



Factors affecting ^{13}C enrichment of vegetation and soil in temperate grasslands in Inner Mongolia, China

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Received: 29 October 2018 / Accepted: 9 January 2019 / Published online: 21 January 2019
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Abstract

Purpose Soil organic carbon (SOC) dynamics are central to understanding the effects of environmental change on the carbon cycle of ecosystems. Vegetation and soil stable carbon isotope composition ($\delta^{13}\text{C}$), especially the difference of $\delta^{13}\text{C}$ between surface soils and source vegetation ($\Delta\delta^{13}\text{C}$), can provide useful information about the SOC dynamics. The variations and patterns of vegetation and soil $\delta^{13}\text{C}$ and $\Delta\delta^{13}\text{C}$ along climatic and edaphic gradients were analyzed to improve the understanding of SOC dynamics in temperate grassland ecosystems.

Materials and methods Soil and plant samples were collected along climatic and edaphic gradients. Meteorological data were extracted from a regional climate database, which was spatially interpolated based on the records at 107 climatic stations located in Inner Mongolia. $\delta^{13}\text{C}$, carbon and nitrogen contents of soil and plant, soil pH, soil clay, silt, and sand contents were determined. $\Delta\delta^{13}\text{C}$ and soil C:N were calculated. The integrative effects of these factors were further estimated using stepwise regression, redundancy analysis (RDA) and *T* value biplots.

Results and discussion As expected, soil $\delta^{13}\text{C}$ was positively related to vegetation $\delta^{13}\text{C}$, and higher than the vegetation $\delta^{13}\text{C}$. Vegetation and soil $\delta^{13}\text{C}$ and $\Delta\delta^{13}\text{C}$ were all related to growing season precipitation (GSP) and growing season temperature (GST). However, when climatic and edaphic factors were considered together, vegetation $\delta^{13}\text{C}$ was positively related to GST and soil C:N, soil $\delta^{13}\text{C}$ was positively related only to GST, and $\Delta\delta^{13}\text{C}$ was negatively related only to soil C:N. Unlike previous research conducted at the species level which only included C_3 species, GST, instead of precipitation, was the dominant controlling factor of vegetation $\delta^{13}\text{C}$.

Conclusions GST influences vegetation $\delta^{13}\text{C}$ mainly through its effect on relative abundance of C_4 plants. Soil C:N rather than climatic factors is the dominant controlling factor of SOC decomposition, and the effects of climatic factors on SOC decomposition are indirect and induced by their effects on soil C:N through influencing species composition and plant C:N. Measuring vegetation $\delta^{13}\text{C}$, soil $\delta^{13}\text{C}$, and $\Delta\delta^{13}\text{C}$ simultaneously is necessary to comprehensively understand how environmental changes influence ecosystem carbon cycles.

Keywords Carbon cycle · Climatic factors · Edaphic factors · Stable carbon isotope composition · Temperate grasslands

Responsible editor: Zhihong Xu

Electronic supplementary material The online version of this article (<https://doi.org/10.1007/s11368-019-02248-z>) contains supplementary material, which is available to authorized users.

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1 Introduction

Soil organic carbon (SOC) stocks greatly exceed the amount of carbon stored in both the atmosphere and terrestrial vegetation (Scharlemann et al. 2014). It has been suggested that CO₂ released from the decomposition of SOC makes an important contribution to climate change. Furthermore, global warming is likely going to increase carbon release from soil to the atmosphere by accelerating decomposition of SOC (Lin et al. 2011; Sanderman et al. 2003). Understanding of SOC dynamics has attracted much attention as it is central to the evaluation of ecosystem carbon balance, and the feedbacks of this to climate change (Kirschbaum 2000; Campbell et al. 2009; Wang et al. 2013; Averill 2014).

It is difficult to detect statistically significant changes of the SOC stock over short-time periods, due to its large pool size, slow turnover rate, and huge spatial heterogeneity (Van Groenigen et al. 2014). Moreover, studies of the microbial processes controlling SOC turnover are necessary to understand the mechanisms governing SOC dynamics (Schimel and Schaeffer 2012); however, these process-based studies can be expensive and time consuming in the field (Campbell et al. 2009). Consequently, carbon stable isotope composition ($\delta^{13}\text{C}$) measurements of more easily collected plant biomass and soil samples offer an alternative and relatively cost-effective method to comprehend SOC dynamics (Garten et al. 2000; Acton et al. 2013; Wang et al. 2013; Yang et al. 2015).

Some studies have estimated SOC turnover and degree of SOC decomposition and explored the mechanisms governing SOC dynamics by investigating the pattern and variation of soil $\delta^{13}\text{C}$ along environmental gradients (Garten et al. 2000; Bai et al. 2012; Peri et al. 2012; Wang et al. 2013; Acton et al. 2013; Angelo and Pau 2015). In general, soil $\delta^{13}\text{C}$ decreases with mean annual precipitation (MAP) (Lee et al. 2005; Feng et al. 2008; Peri et al. 2012), and increases with mean annual temperature (MAT) (Cheng et al. 2011). The effects of climatic factors on soil $\delta^{13}\text{C}$ are mainly attributed to their effects on plant $\delta^{13}\text{C}$, relative abundance of C₄ plants (P_{C_4}) or both (Lee et al. 2005; Feng et al. 2008; Cheng et al. 2011; Peri et al. 2012). Mostly, soil $\delta^{13}\text{C}$ decreased with soil C:N, SOC, and soil silt content (Wang et al. 2015; Yang et al. 2015), and increased with soil sand content (Wang et al. 2013). Edaphic factors may affect soil $\delta^{13}\text{C}$ by changing microbial activities, substrate quality and availability and stabilizing effect (Wynn et al. 2006; Xu et al. 2016).

