Defoliation effects on carbon allocation in grasslands

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Abstract

Grazing represents a multifaceted interaction between livestock and grasslands, encompassing three main mechanisms: defoliation, dung and urine return, and trampling, each of which profoundly affects soil carbon (C) storage processes. To better understand the impact of grazing on soil C dynamics, we conducted an in-situ ^{13}C pulse labeling experiment on a field-simulated grazing platform, incorporating separate or combined treatments of defoliation, excreta return, and trampling. We tracked ^{13}C allocation in dissolved organic carbon (DOC), microbial biomass carbon (MBC) and soil organic carbon (SOC). We found that mowing significantly promoted the accumulation of root-derived DOC and MBC. Trampling also increased root-derived DOC. A positive correlation was observed between root-derived DOC and MBC, whereas no significant correlation was found between root-derived SOC and either root-derived DOC or MBC. Overall, we disentangled the complex grazing behaviors, quantified and tracked the pathways of C among different C pools under different grazing disturbance. Our study also highlights the distinct impact of mowing, trampling, and dung and urine return by ungulates on SOC, and future research should thoroughly consider these mechanisms to improve grassland management practices.

Introduction

Grasslands occupy approximately one-fifth of the Earth's land surface and store an estimated 200–300 Pg of carbon. As a result, carbon allocation dynamics within grasslands play a critical role in shaping the global carbon balance (Bai & Cotrufo, 2022). In these ecosystems, minor shifts in plant and soil carbon pools can affect atmospheric CO_2 concentrations. Investigating the turnover and sequestration of grassland carbon is therefore essential for understanding its impact on global climate change. As active components of the soil carbon pool, dissolved organic carbon (DOC) and microbial biomass carbon (MBC) are critical indicators for predicting soil carbon release and stability.

Grazing represents a complex interaction between livestock and grasslands, encompassing three key mechanisms: mowing, dung and urine return, and trampling. Each of these mechanisms can significantly impact soil carbon storage processes. Although DOC accounts for only a small fraction of soil organic

carbon (SOC) (approximately 0.25%), it represents the most dynamic and mobile component of SOC (Angst et al., 2023). DOC plays a pivotal role in SOC formation and the processes of soil carbon transport and transformation. Labile compounds within DOC contribute directly to SOC through the *in vivo* turnover process of the soil microbial carbon pump, while some recalcitrant components can also form SOC directly through mineral adsorption (Liang et al., 2017). Grazing by livestock can significantly alter the composition and diversity of plant communities, which may, in turn, affect the quantity and composition of root exudates DOC and the leached DOC from surface litter, ultimately influencing the formation of SOC (Wei et al., 2023). Plant-derived DOC plays a crucial role in the process of being converted into stable SOC, with the involvement of soil microorganisms. MBC is an important driving force for the decomposition and transformation of SOC. Microorganisms fix or release carbon into the soil or atmosphere through the decomposition of organic matter. Dung and urine return can increase available nutrients, which is more conducive to the formation of MBC. Overall, grazing promotes SOC formation through living root inputs. However, further evidence is still needed to determine whether the changes in DOC or MBC under grazing conditions will ultimately affect SOC.

Methods

Study site and experimental design

The study was conducted in a semiarid steppe at the Duolun Restoration Ecology Station of the Institute of Botany, Chinese Academy of Sciences (42° 02′ N, 116° 17′ E), located in Inner Mongolia, China. The site experiences a mean annual temperature of 2.1°C and an average annual precipitation of 378 mm.

In May 2015, a simulated grazing experiment was initiated using a randomized block design with factorial treatments. The treatments included mowing to simulate defoliation by livestock (with and without mowing), the addition of a liquid mixture of dung and urine (with and without addition), and simulated trampling (with and without trampling). Trampling was performed by a man who wore hoof-shaped shoes and carried a counterweight walking in the plots. These factors were combined to create eight distinct treatment combinations (Liu et al., 2015). The experiment was replicated across eight blocks, resulting in a total of 64 plots. Detailed information on the experimental design is available in Wei et al. (2023) and Liu et al. (2015).

