Effects of DOM on Soil Microbial Respiration and Its Metabolic Quotient in Different Soil Layers of a Castanopsis carlesii Secondary Forest Postprint

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Abstract

Dissolved organic matter (DOM), as an important source of soil dissolved organic carbon, influences soil mineralization by altering the quantity and activity of soil microorganisms upon entering the soil. Research on the effects of DOM input on soil microbial respiration and entropy values has primarily focused on surface soils, with less attention devoted to its impacts on microbial respiration and entropy values in deep soils. This study employed a laboratory incubation experiment (120 d) to investigate the effects of fresh leaf DOM addition from Castanopsis carlesii on microbial respiration, soil metabolic quotient, and microbial quotient in surface soil (0–10 cm) and deep soil (40–60 cm), providing a theoretical basis for elucidating the influence of DOM input on carbon processes in subtropical forest soils. The results demonstrated that on day 1 of incubation, the instantaneous CO2 emission rates from both surface and deep soils with DOM addition were significantly higher than those of the control ($P < 0.001$), being 3.58 and 6.93 times that of the control (without DOM addition), respectively, after which they declined significantly. In terms of cumulative emissions, surface soil exhibited significantly greater values than deep soil regardless of DOM addition treatment or control; following fresh leaf DOM addition from Castanopsis carlesii, cumulative emissions from surface soil were significantly greater than those from the control surface soil ($P < 0.001$), whereas no significant difference was observed in cumulative emissions between deep soil with DOM addition and the control deep soil. Regarding microbial biomass carbon, the microbial biomass carbon content in surface soil was significantly greater than that in deep soil throughout the incubation period. During the entire DOM addition incubation period, microbial biomass carbon content in surface soil was significantly greater than that in the control surface soil, and microbial biomass carbon content in deep soil was significantly greater than that in the control deep soil (except on day 3). At the end of incubation (120 d), under fresh leaf DOM addition treatment from Castanopsis carlesii, organic carbon

content in surface soil and deep soil decreased by 26% and 19%, respectively, compared with day 3. The metabolic quotient (qCO2) of deep soil after fresh leaf DOM addition from Castanopsis carlesii was significantly lower than that of the control deep soil and the surface soil with DOM addition ($P < 0.001$), indicating that the input of exogenous DOM into deep soil enhanced the carbon utilization efficiency of soil microorganisms. The microbial quotient of deep soil after fresh leaf DOM addition from Castanopsis carlesii was 1.58 times that on day 3 of incubation, significantly greater than the initial incubation period ($P < 0.05$), whereas the microbial quotients of surface soil with DOM addition, control surface soil, and control deep soil were 68%, 79%, and 21% of that on day 3, respectively, demonstrating that DOM addition improved deep soil quality.

Full Text

Preamble

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Effects of Dissolved Organic Matter Addition on Soil Microbial Respiration and Quotient Values in Different Soil Layers of a Secondary Castanopsis carlesii Forest

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Abstract: Dissolved organic matter (DOM) is an important source of soil dissolved organic carbon that affects soil mineralization by altering microbial quantity and activity. However, most studies on DOM impacts on soil microbial respiration and quotient values have focused on surface soils, with less attention paid to deep soil layers. We conducted a 120-day incubation experiment to investigate the effects of DOM from fresh Castanopsis carlesii leaves on microbial respiration and quotient values in surface (0–10 cm) and deep (40–60 cm) soils of a secondary forest. This work provides theoretical insights into the role of DOM input in subtropical forest soil carbon processes.

The results showed that on the first day after DOM addition, instantaneous CO emission rates from both surface and deep soils were significantly higher than controls ($P < 0.001$), being 3.58 and 6.93 times the control values, respectively, but decreased significantly thereafter. In both DOM-added and control treatments, cumulative CO emissions from surface soil were significantly greater than from deep soil. For surface soil, cumulative emissions under DOM addi-

tion were significantly higher than the control ($P < 0.001$), whereas deep soil cumulative emissions showed no significant difference between DOM addition and control treatments.

Microbial biomass carbon (MBC) content in surface soil during the incubation period was significantly greater than in deep soil. Except for day 3, MBC content under DOM addition was higher than control treatments in both surface and deep soils. By the end of incubation (120 days), soil organic carbon contents in surface and deep soils under DOM addition had decreased by 26% and 19%, respectively, compared to day 3. The soil metabolic quotient of DOM-added deep soil was significantly lower than that of deep control soil and DOM-added surface soil ($P < 0.001$), indicating increased carbon use efficiency after DOM addition to deep soil. After 120 days of incubation, the microbial quotient of DOM-added deep soil was 1.58 times that of the initial period (day 3). In contrast, microbial quotients of DOM-added surface soil and control surface and deep soils were 68%, 79%, and 21% of day 3 values, respectively. This demonstrated that DOM addition improved deep soil quality.