Previous studies have demonstrated that climatic and edaphic variables have important roles in shaping patterns of soil $\delta^{13}\text{C}$ and consequently soil carbon dynamics (Peri et al. 2012; Wang et al. 2013; Yang et al. 2015). However, the controlling factors of soil $\delta^{13}\text{C}$ differ for various ecosystems and regions. For instance, Peri et al. (2012) investigated relationships of soil $\delta^{13}\text{C}$ in Southern Patagonia's native forests with 28 climatic and soil variables, and found that MAP, but

not soil properties, was the controlling factor of soil $\delta^{13}\text{C}$. At a continental-scale in Australia, Wynn and Bird (2008) found that climate and soil texture (percent of mineral particles < 63 μm) were the dominant controllers of soil $\delta^{13}\text{C}$. Wang et al. (2013) found that MAT and SOC were the controlling factors of soil $\delta^{13}\text{C}$ in alpine meadow of the Tibetan Plateau.

Compared with soil $\delta^{13}\text{C}$, the difference of $\delta^{13}\text{C}$ between surface soils and source plants ($\Delta\delta^{13}\text{C}$) may be a more direct and profound indicator of soil carbon dynamics along environmental gradients (Yang et al. 2015). This is because soil $\delta^{13}\text{C}$ is not only determined by the rate of SOC decomposition but also by the $\delta^{13}\text{C}$ value of source vegetation (Ehleringer et al. 2000; Peri et al. 2012). Thus, $\Delta\delta^{13}\text{C}$ can eliminate the effect of $\delta^{13}\text{C}$ value of source vegetation on soil $\delta^{13}\text{C}$, and so provide a more accurate indicator of soil carbon dynamics, i.e., large $\Delta\delta^{13}\text{C}$ translates to faster SOC decomposition rates (Yang et al. 2015). However, previous studies have mostly concentrated on soil $\delta^{13}\text{C}$ and rarely on $\Delta\delta^{13}\text{C}$ (Yang et al. 2015; Wang et al. 2018). Although several studies have investigated the relationships of $\Delta\delta^{13}\text{C}$ with climatic and edaphic factors in alpine grassland and temperate forest ecosystems (Garten et al. 2000; Yang et al. 2015), the results indicated that controlling factors of $\Delta\delta^{13}\text{C}$ differed among ecosystems. MAT was the dominant controlling factor of $\Delta\delta^{13}\text{C}$ in temperate forest ecosystems (Garten et al. 2000). Soil silt content and C:N were the dominant controlling factors of $\Delta\delta^{13}\text{C}$ in alpine meadow, while in alpine steppe it was SOC (Yang et al. 2015). Moreover, the soil properties, climatic conditions, and vegetation types of these studies are very different from those of temperate grassland ecosystems. Thus, it is important to conduct related research on a temperate grassland ecosystem to determine if its relationships of $\Delta\delta^{13}\text{C}$ with climate and soil factors differ from those of other ecosystems.

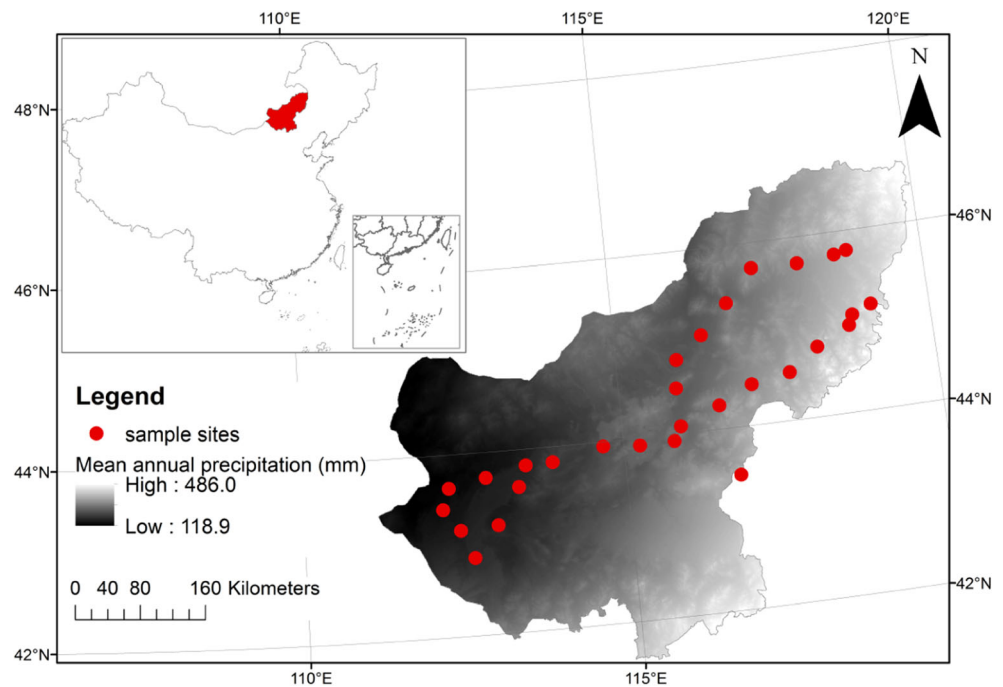
Xilingele League, Inner Mongolia, China, is a region with typical temperate grassland ecosystems. Thus, we conducted research in this region with the following primary objectives: (1) to verify the hypothesis that in temperate grasslands soil $\delta^{13}\text{C}$ is positively correlated to vegetation $\delta^{13}\text{C}$ and is higher than vegetation $\delta^{13}\text{C}$, as soil organic matter is mainly derived from plant inputs and SOC decomposition discriminates ^{13}C ; and (2) to quantify the individual and combined effects of climatic and edaphic variables on vegetation, soil $\delta^{13}\text{C}$ and $\Delta\delta^{13}\text{C}$, in order to determine the controlling factors of soil carbon dynamics in temperate grasslands.