$^{13}CO_2$ pulse labeling

The labeling experiment began on July 29, 2022, during the 8th year of the ongoing experimental treatments. In each of the eight treatments, five replicate subplots were selected for labelling (40 total). Labeling was conducted on consecutive sunny days, with one plot labeled per day, requiring a total of 5 days to label all 5 replicate plots. We constructed a labeling chamber using steel pipe supports and transparent polyethylene film (with a light transmittance of over 92%), measuring 0.5 m × 0.5 m × 0.6 m. The bottom of the chamber was inserted into the soil to a depth of 5 cm.

The $^{13}CO_2$ pulse was generated by slowly injecting a sodium bicarbonate solution, made by dissolving 1.0 g of sodium bicarbonate ($NaH^{13}CO_3$, 99 atom% ^{13}C) in 40 mL of water, and then adding 100 mL of 1.0 M H_2SO_4. To prevent excessive $^{13}CO_2$ concentrations, the sodium bicarbonate solution was added to the sulfuric acid solution in two stages. Twenty-four hours after labeling, we collected soil samples and measured for DOC, MBC and SOC.

MBC and DOC were measured using the chloroform fumigation-extraction method. Two soil subsamples, each equivalent to 7.5 g dry weight and stored at −20°C, were prepared for analysis. One subsample underwent chloroform fumigation in the dark for 24 hours, while the other remained unfumigated as a

control. Following treatment, both subsamples were extracted by shaking them with 30 mL of 0.25 M K_2SO_4 for 1 hour, and the resulting solutions were filtered. Total organic carbon in the extracts was quantified using a TOC/TN analyzer (Multi N/C 3100, Analytik Jena, Jena, Germany). DOC was determined from the unfumigated samples, while MBC was calculated as the difference in organic carbon content between the fumigated and unfumigated samples.

Data analysis was conducted using R version 4.3.0. Initially, we employed linear mixed models (LMMs) with 'treatments' as a fixed effect and 'block' as a random effect to examine the impacts of mowing, trampling, and dung and urine return on ^{13}C-DOC and ^{13}C-MBC. These models were implemented using the lmer function from the lme4 package. Linear regression analysis was performed using R software to explore the relationships between ^{13}C-SOC and ^{13}C-DOC or ^{13}C-MBC.

Results

Defoliation significantly increased root-derived DOC (+33.03%) and MBC (+33.85%) ($p < 0.05$, Fig. 1 a, b). Trampling also increased DOC (+34.43%, $p < 0.05$, Fig. a). MBC was positively correlated with root-derived DOC ($p < 0.05$, Fig. c). While SOC showed no correlation with MBC or DOC (Fig. d, e).