Keywords: soil microbial respiration; microbial biomass carbon; soil metabolic quotient; microbial quotient

1. Study Site Overview

The study area is located at the Chen Da Forest Management Science and Technology Demonstration Base in Sanming City, Fujian Province, northwest of the Daiyun Mountain range (25°59 -27°07 N, 116°22 -118°39 E). The region features low mountain and hilly terrain with a mid-subtropical maritime monsoon climate. The mean annual temperature is 19.1°C, with a frost-free period of 300 days. Annual precipitation is 1749 mm and annual evaporation is 1585 mm. Soils are red and yellow soils developed from granite, with thickness exceeding 330 m. The area contains the largest evergreen broad-leaved forest in China, with a forest coverage rate of 76.8% and rich species diversity.

The secondary Castanopsis carlesii forest formed through secondary succession after selective logging of primary forest. Stand density is 10.8 m, with an average diameter at breast height of 12.2 cm. The shrub layer is dominated by Ormosia xylocarpa, Neolitsea aurata, and Litsea elongata. The herbaceous layer mainly consists of Woodwardia japonica, Pourt hiaea hirsuta, and Gahnia tristis.

2. Soil Collection and Processing

We established 20 m × 20 m standard plots on upper, middle, and lower slopes of the Castanopsis carlesii secondary forest. Within each plot, we collected 0–10 cm and 40–60 cm soil layers. Fresh soil samples were rapidly refrigerated and transported to the laboratory. Visible roots and animal/plant residues were removed, and soils were passed through a 2 mm sieve. To eliminate soil heterogeneity, soils from each layer were thoroughly mixed. One portion was used for

basic physicochemical property determination, while another was refrigerated for subsequent incubation experiments. Basic chemical properties of test soils are shown in .

** Basic Chemical Properties of Test Soils (mean±SE)**

Soil layer (cm)	Dissolved organic carbon (mg/kg)	Soil organic carbon (g/kg)	Total nitrogen (g/kg)	C/N ratio
0–10	127.4±7.1a	19.4±1.2a	1.31±0.71a	14.8±0.17a
40–60	11.3±0.9b	4.7±0.14b	0.59±0.01b	7.9±0.18b

Different letters after values indicate significant differences ($P < 0.05$).

3. Experimental Design

The experiment included four treatments: surface soil without DOM addition (Ts), surface soil with DOM addition (TsTo), deep soil without DOM addition (Ss), and deep soil with DOM addition (SsTo). DOM was extracted from fresh Castanopsis carlesii leaves (100 g) with 500 mL deionized water for 24 hours, then filtered through 0.45 m glass fiber filter membrane. The extract contained total organic carbon (TOC) of 18.7 g/kg, total nitrogen (TN) of 0.022 g/kg, and C/N ratio of 26.3. DOM was added to soils at a rate of 1 g C/kg dry soil weight.

Fresh soil (equivalent to 1 kg dry weight) was placed in 1000 mL incubation bottles. After 15 days of pre-incubation at 60% water holding capacity to stabilize soil properties, DOM solution or deionized water was added and soil moisture was adjusted to field capacity. Gas samples were collected on days 1, 3, 6, 11, 21, 33, 58, 84, and 120. Before each collection, bottles were flushed with known-concentration CO -free air. Headspace gas was extracted and injected into a gas chromatograph-mass spectrometer (GC-MS, GC-2014, Shimadzu, Kyoto, Japan) to measure CO concentrations.

Destructive sampling was performed on days 3, 33, and 120 to determine microbial biomass carbon (MBC) and soil organic carbon (SOC). MBC was measured using the chloroform fumigation-extraction method and calculated as the difference in organic carbon between fumigated and non-fumigated samples (MBC = E/K), where E is the extractable organic carbon difference and K is a conversion factor (0.45). SOC was analyzed using a carbon-nitrogen elemental analyzer (Elementar Vario EL III, Elementar).

4. Calculation Methods and Data Processing

The CO production rate was calculated using the formula: $F = v/m \times k \times dc/dt \times 273/(273+T)$, where F is the gas emission rate (mg/kg/h), v is

the incubation bottle volume (m^3), m is soil weight (kg), dc/dt is the slope of gas concentration change over time, T is incubation temperature (°C), and k is a constant (1.964 kg/m^3). Cumulative CO emissions were calculated by multiplying the average of adjacent production rates by the time interval.

Soil metabolic quotient (qCO) was calculated as: qCO = MR/MBC, where MR is soil microbial respiration (g CO -C/mg MBC/h) and MBC is microbial biomass carbon. Microbial quotient was calculated as: Microbial quotient = MBC/SOC, reflecting the proportion of soil organic carbon converted to microbial biomass.

All data were analyzed using SPSS 19.0 statistical software. Figures were prepared using Origin 9.0. One-way ANOVA was used to test differences in cumulative CO emissions, soil metabolic quotient, and microbial quotient among treatments and soil layers. Significance level was set at < 0.05.

5. Results and Analysis

5.1 Effects of DOM Addition on Soil CO Emission Rates

On the first day after DOM addition, instantaneous CO emission rates from both surface and deep soils were significantly higher than controls ($P < 0.001$), being 3.58 and 6.93 times control values, respectively. Emission rates decreased significantly thereafter. During the incubation period, CO emission rates in DOM-added treatments gradually exceeded controls and stabilized. Deep soil showed a more sensitive response to DOM addition than surface soil, with a larger initial increase but shorter duration.