2 Materials and methods

2.1 Study area

Plant and soil samples were obtained from 29 grassland sites along northeast–southwest transects located at longitude 111°

Fig. 1 Locations of the sampling sites on the Xilingele League, Inner Mongolia, China



25'~119° 58', latitude 41° 3'~46° 45' in the Xilingele League, Inner Mongolia, China (Fig. 1).

The Xilingele League has an average elevation of about 1200 m. It has an arid and semi-arid continental climate with water availability being the major abiotic factor limiting plant growth. MAP ranges from 100 to 450 mm, MAT ranges from – 3 to 6 °C. There is a significant negative correlation between MAP and MAT ($r^2 = 0.74$, $P < 0.001$). The natural vegetation cover is temperate grassland. The main grassland types, in transition from east to west, are temperate meadow steppe, temperate typical steppe, and temperate desert steppe (DAHV and CISNR 1996). Dominant plant species are as follows: *Stipa grandis*, *Stipa baicalensis*, *Stipa krylovii*, *Stipa tianschanica*, *Leymus chinensis*, *Agropyron cristatum*, *Cleistogenes squarrosa*, *Cleistogenes songorica*, *Artemisia frigida*, *Salsola passerine*, *Salsola arbuscula*, *Chenopodium glaucum*, *Allium bidentatum*, and *Allium mongolicum* (DAHV and CISNR 1996). For more detailed information, see Table A1 - ESM. The soils are mostly chernozems, kastanozems, calcisols, and cambisols (Fu et al. 2011).

2.2 Sampling

Plant and soil samples were obtained in August 2015. To minimize influences of short-term human disturbance, such as grazing, sampling sites were at least 500 m away from human habitation, fenced, and not grazed in the sampling year. Sites were separated from each other by about 50 km and their geographic positions were recorded using GPS (Garmin, Montana 650, USA). At each site, an approximately 10 m × 10 m plot was established, and within that plot, three 1 m × 1 m quadrats were

randomly located. All aboveground standing plant biomass in each quadrat was cut to ground level and put in a large envelope. Three replicate soil samples from the top 10 cm of each quadrat were collected using a 70-mm inner diameter soil auger.

2.3 Sample analysis

All aboveground plant biomass samples were washed with deionized water to remove dust particles, de-enzymed at 105 °C for 15 min, oven-dried at 80 °C to constant weight, and then were ground and thoroughly mixed using a plant grinding machine (FW100, China). Finally, in preparation for analysis, subsamples of about 20 g were finely ground using a ball mill (Retsch MM400, Germany). After air-drying, soil samples were sieved to 2 mm, and plant residues were removed. Before isotope measurements, soil samples were ground to fine powder as for plant samples, and treated with 0.1 N HCl solution for 24 h at room temperature to remove any carbonates, then washed with distilled water and finally oven-dried at 65 °C to constant weight.

The carbon and nitrogen concentrations and carbon isotope compositions ($\delta^{13}\text{C}$, ‰) of plant and soil samples were determined by a coupled elemental analyzer (FlashEA1112; Thermo Electron) and isotope ratio mass spectrometer (Finnigan MAT-253; Thermo Electron). $\delta^{13}\text{C}$ values of samples were calculated as:

$$\delta^{13}\text{C}(\text{‰}) = \left[\frac{(R_{\text{samples}} - R_{\text{standard}})}{R_{\text{standard}}} \right] \times 1000 \quad (1)$$

where R_{sample} and R_{standard} , respectively, are the $^{13}\text{C}/^{12}\text{C}$ ratios in the sample and the standard. The universally accepted

standard of Vienna Pee Dee Belemnite (VPDB) was used. The precision for isotope composition measurement was 0.1‰.

Percentages of clay, silt, and sand contents in the soil samples were measured using a particle size analyzer (Malvern Mastersizer 2000; Malvern Instruments Ltd., Worcestershire, UK) after removal of organic matter and calcium carbonates by adding 30% H₂O₂ and heating at 100 °C for 45 min. Soil pH was determined using a pH electrode in a mixture of soil and water, with a water:soil ratio of 2.5:1 (Bao 2005).

2.4 Meteorological data

Mean monthly temperature (MMT), MAT, mean monthly precipitation (MMP), and MAP for each sampling site were extracted from a regional climate database with 1-km spatial resolution. This database was spatially interpolated based on the records of 107 climatic stations located in Inner Mongolia using the software package Anuspline (ver. 4.2). Using MMT and MMP, we then calculated the growing season temperature (GST) and growing season precipitation (GSP) of each sample site.

2.5 Data analysis

Paired-sample *T* tests were conducted to compare the mean values of vegetation and soil δ¹³C. Simple regression analysis was applied to investigate the relationship between vegetation and soil δ¹³C and also the relationships of vegetation δ¹³C, soil δ¹³C, and Δδ¹³C with climatic and edaphic variables (that is, GSP, MAP, GST, MAT, soil pH, clay content, silt content, sand content, soil organic carbon, and soil C:N). Then, stepwise regression was used to evaluate integrative effects of climatic and edaphic variables on vegetation δ¹³C, soil δ¹³C, and Δδ¹³C. Further, redundancy analysis (RDA) was conducted and *T* value biplots were drawn to explore primary controlling factors for vegetation δ¹³C, soil δ¹³C, and Δδ¹³C. Data analyses were conducted using SPSS (ver.18.0; SPSS Inc., USA) and Canoco (ver. 5.0; Microcomputer Power, Ithaca, NY, USA).

