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Short Communication

Soil organic carbon turnover recovers faster than plant diversity in the grassland when high nitrogen addition is ceased: Derived from soil ^{14}C evidences

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ABSTRACT

Responses of terrestrial ecosystems to decreased nitrogen (N) deposition has brought considerable attention. Soil organic carbon (SOC) turnover is an important parameter in the terrestrial ecosystem model and ecosystem management. However, how SOC turnover responds to decreased N deposition has not yet been evaluated. Here we addressed this issue by the comparison of the SOC turnover in the treatment that receiving high N addition for 4 years and then none for 6 years (48 g $N \cdot m^{-2} \cdot yr^{-1}$, N48-cessation) with that derived from the control treatment (0 g $N \cdot m^{-2} \cdot yr^{-1}$, N0) in Inner Mongolia grassland, China. In this study, ^{14}C isotope technology was used to directly reveal SOC turnover time. Our results showed that SOC turnover time in the N48-cessation treatment had no statistical difference compared with that in the N0 treatment after 6 years of N cessation, indicating that its SOC turnover had recovered from high N enrichment. The reduction of SOC storage (274 g/m^2) in N48-cessation treatment from 2010 to 2014 also proved this recovery. Moreover, we also found that the species richness and Shannon diversity index in the N48-cessation treatment were significantly lower than those in the N0 treatment. It indicated that the recovery of SOC turnover was faster than plant diversity in the grassland when high N addition was ceased. This study suggested that the grasslands experienced high N deposition should reduce grazing and mowing in order to increase soil carbon input and offset the negative effect of the rapid recovery of SOC turnover on soil C storage when N deposition was decreased.

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1. Introduction

Nitrogen (N) deposition has brought profound effects on terrestrial ecosystems (Galloway et al., 2008). However, due to the enforcement of environmental policy and the transformation of economic structure, the increasing trend of N deposition has been slowed down (Yu et al., 2019). And even in some regions, such as Europe and North America, scientists have observed a decrease in N deposition (Gilliam et al., 2019; Schmitz et al., 2019). Thus, the potential changes of ecosystem processes with reduced N deposition have received considerable attention (Clark and Tilman, 2008; Gilliam et al., 2019; Schmitz et al., 2019). Soil organic carbon (SOC) turnover is a key parameter in the terrestrial ecosystem model and ecosystem management (Tan et al., 2020), and SOC pool is the largest terrestrial C reservoir (Amundson, 2001). Thus, the changes in SOC turnover caused by decreased N deposition might significantly affect global C cycle and further exerted an impact on climate change. However, how SOC turnover will respond when N input declines, to our knowledge, has not yet been evaluated, although some scholars mentioned this issue in their articles (Stevens et al., 2012; Gilliam et al., 2019).

Previous studies mainly used the manipulative experiment with N cessation to explore the responses of ecosystem processes to decreased N deposition, and their results showed that plant diversity and species composition would take decades or more to recover from N enrichment (Isbell et al., 2013; Stevens, 2016). Since SOC turnover has been found to be inhibited by high N addition (Tan et al., 2017; Zak et al., 2017), thus, we are eager to know whether the recovery of SOC turnover from high N enrichment would also take decades or more. To address this question, we conducted a N manipulative experiment that receiving high N addition for 4 years and then none for 6 years in Inner Mongolia Grassland, China.

Many previous field and lab experiments evaluated SOC turnover by measuring CO_2 efflux, however, this could only reflect the turnover of labile C pools in soil, which is just a small part of SOC stock (Luo et al., 2001). In the present study, we used ^{14}C isotope technology to directly reveal the turnover rate of SOC. ^{14}C isotope technology was thought to be a perfect tool to research SOC turnover on yearly to millennial timescales, because soil ^{14}C content, when combined with mathematic model, can be well used to calculate the turnover time of bulk SOC including all soil C pools (Trumbore, 2009).

