Response of Labile Organic Carbon Content in Surface Soil Aggregates to Short-term Nitrogen Addition in a Planted Pinus tabuliformis Forest (Postprint)

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Abstract

Atmospheric nitrogen deposition is significantly affecting soil carbon cycling processes in forest ecosystems. Currently, how atmospheric nitrogen deposition affects the content of labile organic carbon within soil aggregates of different particle sizes is not well understood, which constrains people' s understanding of forest soil carbon cycling and the development of related carbon cycling models. Through a nearly 2-year gradient nitrogen addition experiment in forest land (0, 3, 6, 9 g N m-2 a-1), the effects of short-term nitrogen addition on the content of different labile organic carbons in surface soil aggregates of a planted Pinus tabuliformis forest were studied. The results showed that short-term nitrogen addition had no significant effect on the distribution of water-stable aggregates in surface soil (0-10 cm); with increasing nitrogen addition levels, the organic carbon content of macro- and micro-aggregates, as well as the labile and highly labile organic carbon contents in macro- and micro-aggregates, showed a pattern of first increasing and then decreasing, with the above indicators reaching their maximum under the N6 treatment (6 g N m-2 a-1); under the same treatment, the labile organic carbon content in soil macro- and micro-aggregates all exhibited the pattern of highly labile organic carbon > moderately labile organic carbon > lowly labile organic carbon; compared with the CK treatment, the N6 treatment increased the lowly, moderately, and highly labile organic carbon contents in macro-aggregates by 115.06%, 178.73%, and 79.61%, respectively, and increased the lowly, moderately, and highly labile organic carbon contents in micro-aggregates by 32.84%, 166.79%, and 62.05%. The moderately labile organic carbon content in macro- and micro-aggregates showed the greatest increase, indicating that moderately labile organic carbon in aggregates responded most significantly to nitrogen addition. The study found that short-term nitrogen addition mainly affects surface soil organic carbon content by influencing moderately labile organic carbon in surface macro- and micro-aggregates. Principal component analysis indicated that N addition altered soil physicochemical properties, thereby leading to increased root biomass and promoted litter decomposition, which are the main causes of changes in labile organic carbon in surface soil aggregates.

Full Text

Preamble

Response of Labile Organic Carbon Content in Surface Soil Aggregates to Short-Term Nitrogen Addition in Artificial *Pinus tabuliformis* Forests

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Abstract

Atmospheric nitrogen (N) deposition is significantly affecting soil carbon cycling processes in forest ecosystems. However, how N deposition influences the content of labile organic carbon within different soil aggregate size fractions remains poorly understood, which constrains our understanding of forest soil carbon cycles and the development of carbon cycle models. This study investigated the effects of short-term N addition on different labile organic carbon fractions in surface soil aggregates of an artificial *Pinus tabuliformis* forest through a two-year gradient N addition experiment. Three N treatments—N3 (3 g N m ^{2}), N6 (6 g N m ^{2}), and N9 (9 g N m ^{2})—were applied, along with a control (CK) receiving no N addition, with six replicates per treatment.

Short-term N addition had no significant effect on the distribution of water-stable aggregates in the surface soil (0–10 cm), but significantly affected soil organic carbon content. Soil organic carbon content was higher in all three N addition treatments compared to the control, reaching its maximum in the N6 treatment. Within each treatment, organic carbon content in macro-aggregates was highest, followed by micro-aggregates, with the lowest content in silt-clay fractions. As N addition levels increased, organic carbon content in both macro- and micro-aggregates followed the same pattern as total soil organic carbon,

initially increasing and then decreasing, with peak values observed in the N6 treatment.

Within soil aggregates, the content of highly labile organic carbon was greatest, followed by moderately labile carbon, with minimally labile carbon being lowest. Compared to the control, the N6 treatment increased minimally, moderately, and highly labile organic carbon in macro-aggregates by 115.06%, 178.73%, and 79.61%, respectively. In micro-aggregates, these same fractions increased by 32.84%, 166.79%, and 62.05%, respectively. Among the three labile organic carbon categories, moderately labile organic carbon showed the greatest change in both macro- and micro-aggregates, indicating that this fraction was most sensitive to short-term N addition.

These results demonstrate that short-term N addition affects surface soil organic carbon content primarily by altering the amount of moderately labile organic carbon in macro- and micro-aggregates. Principal component analysis revealed that N addition changed soil total N and pH, which also caused changes in soil texture and total P. These alterations in soil physicochemical properties may have increased root biomass and promoted litter decomposition, which were the main drivers of changes in active organic carbon content in surface soil aggregates.