5.2 Effects of DOM Addition on Cumulative CO Emissions

Cumulative CO emissions from surface soil under DOM addition were significantly higher than the control ($P < 0.001$), while deep soil cumulative emissions showed no significant difference from the deep control. Throughout the incubation, cumulative emissions from surface soil were significantly greater than from deep soil in both control and DOM-added treatments ($P < 0.001$). Initially, deep soil cumulative emissions under DOM addition were higher than the deep control, but the difference was not significant. Over time, the difference gradually increased.

5.3 Effects of DOM Addition on Microbial Biomass Carbon

During the entire incubation period, MBC content in surface soil was significantly greater than in deep soil ($P < 0.001$). Except for day 3, MBC content under DOM addition was significantly higher than control treatments in both surface and deep soils. With increasing incubation time, MBC content in both surface and deep soils under DOM addition decreased significantly. By day 120, MBC content under DOM addition had decreased by 26% in surface soil and 19% in deep soil compared to day 3.

5.4 Effects of DOM Addition on Soil Metabolic Quotient and Microbial Quotient

DOM addition affected soil metabolic quotients differently across soil layers. During early incubation, the metabolic quotient of deep soil under DOM addition was significantly higher than that of surface soil under the same treatment. However, by day 120, the metabolic quotient of DOM-added deep soil was significantly lower than that of DOM-added surface soil and deep control soil ($P < 0.001$), indicating improved carbon use efficiency.

The microbial quotient of DOM-added deep soil at the end of incubation was 1.58 times that of the initial period (day 3). In contrast, microbial quotients of DOM-added surface soil and control surface and deep soils were 68%, 79%, and 21% of day 3 values, respectively. Throughout the incubation, DOM addition resulted in significantly lower metabolic quotients in both surface and deep soils compared to their respective controls, suggesting that DOM input enhanced microbial carbon use efficiency, particularly in deep soil.

6. Discussion

Previous studies have shown that DOM addition promotes soil mineralization. Fanin et al. found that DOM had more significant effects on microbial respiration than litter addition, consistent with our results. DOM addition increases soil dissolved organic carbon content, providing readily available carbon sources for microbial respiration and metabolism, which significantly increases mineralization rates initially.

In this study, both surface and deep soils showed significantly higher instantaneous CO emission rates after DOM addition. For surface soil, which regularly receives litter inputs, the relative limitation of available nutrients is less severe than in deep soil. Deep soil, being chronically nutrient-poor, showed a more pronounced response to external DOM input. During early incubation, deep soil instantaneous emission rates were significantly higher than deep controls, though cumulative emission differences were not significant initially. As incubation progressed, cumulative emissions from DOM-added deep soil gradually exceeded controls, indicating that prolonged DOM addition increased microbial abundance and organic matter utilization capacity.

The difference in cumulative emissions between surface and deep soils was significant regardless of DOM addition, likely because surface soil contains larger quantities of organic matter and has greater nutrient supply capacity. Substrate quantity and quality are primary factors affecting soil microbial respiration. As incubation time extended, nutrient depletion weakened microbial respiration in deep soil, while surface soil maintained higher activity due to richer nutrient conditions.

Soil metabolic quotient reflects microbial activity responses to environmental factors and indicates carbon use efficiency. A higher metabolic quotient suggests

more carbon is used for respiration rather than biomass construction, indicating low efficiency and poor soil quality improvement. Deep soil microorganisms experience more severe nutrient limitations, so DOM addition significantly improved their nutritional environment, increasing microbial numbers and activity. This caused deep soil metabolic quotient to be significantly higher than surface soil initially, but as active carbon sources decreased, microbial activity declined and respiration weakened. By day 120, deep soil metabolic quotient under DOM addition was significantly lower than surface soil and deep control, indicating enhanced carbon use efficiency.

Microbial quotient reflects the proportion of active organic carbon in soil and indicates the availability of the total carbon pool. Higher microbial quotient suggests a larger proportion of easily decomposable organic carbon in the total pool. At the end of incubation, DOM-added deep soil microbial quotient was significantly higher than surface soil and controls, indicating that DOM addition improved deep soil quality by increasing the proportion of active organic carbon.

7. Conclusion

DOM addition significantly affected soil microbial respiration. In surface soil, DOM addition significantly increased cumulative CO emissions compared to controls ($P < 0.001$). In deep soil, cumulative emissions under DOM addition showed no significant difference from deep controls, but were significantly lower than surface soil emissions ($P < 0.001$). Both surface and deep soil metabolic quotients under DOM addition were significantly lower than their respective controls, indicating that DOM input improved microbial carbon use efficiency, particularly in deep soil. At the end of incubation, the microbial quotient of DOM-added deep soil was significantly higher than that of surface soil and controls, demonstrating that DOM addition improved deep soil quality.

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