3 Results

3.1 Variation of vegetation and soil δ¹³C among sampling sites

Soil δ¹³C values ranged from −24.7 to −19.5‰, with a mean of −22.4 ± 0.3‰, and with 55% of these values within the range of −24 to −22‰ (Fig. A1a, Electronic Supplementary Material - ESM). Vegetation δ¹³C values ranged from −26.4 to −21.6‰, with a mean of −24.5 ± 0.2‰, and with 69% of these values within the range of −26 to −24‰ (Fig. A1b - ESM).

3.2 Relationship between vegetation and soil δ¹³C

Soil δ¹³C increased linearly with vegetation δ¹³C (Fig. 2). There was a clear pattern of soil being ¹³C enriched relative to the source vegetation, and this was more pronounced at sites with lower vegetation δ¹³C (Fig. 2). On average, Δδ¹³C was 2.1‰ and higher than 1.3‰ for 59% of the sample sites.

3.3 Variation of vegetation δ¹³C, soil δ¹³C, and Δδ¹³C along climatic gradients

All soil δ¹³C, vegetation δ¹³C, and Δδ¹³C showed linear increasing trends with GST and MAT, and decreasing trends with GSP and MAP (Fig. 3 and Fig. A2 - ESM). Moreover, the relationships of soil δ¹³C with climate factors were stronger than those of vegetation δ¹³C (Fig. 3).

3.4 Variation of vegetation δ¹³C, soil δ¹³C, and Δδ¹³C along edaphic gradients

Soil δ¹³C was positively related to soil pH (Fig. 4a), and negatively related to SOC content and soil C:N (Fig. 4b, c). Vegetation δ¹³C was not related to soil pH, SOC content, and soil C:N (Fig. 4d–f). Δδ¹³C was negatively related to SOC content and soil C:N (Fig. 4h, i), but was not related to soil pH (Fig. 4g). None of vegetation δ¹³C, soil δ¹³C, and Δδ¹³C were related to soil texture characteristics (clay, silt, and sand content; Fig. A3 - ESM).

3.5 Integrative effects of climatic and edaphic variables on vegetation δ¹³C, soil δ¹³C, and Δδ¹³C

When the integrative effects of climatic and edaphic variables were evaluated in stepwise regression analyses, only GST

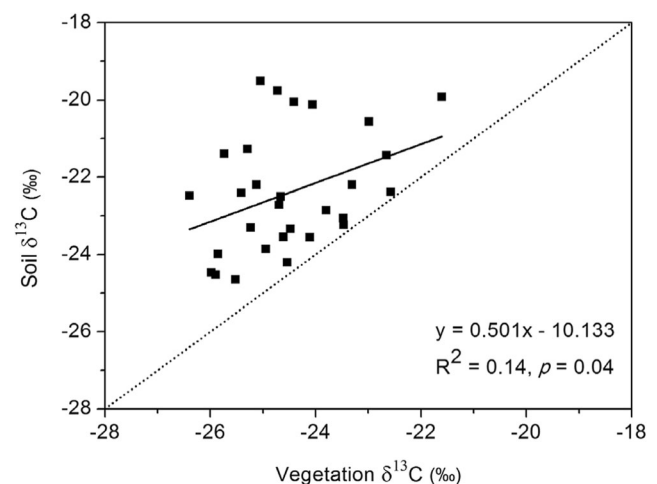
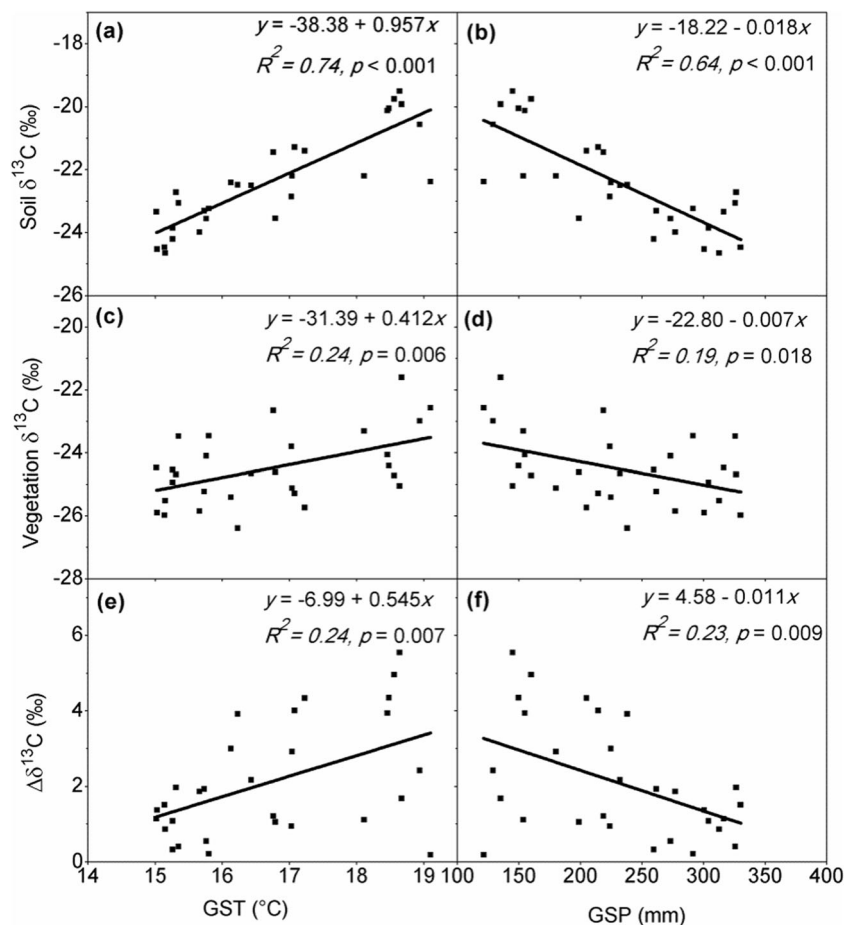