Keywords: nitrogen deposition; surface soil aggregates; labile organic carbon; *Pinus tabuliformis* forest

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1. Study Area Overview

The experimental site was located at Tielongwan Forest Farm in Yichuan County, Shaanxi Province, on the eastern side of the Huanglong Mountain Forest Region (35°39 N, 110°06 E). The site is characterized by loess ridge hills at an elevation of 860–1200 m, with slopes ranging from 20° to 25°. The region has a mean annual temperature of 9.7°C and mean annual precipitation of 584.4 mm, with approximately 60% of rainfall occurring between July and September. The frost-free period lasts about 180 days.

The zonal vegetation is warm temperate deciduous broadleaf forest, with *Pinus tabuliformis* and oak forests as the climax community. The soil is derived from primary loess, with a surface bulk density of 1.1 g cm^{3}. Understory shrubs primarily include *Lespedeza davurica*, *Spiraea salicifolia*, *Lonicera japonica*, and *Elaeagnus umbellata*. The herbaceous layer is dominated by *Carex lanceolata*. The forest floor litter layer thickness ranges from 3 to 5 cm. The experimental *Pinus tabuliformis* forest was established in [year not specified in original text].

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2. Experimental Design

Previous studies have shown that N deposition in China ranged from 0.1 to 7.43 g N m ^{2} yr ^{1}, with total N deposition increasing at an average rate of 0.041 g N m ^{2} yr ^{1} in 2010, a trend projected to continue for several decades. Based on this background, we established four N addition treatments: control (CK, 0 g N m ^{2}), low N (N3, 3 g N m ^{2}), medium N (N6, 6 g N m ^{2}), and high N (N9, 9 g N m ^{2}). Each treatment was replicated six times in 10 m × 10 m plots separated by 10 m buffers.

Urea was used as the N fertilizer, dissolved in water and sprayed evenly onto the forest floor using a sprayer to simulate N deposition. To minimize ammonia volatilization, fertilizer was applied before rainfall events. The application was conducted [frequency not fully specified] during the growing season. The final N application before sampling occurred in [month not specified]. Control plots received equivalent amounts of water without urea.

In October [year not specified], vegetation surveys were conducted in each plot. Stand characteristics are summarized in Table 1 . The results showed no significant differences in tree diameter at breast height (DBH) or height among treatments, indicating uniform site conditions.

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3. Sample Collection

Within each plot, three 1 m × 1 m subplots were randomly established. Litter was collected from each subplot, and soil samples were excavated from 0–10 cm depth. Visible plant roots were separated into coarse (>2 mm) and fine (<2 mm) fractions. Soil samples were placed in rigid aluminum boxes and transported to the laboratory. Visible animal and plant residues were removed, and samples were air-dried. Each sample was divided into two portions: one for aggregate fractionation and the other ground to pass through a sieve for soil organic carbon determination.

Based on previous research showing that short-term N addition primarily affects surface soil properties, we focused on the 0–10 cm layer. Soil mechanical composition was determined using a laser particle size analyzer after pretreatment. According to the USDA classification system, particles were categorized as clay (<0.002 mm), silt (0.002–0.05 mm), and sand (0.05–2 mm).

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4. Measurement Methods

Soil aggregate fractionation was performed using the wet sieving method. One hundred grams of air-dried soil were immersed in deionized water for 10 minutes, then transferred to a nested set of sieves (250 m and 53 m). The samples were oscillated vertically at 100 cycles per minute for 2 minutes using a mechanical shaker. Floating debris was removed, and soil particles in the water were separated by centrifugation to obtain macro-aggregates (>0.25 mm), micro-aggregates (0.053–0.25 mm), and silt-clay fractions (<0.053 mm). All aggregate fractions were washed into aluminum boxes, oven-dried at 65°C for 48 hours, and weighed.

Soil organic carbon content was determined using the potassium dichromate oxidation method. Different labile organic carbon fractions were measured using a modified potassium permanganate oxidation method. Briefly, 15–30 mg of soil aggregates were placed in 50 mL centrifuge tubes with 25 mL of KMnO solutions of three concentrations (33, 167, and 333 mmol L ^{1}). After shaking for 1 hour, samples were centrifuged at 4000 rpm for 10 minutes. The supernatant was diluted and absorbance measured at 565 nm. Carbon oxidized by 333 mmol L ^{1} KMnO was defined as highly labile organic carbon, by 167 mmol L ^{1} as moderately labile organic carbon, and by 33 mmol L ^{1} as minimally labile organic carbon.