2. Materials and methods

This field experiment was carried out in a semiarid temperate steppe (42°02′ N, 116°17′ E), which was fenced off since 2001. Prior to this, it was grazed, and after that no grazing was allowed. Mean annual temperature and mean annual precipitation are 2.1 °C and 386 mm. Dominant species are *Agropyron cristatum, Leymus chinensis* and *Stipa krylovii* (Fig.S1). The soil is classified as a Calcic Luvisol.

The N addition experiment was started since 2005. Urea was added in 2005, and then NH_4NO_3 was added after 2005. This experiment consisted of two treatments with five replicates: 0 g $N \cdot m^{-2} \cdot yr^{-1}$ (N0) and 48 g $N \cdot m^{-2} \cdot yr^{-1}$ (N48-cessation). Each plot is 5 × 5 m. N48-cessation treatment received N input from 2005 to 2008, and then ended since 2009 to observe the possible restoration of grassland ecosystem (Hao et al., 2018). To reduce the cost of soil ^{14}C measurements, sampling was conducted in only three of the five (randomly selected) replicates in the N0 and N48-cessation treatments.

Soil and plant sampling were finished in late August of 2014. A quadrat of 1 m^2 (1 × 1 m) was randomly set in each plot. The plants at ground level were clipped and mixed as the aboveground plant samples. The roots (i.e. belowground plant samples) were collected to depth 50 cm using a 5-cm diameter core. We collected soil samples using soil auger (3.5 cm diameter, 0–5 cm depth). Soil ^{14}C content, soil pH, SOC content, soil microbial composition and the lignin content and C and N concentrations in plants were analyzed in this study. Details on analytical methods of soil and plant samples could refer to Tan et al. (2018). The calculation of SOC turnover time (TT) followed Cherkinsky and Brovkin (1993) and Herold et al. (2014). Data on species richness and Shannon diversity index were obtained from our previous study (Hao et al., 2018). One-way ANOVAs ($p < 0.05$) were conducted to compare the differences in soil and plant properties between N0 and N48-cessation treatments. And the effect size (ES) of these comparisons were also recorded. To further examine the SOC dynamics when high N addition was ceased, we calculated the SOC storage of the N48-cessation treatment in the years of 2010 and 2014 according to the method reported in Tan et al. (2020). Then we conducted Paired-Samples T Test to compare the difference in SOC storage of the N48-cessation treatment between 2010 and 2014. All statistical analyses were conducted using software SPSS 20.0.

3. Results and discussion

The difference in SOC TT between N0 and N48-cessation treatments was statistically insignificant (ES = 0.083 and $p > 0.05$) (Fig. 1a and Table S1). The species richness and Shannon diversity index in the N48-cessation treatment were significantly lower than that in the N0 treatment (ES = 0.910 and $p < 0.05$ for species richness and ES = 0.188 and $p < 0.05$ for Shannon diversity index) (Fig. 1b and c and Table S1). The SOC storage of the N48-cessation treatment in 2014 was basically lower than that in 2010 ($p = 0.06$) (Fig. 2). Soil pH and the abundances of total phospholipid fatty acids (PLFAs) and bacteria PLFAs in the N48-cessation treatment were significantly lower than that in the N0 treatment (ES = 0.967 and $p < 0.05$ for soil pH, ES = 0.749 and $p < 0.05$ for total PLFAs and ES = 0.861 and $p < 0.05$ for bacteria PLFAs) (Table 1 and Table S1), whereas SOC content and the abundance of fungi PLFAs showed no statistical difference between them (ES = 0.266 and $p > 0.05$ for SOC content and ES = 0.150 and $p > 0.05$ for fungi PLFAs) (Table 1 and Table S1). The lignin content and C/N ratio in aboveground and belowground parts had no statistically significant differences between N0 and N48-cessation treatments (ES = 0.045 and $p > 0.05$ for the lignin content of aboveground part, ES = 0.379 and $p > 0.05$ for the C/N ratio of aboveground part, ES = 0.094