Fig. 2 Relationship between soil δ¹³C and vegetation δ¹³C in temperate grasslands on the Xilinge League, Inner Mongolia, China

Fig. 3 Relationships of soil $\delta^{13}\text{C}$, vegetation $\delta^{13}\text{C}$, and $\Delta\delta^{13}\text{C}$ with GST and GSP in temperate grasslands on the Xilingele League, Inner Mongolia, China. GST growing season temperature, GSP growing season precipitation



entered the model for soil $\delta^{13}\text{C}$ and explained 74% of the variation in soil $\delta^{13}\text{C}$ (soil $\delta^{13}\text{C} = -38.38 + 0.96 \times \text{GST}$, $r^2 = 0.74$). GST and soil C:N entered the model for vegetation $\delta^{13}\text{C}$ and together explained 45% of the variation in vegetation $\delta^{13}\text{C}$ (vegetation $\delta^{13}\text{C} = -41.59 + 0.74 \times \text{GST} + 0.61 \times \text{C:N}$, $r^2 = 0.45$). Only soil C:N entered the model for $\Delta\delta^{13}\text{C}$ and explained 51% of the variation in $\Delta\delta^{13}\text{C}$ ($\Delta\delta^{13}\text{C} = 9.71 - 0.97 \times \text{C:N}$, $r^2 = 0.51$).

The ordination diagram of RDA showed that most variation of $\delta^{13}\text{C}$ was explained by climatic factors, soil C:N and SOC, but not by soil texture (Fig. 5a). Further, *T* value biplots of RDA showed that GST was related positively to soil and vegetation $\delta^{13}\text{C}$, but was not related significantly to $\Delta\delta^{13}\text{C}$ (Fig. 5b), soil C:N was related negatively to $\Delta\delta^{13}\text{C}$ and positively to vegetation $\delta^{13}\text{C}$, but was not related significantly to soil $\delta^{13}\text{C}$ (Fig. 5e). None of GSP, SOC, clay content, silt content, and soil pH were related significantly to soil $\delta^{13}\text{C}$, vegetation $\delta^{13}\text{C}$ or $\Delta\delta^{13}\text{C}$ (Fig. 5 c, d, and f-h). There was no *T* value biplot for sand content as the relationships between sand content and $\delta^{13}\text{C}$ was too weak. Thus, the results of RDA confirm the results of stepwise regression analyses.

4 Discussion

4.1 Potential mechanisms for soil ^{13}C enrichment relative to vegetation

As expected, soil $\delta^{13}\text{C}$ was higher than vegetation $\delta^{13}\text{C}$ and this is consistent with other findings (Peri et al. 2012; Yang et al. 2015). An explanation for soil ^{13}C enrichment relative to vegetation is the Suess effect (Revelle and Suess 1957). This arises from a shift to lighter C in the atmosphere due to the burning of fossil fuels (Garten et al. 2000). The maximum of the Suess effect on $^{13}\text{C}\text{-CO}_2$ is about 1.3‰ (Graven et al. 2017). But, the mean $\Delta\delta^{13}\text{C}$ of our study was 2.1‰, suggesting that the Suess effect cannot be the only mechanism for soil ^{13}C enrichment in our study. Thus, other mechanisms, such as isotopic fractionation during SOC decomposition, could also play an important role (Yang et al. 2015).

Soil $\delta^{13}\text{C}$ increased linearly with vegetation $\delta^{13}\text{C}$ (Fig. 2), a result in accord with previous observations in alpine grasslands and temperate forests (Peri et al. 2012; Yang et al. 2015). This indicates that the positive relationship between soil $\delta^{13}\text{C}$ and vegetation $\delta^{13}\text{C}$ is common to various

