesting for the purpose of improving soil fertility and increasing carbon sequestration in the mountainous region of eastern Liaoning, alder introduction should be considered, though precautions should be taken to prevent soil acidification.

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Note: Figure translations are in progress. See original paper for figures.