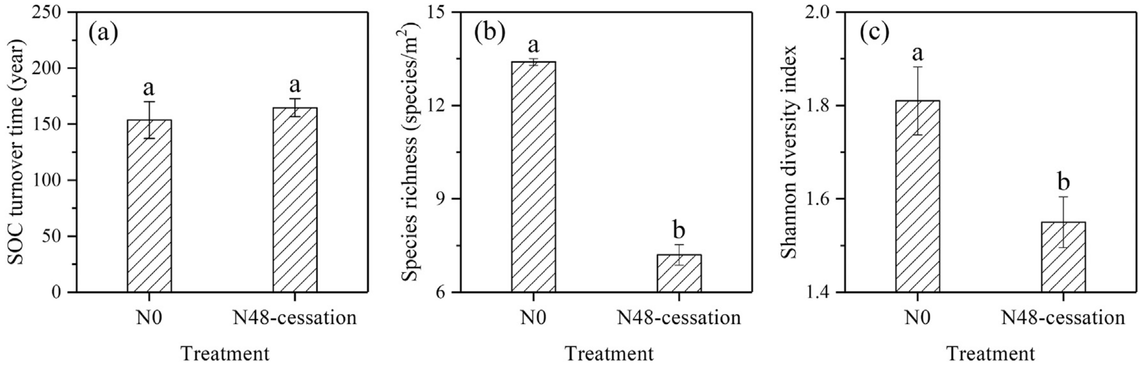

Fig. 1. Comparisons of SOC turnover time (a), species richness (b) and Shannon diversity index (c) between N0 and N48-cessation treatments. Values are mean ± standard error (SE). Different letters indicate there is significant difference between the two treatments (one-way ANOVA, $p < 0.05$).

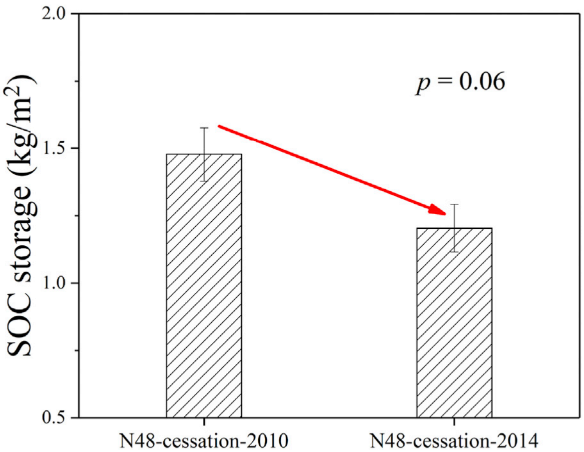

Fig. 2. Difference in SOC storage of the N48-cessation treatment between 2010 and 2014.

Table 1
Comparison of soil pH, SOC content and soil microbial composition between the N0 treatment and the N48-cessation treatment. Each value is mean ± standard error (SE). Different letters indicate significant differences at the 0.05 level.

Treatment	Soil pH	SOC content	Total PLFAs (nmol·g^{-1}DW)	Fungi PLFAs (nmol·g^{-1}DW)	Bacteria PLFAs (nmol·g^{-1}DW)
N0	7.16 (±0.08)^a	17.60 (±1.38)^a	41.97 (±9.57)^a	2.04 (±1.12)^a	32.99 (±6.82)^a
N48-cessation	5.40 (±0.14)^b	19.58 (±0.89)^a	16.11 (±0.04)^b	1.34 (±0.35)^a	8.84 (±0.63)^b

Note: DW means soil dry weight.

and $p > 0.05$ for the lignin content of belowground part and ES = 0.013 and $p > 0.05$ for the C/N ratio of belowground part) (Table 2 and Table S1).

Previous studies had found that SOC turnover was suppressed by high N addition (Tan et al., 2017; Zak et al., 2017). However, this study showed that the difference in SOC TT between N0 and N48-cessation treatments was statistically insignificant (Fig. 1a), indicating that after six years of stopping fertilization, the SOC turnover rate could return to the level

Table 2
Lignin content and C/N ratio in aboveground and belowground parts for the N0 treatment and the N48-cessation treatment. Values (±SE) and letters have the same meaning as in Table 1.