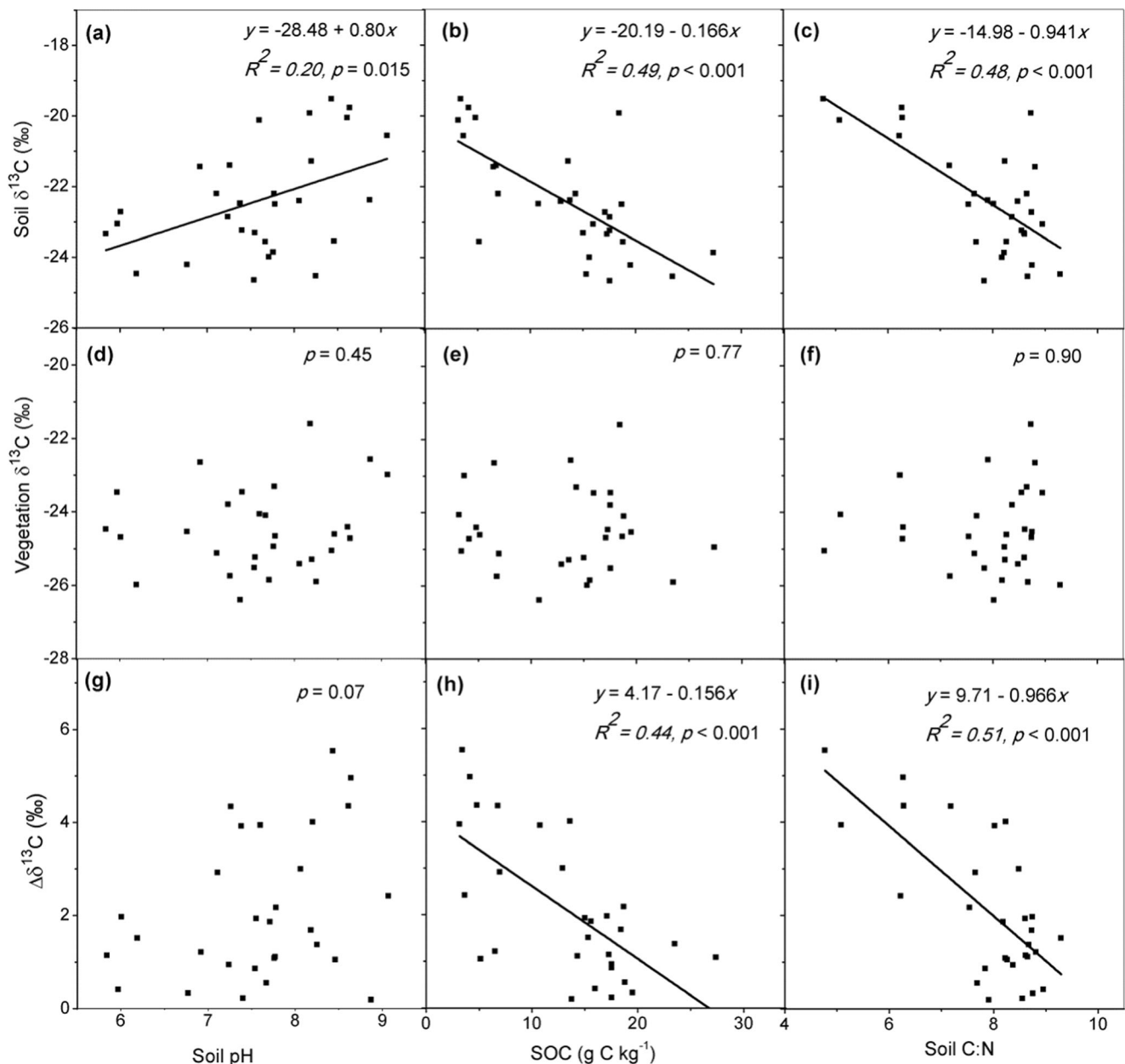


Fig. 4 Relationships of soil $\delta^{13}\text{C}$, vegetation $\delta^{13}\text{C}$, and $\Delta\delta^{13}\text{C}$ with soil pH, SOC, and soil C:N in temperate grasslands on the Xilingele League, Inner Mongolia, China

ecosystems and that soil $\delta^{13}\text{C}$ reflects the $\delta^{13}\text{C}$ of source plants due to soil organic matter being derived mainly from plant inputs.

4.2 Effects of climate and edaphic factors on vegetation $\delta^{13}\text{C}$

Although GST, MAT, GSP, and MAP were all related significantly to vegetation $\delta^{13}\text{C}$ (Fig. 3 and Fig. A2 - ESM), when they were considered together, only GST was related to vegetation $\delta^{13}\text{C}$. Firstly, MAT and GST are both expressions of temperature, but GST can more accurately reflect the effect of

temperature on vegetation $\delta^{13}\text{C}$ than MAT, because MAT largely depends on winter temperatures which always remain below biological zero in the region of the study and have no effect on growing vegetation. Secondly, this result indicated that temperature is the dominant controlling factor for vegetation $\delta^{13}\text{C}$, a result inconsistent with those showing that precipitation is the dominant controlling factor for $\delta^{13}\text{C}$ of C_3 species in Inner Mongolia (Wittmer et al. 2008; Zhou et al. 2016). The reason for this inconsistency is that the previous research was conducted at the species-level and only included C_3 species, whereas the present study was conducted at the community-level and included both C_3 and C_4 species. There