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5. Results Calculation and Statistical Analysis

The mass percentage of each aggregate fraction was calculated as: (mass of the aggregate fraction / total soil mass) × 100%. Data were processed using SPSS 20 and SigmaPlot 12.5 software. One-way ANOVA followed by Duncan' s test was used to compare differences among N treatments (= 0.05). Principal component analysis (factor analysis) was performed on soil and vegetation factors to identify key drivers of variation in aggregate labile organic carbon.

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6. Results and Analysis

6.1 Effects of N Addition on Water-Stable Aggregate Distribution

Short-term N addition had no significant effect on the distribution of water-stable aggregates in surface soil ($P > 0.05$). Across all treatments, macro-aggregates (>0.25 mm) dominated, accounting for 65.36–73.65% of the total mass. Micro-aggregates (0.053–0.25 mm) comprised 21.88–28.49%, and silt-clay fractions (<0.053 mm) accounted for only 4.48–6.16% (Table 2).

6.2 Effects of N Addition on Soil Organic Carbon and Aggregate Organic Carbon

N addition significantly affected soil organic carbon content ($P < 0.05$). As N addition levels increased, soil organic carbon content in the 0–10 cm layer initially increased then decreased, reaching its maximum in the N6 treatment. Compared to the control, soil organic carbon increased by 61.1%, 74.4%, and

47.6% in the N3, N6, and N9 treatments, respectively (Figure 1 [Figure 1: see original paper]).

Within each treatment, organic carbon content was highest in macro-aggregates, intermediate in micro-aggregates, and lowest in silt-clay fractions. With increasing N addition levels, organic carbon in macro- and micro-aggregates followed the same pattern as total soil organic carbon, peaking in the N6 treatment. Compared to pre-treatment values, organic carbon in macro-aggregates increased by 51.63% in N6, while micro-aggregate organic carbon increased by 56.85% in N6. These results indicate that the three aggregate fractions responded differently to N addition.

6.3 Effects of N Addition on Labile and Non-Labile Organic Carbon in Aggregates

Short-term N addition significantly increased all three labile organic carbon fractions in both macro- and micro-aggregates, but had no significant effect on non-labile organic carbon ($P > 0.05$). In macro-aggregates, the content of highly labile organic carbon was highest, followed by moderately labile carbon, with minimally labile carbon being lowest. As N addition increased, minimally and moderately labile carbon increased initially then decreased, peaking in the N6 treatment. Specifically, minimally, moderately, and highly labile carbon increased by 86.67%, 115.06%, and 79.61% in N3; 115.06%, 178.73%, and 104.22% in N6; and 144.60%, 148.65%, and 72.54% in N9, respectively (Figure 2 [Figure 2: see original paper] and Figure 3 [Figure 3: see original paper]).

In micro-aggregates, the same pattern was observed: highly labile carbon > moderately labile carbon > minimally labile carbon. Minimally and moderately labile carbon increased initially then decreased with N addition, while changes in highly labile carbon were less consistent. Compared to the control, minimally, moderately, and highly labile carbon increased by 65.69%, 32.84%, and 115.02% in N3; 32.84%, 166.79%, and 166.79% in N6; and 64.46%, 59.18%, and 59.35% in N9, respectively (Figure 4 [Figure 4: see original paper]).

Notably, moderately labile organic carbon showed the greatest increase in both aggregate types, indicating it was the most sensitive fraction to short-term N addition. Non-labile organic carbon in both macro- and micro-aggregates showed no significant differences among treatments (Figure 5 [Figure 5: see original paper]).

6.4 Principal Component Analysis of Factors Influencing Aggregate Labile Organic Carbon

Soil labile organic carbon is influenced by both soil physicochemical properties and biological factors. N addition can alter forest ecosystem processes, affecting litter decomposition and soil respiration, which subsequently influences the soil carbon pool. To clarify the relative importance of these factors, we performed principal component analysis on 11 soil physicochemical factors and 3 vegetation factors.