Treatment	Lignin content of aboveground part (mg/g)	C/N ratio of aboveground part (unitless)	Lignin content of belowground part (mg/g)	C/N ratio of belowground part (unitless)
N0	109.8 (±9.9)^a	37.00 (±0.57)^a	293.1 (±60.6)^a	30.01 (±2.01)^a
N48-cessation	102.5 (±13.5)^a	34.15 (±1.59)^a	333.7 (±16.7)^a	30.90 (±3.25)^a

when no fertilization was applied. The comparison of the SOC storage of the N48-cessation treatment between 2010 and 2014 also provided an indirect support for this possibility, because the SOC storage of the N48-cessation treatment in 2014 was lower than that in 2010 (Fig. 2). The decrease in SOC storage between these two years was 274 g/m^2. The change in SOC storage should be controlled by two processes: the plant litter input and the SOC decomposition. Given that the time that aboveground biomass in grassland changes from live plant to soil organic matter is usually one year, we compared the difference in aboveground biomass of the N48-cessation treatment between 2009 and 2013. The aboveground biomass in 2013 was 53 g/m^2 less than that in 2009 (Hao et al., 2018). Thus, the decrease in SOC storage between 2010 and 2014 was 5 times of the decrease in aboveground biomass between 2009 and 2013. Hence, the decrease in SOC storage should be largely due to the accelerated SOC turnover after high N cessation. Furthermore, our previous work had reported that the plant diversity (i.e. species richness and Shannon diversity index) in the N48-cessation treatment was significantly lower than that in the N0 treatment (Fig. 1b and c) (Hao et al., 2018). This meant that the recovery in plant diversity showed limited response to the cessation of high N addition. Therefore, our results suggested that SOC turnover recovered faster than plant diversity in the grassland when high N addition was ceased.

Microbial composition and plant litter quality have been suggested to be the main factors regulating SOC turnover (Tan et al., 2018). Thus, the rapid recovery of SOC turnover observed in this study could also be associated with these two factors. Lignin content and C/N ratio are two key indicators of plant litter quality. The lignin content and C/N ratio in both aboveground and belowground parts in the N48-cessation treatment showed no statistical difference from those in the N0 treatment (Table 2). Thus, the observed quick recovery in SOC turnover after stopping high N addition could be partially explained by plant litter quality. Although the total PLFAs and the bacteria PLFAs in the N48-cessation treatment were found to be significantly lower than that in the N0 treatment, the fungi PLFAs did not showed statistical difference between the two treatments after six years of the cessation of N fertilization (Table 1). Fungi is mainly responsible for the decomposition of the lignin in SOC (De Boer et al., 2005), and lignin is passive in the dynamic of SOC (Melillo et al., 1982). Hence, the recovery of SOC turnover could also be associated with the undifferentiated fungi PLFAs between the two treatments after stopping high N addition.

Recovery of SOC turnover after the decrease of N deposition will weaken the function of C sink of soil. Thus, based on our results, it should be considered in the climate prediction model that the relatively rapid recovery of SOC turnover as high N deposition lowered would exert a negative effect on soil C storage, which might affect global C cycle and climate change. In addition, Storkey et al. (2015) suggested that grassland ecosystem needed some good management to maintain their benign turnover. Hence, for those grasslands experienced high N deposition, we recommend reducing grazing and mowing in order to increase soil carbon input and offset the negative effect of the rapid recovery of SOC turnover on soil C storage when high N deposition was decreased.

4. Conclusion

This study found that SOC turnover recovered faster than plant diversity in the grassland when high N addition was ceased. Fungal activity and plant litter quality mainly regulated the recovery of SOC turnover. Our results indicated that decreased high N inputs might have a negative effect on soil C storage, which should attract the attention of scientists working on climate prediction models. Furthermore, future N cessation experiments should explore the effects of N addition rates and ecosystem types on the recovery of SOC turnover when N addition was ceased.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.gecco.2020.e01229.

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