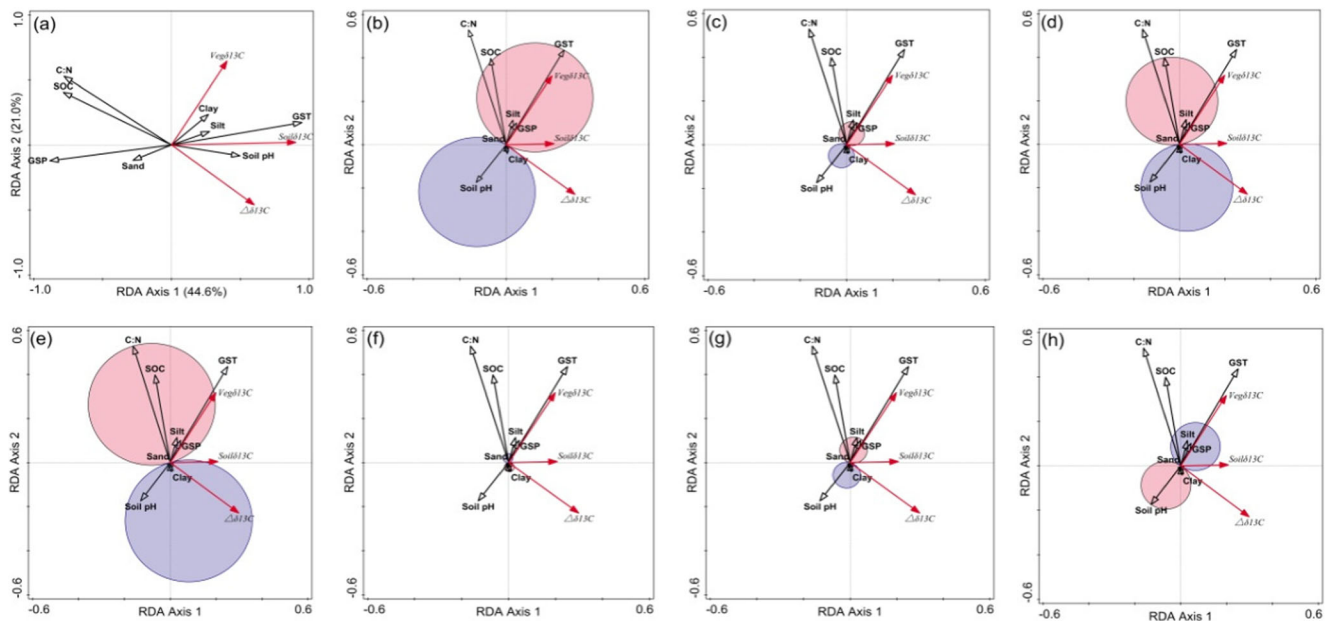


Fig. 5 Ordination diagram and T value biplots of redundancy analysis (RDA) plotted with the first axis shown in horizontal (X) direction and second axis shown in vertical (Y) direction. Response variables (soil $\delta^{13}\text{C}$, vegetation $\delta^{13}\text{C}$, and $\Delta\delta^{13}\text{C}$) were displayed as solid arrows, explanatory variables (GST, growing season temperature; GSP, growing season precipitation; SOC, soil organic carbon content; C:N, soil carbon:nitrogen

ratio; clay, soil clay content; silt, soil silt content; sand, soil sand content; soil pH) were displayed as hollow arrows. Ordination diagram (a); T value biplots with Van Dobben Circle drawn for GST (b), GSP (c), SOC (d), C:N (e), clay (f), silt (g), and soil pH (h). Response variables with arrows that end in Van Dobben Circles had significant relationships with particular explanatory variables

is a significant difference of $\delta^{13}\text{C}$ between C_3 and C_4 species, with mean $\delta^{13}\text{C}$ values of -12‰ for C_4 species, and -26‰ for C_3 species (O'Leary 1981). Thus, when there are only C_3 species present, precipitation is the major determinant of plant $\delta^{13}\text{C}$ in the Inner Mongolia region (Zhou et al. 2016). However, when both C_3 and C_4 species are present, the relative abundance of C_4 plants (P_{C_4}) is the major determinant of vegetation $\delta^{13}\text{C}$ in the region (Chen et al. 2007). Moreover, there is abundant evidence that P_{C_4} is primarily controlled by temperature and it has been found that P_{C_4} increased with GST in Inner Mongolia grassland ecosystems (Wittmer et al. 2010; Auerswald et al. 2012). Thus, at the community-level, GST is the dominant climatic factor determining vegetation $\delta^{13}\text{C}$ in the temperate grasslands of Inner Mongolia.

Although there was no correlation between vegetation $\delta^{13}\text{C}$ and soil C:N in simple regression (Fig. 4f), when the climate and edaphic factors were considered together, vegetation $\delta^{13}\text{C}$ was positively related to soil C:N (Fig. 5e). The mechanisms underlying the positive correlation between vegetation $\delta^{13}\text{C}$ and soil C:N are not clear. Possibly it is because soil C:N is positively related to P_{C_4} . To test this hypothesis, data on P_{C_4} across soil C:N gradients in our study area is required. However, there was significantly positive correlation between plant and soil C:N ($P=0.007$) in our study, and leaf C:N of C_4 species was significantly higher than that of C_3 species (Zheng and Shangguan 2007), which supports our hypothesis above.

4.3 Effects of climate and edaphic factors on $\Delta\delta^{13}\text{C}$

Although there were significant positive relationships of $\Delta\delta^{13}\text{C}$ with GST, MAT, GSP, and MAP in simple regression (Fig. 3 and Fig. A2 - ESM), when the climate and edaphic factors were considered together, the climate factors were not significantly related to $\Delta\delta^{13}\text{C}$ (Fig. 5b, c). This indicates that compared with edaphic factors, climate factors are not the predominant controlling factors of SOC decomposition. This result is inconsistent with the finding of Peri et al. (2012), but is in accord with the finding of Yang et al. (2015) that edaphic rather than climatic factors controlled over $\Delta\delta^{13}\text{C}$ in alpine grasslands on the Tibetan Plateau, i.e., $\Delta\delta^{13}\text{C}$ regulated by soil C:N and silt content. We found that $\Delta\delta^{13}\text{C}$ was regulated only by soil C:N as shown by a negative association between C:N and $\Delta\delta^{13}\text{C}$ (Fig. 5). This negative association could be largely driven by the higher rate of SOC decomposition in soils with lower C:N ratios (Yang et al. 2015).

It is known that in many ecosystems, soil texture can play an important role in regulating SOC dynamics. Generally, fine textured soils can increase physical and hydrological protection of SOC by inhibiting decomposition, and stabilizing SOC (Schimel et al. 1994; Wynn et al. 2006; Xu et al. 2016). This would reduce gaseous carbon losses to the atmosphere and the leaching of dissolved organic carbon leading to lower $\Delta\delta^{13}\text{C}$. Indeed, previous studies have found that $\Delta\delta^{13}\text{C}$ was negatively related to soil silt content (Powers and Schlesinger 2002; Yang et al. 2015). However, $\Delta\delta^{13}\text{C}$ was not related to soil silt

content or soil clay or sand content in our study (Fig. A3 - ESM). Wynn et al. (2006) found that SOC decomposition was regulated by soil texture in some climatic regions, but not in others. Furthermore, previous studies have demonstrated that the importance of clay content on SOC decomposition could vary across soils with different clay mineralogy (Spain 1990; Percival et al. 2000). Thus, the absence of association between $\Delta\delta^{13}\text{C}$ and soil texture might be attributed to the climate and the physical and chemical properties of clay and silt minerals in our study area. Clearly, these hypotheses need to be further considered and tested in future studies.