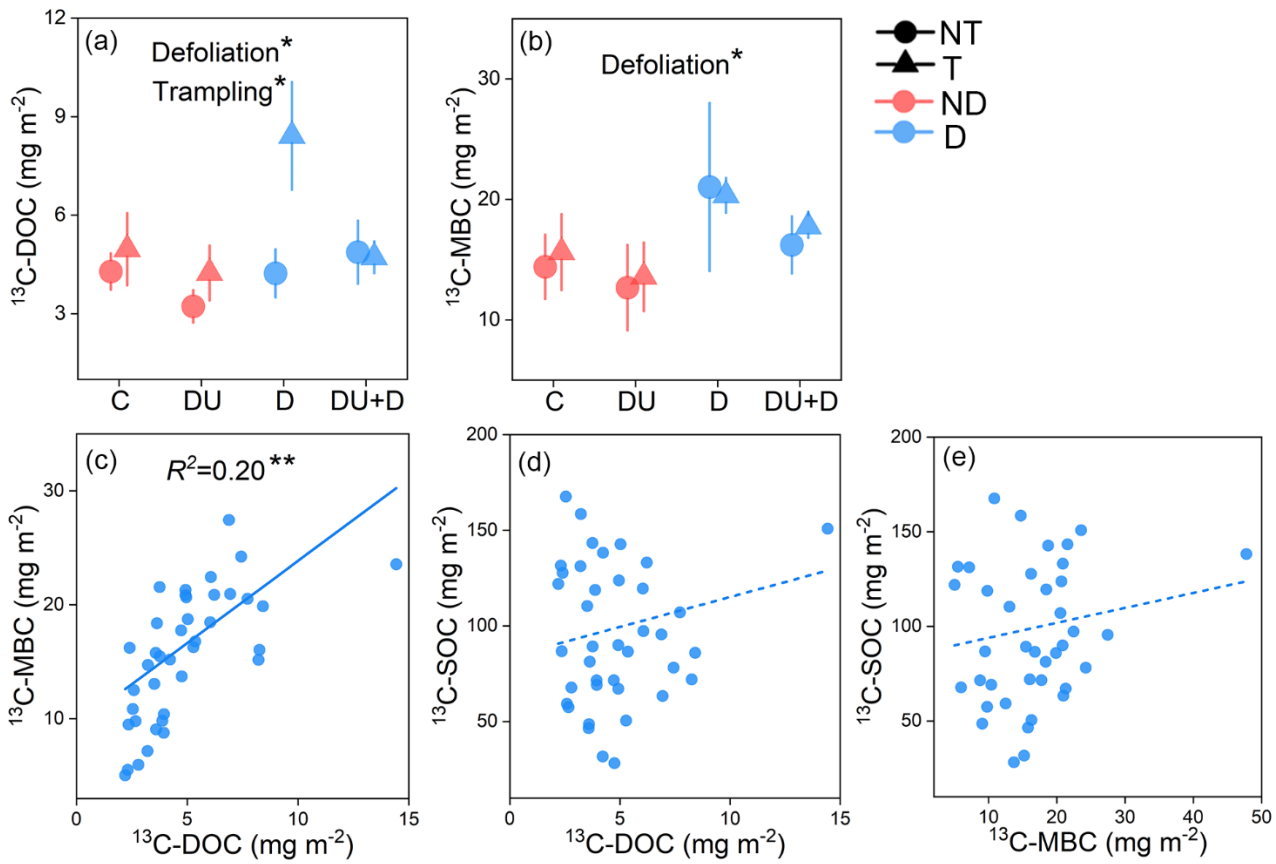

Fig. 1 The effects of grazing treatments on root-derived DOC (a), MBC (b). The relationship between ^{13}C-MBC and ^{13}C-DOC (b), ^{13}C-SOC and ^{13}C-DOC (d), ^{13}C-MBC (e). Note: C: control, D: defoliation, DU: Dung and urine addition, T: trampling.

Discussion

This study employed a ^{13}C isotope pulse labeling to quantitatively analyze the impact of living root-derived carbon inputs under grazing conditions on DOC and MBC. It also explored the relationship between root-derived DOC, MBC, and root-derived SOC under grazing.

Defoliation leads to increased root biomass (or specific root area). The positive correlation between root exudation and SRL suggests that changes in root functional traits enhance plant tolerance to defoliation, promoting the formation of root-derived DOC (Huang et al., 2021). DOC, as an unstable carbon source, is more readily utilized by microorganisms, serving as a carbon source for microbial activity. The positive correlation we found between ^{13}C-DOC and ^{13}C-MBC supports this hypothesis. However, the lack of a relationship between ^{13}C-SOC and either DOC or MBC indicate that other pathways may be ultimately dominating SOC formation under grazing, such as soil microbial necromass carbon. Liang et al. (2019) reported that microbial necromass carbon contributes more than 50% to SOC in grasslands. Therefore, future research could focus more on the changes in soil microbial necromass carbon to better understand SOC formation.

Livestock trampling compacts soil and promotes the contact between soil and roots, which may destroy soil aggregates to a certain extent, promote root growth and produce more root-derived DOC (Wei et al., 2021). Trampling has been shown to break plant material into fragments, facilitating the incorporation of litter into the soil (Mancilla-Leyton et al., 2013). This process, in turn, may enhance litter decomposition by stimulating microbial activity.

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