The analysis revealed significant multicollinearity among factors. Three principal components with eigenvalues >1 were extracted, cumulatively explaining 77.16% of the variance (Table 3). The first component (42.62% variance) was strongly positively correlated with sand content and negatively correlated with clay content, reflecting changes in soil texture induced by N addition. The second component (18.96% variance) was positively correlated with coarse root biomass and negatively correlated with litter biomass, representing vegetation factors. The third component (15.58% variance) was positively correlated with fine root biomass, indicating the combined influence of soil and vegetation. These results suggest that N-induced changes in soil physicochemical properties enhanced root biomass and promoted litter decomposition, which were the primary drivers of changes in aggregate labile organic carbon.

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7. Discussion

Our two-year N addition experiment demonstrated that N addition significantly affected surface soil organic carbon content in the artificial *Pinus tabuliformis* forest on the Loess Plateau. Low and medium N additions (3 and 6 g N m ^{2}) significantly promoted soil organic carbon accumulation, while high N addition (9 g N m ^{2}) showed less pronounced effects, indicating a non-linear response with a potential saturation point. Soil organic carbon dynamics depend on the balance between carbon inputs from net primary production and carbon losses through decomposition and mineralization. The Loess Plateau is an N-limited region, and moderate N addition can stimulate plant growth, increasing carbon input to soil. However, excessively high N deposition rates may reduce plant growth and cause toxicity, decreasing carbon return via roots and root exudates. Our results align with findings from Shaoshan et al., suggesting that 6 g N m ^{2} may be the optimal N addition rate for *Pinus tabuliformis* forests in this region.

Short-term N addition did not significantly affect the distribution of water-stable aggregates, consistent with findings that aggregate structure is relatively stable over short periods. However, N addition significantly increased organic carbon content in both macro- and micro-aggregates, following the same pattern as total soil organic carbon. Macro-aggregates contained the highest organic carbon content because they are formed from micro-aggregates cemented by newly input organic matter such as carbohydrates and root exudates, in addition to existing humus.

Soil organic carbon fluctuations occur primarily in labile fractions, which include microbial carbon, water-soluble carbon, and easily oxidizable compounds from litter decomposition and root exudates. Our study found that short-term N addition significantly increased all three labile carbon fractions in both aggregate types but did not affect non-labile carbon, confirming that N addition influences soil carbon primarily through labile pools. The consistent pattern of highly > moderately > minimally labile carbon across treatments indicates that high-lability carbon dominates the active carbon pool in this region.

The most notable finding was that moderately labile organic carbon showed the greatest increase in both macro- and micro-aggregates, suggesting it is the most sensitive indicator of N addition effects. This sensitivity may reflect the complex nature of soil labile carbon and its response to changing soil conditions. Previous studies have shown that N addition promotes litter decomposition and root exudation, producing more simple compounds like soluble sugars. Our principal component analysis supports this mechanism, indicating that N-induced changes in soil properties enhanced root biomass and litter decomposition, driving the observed changes in aggregate labile organic carbon.

Interestingly, we found no significant difference in labile organic carbon distribution between macro- and micro-aggregates, which contrasts with some previous studies. For example, Hua et al. found that vegetation restoration primarily affected macro-aggregate labile carbon in the Yunnwushan grassland region, and Li et al. reported similar results. Wang et al., however, found that labile organic carbon decreased with increasing aggregate size. These discrepancies likely arise from differences in soil parent material, vegetation type, ecological conditions, and aggregate separation methods across studies, highlighting the complexity of labile carbon distribution among aggregate size fractions.

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8. Conclusion

Short-term N addition significantly affected organic carbon and labile organic carbon content in surface soil macro- and micro-aggregates of artificial *Pinus tabuliformis* forests, but had no significant effect on non-labile organic carbon. The N6 treatment (6 g N m ^{2}) produced the strongest effects, increasing all three labile carbon fractions in both aggregate types. Moderately labile organic carbon showed the most pronounced response, indicating its high sensitivity to N addition.

Within each treatment, labile organic carbon content followed the pattern: highly labile > moderately labile > minimally labile. The greatest increase occurred in moderately labile carbon within micro-aggregates, suggesting that short-term N addition affects surface soil organic carbon primarily through this fraction. Principal component analysis revealed that N-induced changes in soil physicochemical properties promoted root biomass increase and litter decomposition, which were the main drivers of changes in aggregate labile organic carbon.

Future research should investigate the specific organic compounds and mechanisms through which N addition influences labile organic carbon in soil aggregates.

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