Based on the results and analysis above, we suggest that climate factors might indirectly affect SOC decomposition through their effects on species composition and plant C:N, and subsequently soil C:N. The significant positive relationships between plant and soil C:N ($P = 0.007$), and significant relationships between climate factors and plant and soil C:N (GST vs. plant C:N, $P < 0.001$; GSP vs. plant C:N, $P < 0.001$; GST vs. soil C:N, $P < 0.001$; GSP vs. soil C:N, $P < 0.001$) support this suggestion.

It is relevant to note that nitrogen deposition may affect $\Delta\delta^{13}\text{C}$ by changing plant species composition and microbial activity (Berendse et al. 2001; Waldrop et al. 2004). Thus, it is necessary to investigate the relationship between nitrogen deposition and $\Delta\delta^{13}\text{C}$ in future. Moreover, another factor requiring attention is that: as it is difficult to obtain source vegetation $\delta^{13}\text{C}$ information, present vegetation $\delta^{13}\text{C}$ is mostly used as proxy for source vegetation $\delta^{13}\text{C}$. In this context, using $\Delta\delta^{13}\text{C}$ to accurately assess soil carbon dynamic is based on the assumption that the present vegetation $\delta^{13}\text{C}$ reflects the source vegetation $\delta^{13}\text{C}$ of soil. Thus, in certain situations, i.e., where there has recently been large plant composition (especially C_4/C_3 abundance) transformation, this approach is not entirely satisfactory for understanding SOC dynamics. In regard to consideration of interannual variation of vegetation $\delta^{13}\text{C}$, the average value of vegetation $\delta^{13}\text{C}$ for several years should more accurately reflect the source vegetation $\delta^{13}\text{C}$. Unfortunately, the present study only considered vegetation $\delta^{13}\text{C}$ for 1 year.

4.4 Effects of climate and edaphic factors on soil $\delta^{13}\text{C}$

We found that GST had a positive effect on soil $\delta^{13}\text{C}$. An explanation for this is that as SOC is derived mainly from plant inputs, SOC largely preserves the isotopic signal of the plants it derives carbon from, and that GST has a positive effect on vegetation $\delta^{13}\text{C}$ by its effect on P_{C4} (Figs. 3c and 5b). Thus, GST can indirectly affect soil $\delta^{13}\text{C}$ through its effect on vegetation $\delta^{13}\text{C}$. However, unexpectedly the relationship between soil $\delta^{13}\text{C}$ and GST was stronger than that between vegetation $\delta^{13}\text{C}$ and GST. This indicates that GST controls soil $\delta^{13}\text{C}$ not only through its effects on vegetation $\delta^{13}\text{C}$ but also through other mechanisms, e.g., SOC

decomposition which can, to some extent, lead to ^{13}C enrichment, resulting in higher soil $\delta^{13}\text{C}$ (Garten et al. 2000). Based on the relationships of $\Delta\delta^{13}\text{C}$ with climatic and edaphic factors, we suggest that climate factors might indirectly affect SOC decomposition through their effects on plant species composition and plant C:N, and then soil C:N. This suggestion is supported by the negative relationship between GST and soil C:N ($P < 0.001$).

As there was a significant relationship between soil C:N and $\Delta\delta^{13}\text{C}$ (Figs. 4c and 5e), soil C:N should have an effect on SOC decomposition, and then to have an effect on soil $\delta^{13}\text{C}$. However, when the climatic and edaphic factors were considered together, there was no relationship between soil $\delta^{13}\text{C}$ and soil C:N (Fig. 5e). This may be because soil C:N had a positive effect on vegetation $\delta^{13}\text{C}$ and a negative effect on SOC decomposition (Fig. 5e), i.e., the effect of soil C:N on soil $\delta^{13}\text{C}$ was eliminated, due to its offsetting effects on $\delta^{13}\text{C}$ value of source vegetation and SOC decomposition.

5 Conclusions

Firstly, this study finds a positive association between soil and plant $\delta^{13}\text{C}$ and soil ^{13}C enrichment relative to vegetation in temperate grassland in the Inner Mongolia, that is similar to previously observed isotopic patterns in alpine grasslands. Secondly, the study demonstrates that GST is the dominant controlling factor of vegetation $\delta^{13}\text{C}$, most likely through its effect on P_{C4} . More importantly, by exhibiting that $\Delta\delta^{13}\text{C}$ was regulated by soil C:N, our results indicates that edaphic rather than climatic factors are the dominant controlling factor of SOC decomposition. Further, the effects of climatic factors on $\Delta\delta^{13}\text{C}$ are indirect and induced by their effects on soil C:N through influencing species composition and plant C:N. Finally, we suggest that compared with soil $\delta^{13}\text{C}$, $\Delta\delta^{13}\text{C}$ may provide more accurate information about soil carbon dynamics, i.e., larger $\Delta\delta^{13}\text{C}$ means faster SOC decomposition rate, as soil $\delta^{13}\text{C}$ may either underestimate or overestimate the effects of some factors on SOC decomposition. This emphasizes that measuring plant $\delta^{13}\text{C}$, soil $\delta^{13}\text{C}$, and $\Delta\delta^{13}\text{C}$ simultaneously are necessary to comprehensively understand how environmental change influences the carbon cycles of ecosystems.

Acknowledgements We thank Hongli Zhang for her assistance with field sampling and laboratory analyses.

Funding information The study was supported by the National Natural Science Foundation of China (No. 31400413); the Fundamental Research Funds for the Central Universities of China (No. N130301001, N160102001); the Doctoral Startup Foundation of Liaoning Province (No. 20141017); and the Visiting Scholar Program of China Scholarship Council (No. 201706085003).

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