Climatic controls on stable carbon and nitrogen isotope compositions of temperate grasslands in northern China

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Abstract
Aims The natural abundances of stable carbon (C) and nitrogen (N) isotopes (δ13C and δ15N) are extensively used to indicate the C and N biogeochemical cycles at large spatial scales. However, the spatial patterns of δ13C and δ15N in plant-soil systems of grasslands in northern China and their main driving factors across regional climatic gradient are still not well understood.
Methods We measured plant and soil δ13C and δ15N compositions as well as their associated environmental factors across 2000 km climatic gradient (-0.2 to 9 °C; 152 to 502 mm) in grasslands of northern China.

Results The soil δ13C and δ15N values in surface were lower than those in bottom for temperate typical steppe but had no significant differences for temperate meadow steppe and temperate desert steppe. Soil δ13C values declined with increasing soil organic carbon (SOC) but increased as mean annual temperature (MAT). These changes were attributed to the microbial decomposition rate. The δ15N values in soil and plant were negatively correlated with MAT and mean annual precipitation (MAP), which were mainly related to the low soil organic matter mineralization rate and the shift of dominant species from C4 to C3.

Conclusions Our results indicate the spatial patterns and different influencing factors on δ13C and δ15N.

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values along the climatic gradient in grasslands of northern China. The findings will provide scientific references for future research on the C and N biogeochemical cycles of temperate grasslands.

Keywords Carbon isotope · Nitrogen isotope · Northern grasslands · SOC turnover

Introduction

Grasslands, comprising open grassland, grassy shrublands and savannas, cover nearly 40% of the world’s land area and provide a wide range of ecosystem services to humans (O’Mara 2012; Bardgett et al. 2021). They store approximately one-third of the total carbon (C) in terrestrial ecosystems and most of the C is stored within 1 m soil layers, which contributes significantly to the mitigation of global climate change (Wang et al. 2016; Xu et al. 2015). In addition, grasslands also play an important role in the global nitrogen (N) biogeochemical cycle (Chen et al. 2021; Risch et al. 2019). Grasslands are vulnerable terrestrial ecosystems due to overexploitation (Liu et al. 2020). C and N cycles in global grassland ecosystems are sensitive to global climate change and land-use change, especially extreme precipitation and global warming in the temperate zone (Wang et al. 2016). However, it is difficult to explore the C and N dynamics by directly measuring the change of C and N stocks because of their relatively slow change processes. Recently, with the rapid development of stable isotope ratio mass spectrometry, stable C (δ13C) and N (δ15N) isotope compositions, reflecting C and N transformation processes in plant-soil systems, have become an important tool to study the C and N biogeochemical cycles in terrestrial ecosystems (Dong et al. 2018; Han et al. 2020; Xia et al. 2021).

The stable C isotope composition in plants and soil has emerged as a useful tool to assess the magnitude and distribution of plant productivity, water use efficiency and soil C turnover rate (Mcdowell et al. 2010; Wu et al. 2018). Previous studies have indicated that plant δ13C composition is mainly controlled by plant’s photosynthetic pathway, while soil δ13C composition mainly depends on the plant-derived organic C and SOC decomposition (An and Li 2015; Dixon et al. 2010). Plant community composition has a distinct influence on the plant δ13C (Chen et al. 2021; Luo et al. 2018). For example, the plant community composition with more C4 species will lead to higher δ13C values than C3 species (Wu et al. 2019). In addition, forbs with higher water use efficiency also have relatively higher δ13C values compared to graminoids and sedges (Liu et al. 2018). Besides, the soil δ13C composition has become an important integrative measure of soil organic carbon (SOC) input and output (Bird et al. 1996; Wang et al. 2017). Soil δ13C composition depends not only on that of plant residuals, but also on synthetic action of abiotic and biotic factors (e.g., SOC decomposition, microbial mobilization and immobilization) (Wu et al. 2019; Yang et al. 2015). Besides, the δ13C composition can differ significantly among various layers within the same soil profiles due to various microbial decomposition rates and soil physicochemical properties (Brunn et al. 2014; Carvalhais et al. 2014; Wang et al. 2017). Up-to-date, studies in tropical, temperate and tundra regions have demonstrated that soil physicochemical properties (e.g., pH, C/N ratio and soil moisture) and climatic factors (e.g., mean annual temperature and mean annual precipitation) regulate biogeochemical processes in soil and influence interactions between soil and plants, and can shape the spatial and temporal distribution of δ13C composition in the terrestrial ecosystem (Nel et al. 2018; Yang et al. 2015).

Compared to the C cycle, the N cycle is more complex due to the various influencing factors along different environment gradients (Craine et al. 2015a). Numerous studies have demonstrated that δ15N values in terrestrial ecosystems are positively correlated with mean annual temperature (MAT) but negatively related to mean annual precipitation (MAP) (Craine et al. 2015a; Nel et al. 2018). Besides, the δ15N composition is also influenced by soil C and N contents and other soil physicochemical properties (Craine et al. 2015a, b; Yang et al. 2013). For example, ammonia (NH3) volatilization will accelerate when soil pH is high, which leads to an abiotic gaseous N loss and higher soil δ15N values (Booth et al. 2005; Chen et al. 2021; Yang et al. 2013). Generally, δ15N signals the openness of the N biogeochemical cycle in terrestrial ecosystems (Boeckx et al. 2005). The N input in terrestrial ecosystems by livestock manure, biological N fixation and N deposition could alter the δ15N composition in plant-soil system (Fang et al. 2011). The δ15N composition of plant also depends on the various preferences of plant species to the available
N forms and the fractionation during the transfer of N from mycorrhiza to plants (Chen et al. 2021; Wu et al. 2019; Xu et al. 2011). The soil δ15N is therefore mainly controlled by plant N uptake and microbial mediated N-cycling processes (Golluscio et al. 2009).

Being one of the most widely-distributed terrestrial ecosystems, grasslands play a crucial role in the global terrestrial biogeochemical cycles of C and N (Yan et al. 2017; Yao et al. 2018). Grasslands in China are mainly distributed in arid and semi-arid regions, covering an area of approximately 4 × 10⁸ ha and accounting for 41.7% of the country’s territory (Shen et al. 2016), and contain different grassland types adapted to various climatic conditions and altitudes. Previous studies have shown that the δ13C and δ15N compositions of plant and soil are mainly controlled by climatic variables and soil characteristics (Wu et al. 2018) and increased our understanding of C and N cycles at both regional and global scales. However, the widely change patterns of δ13C and δ15N in China’s grasslands were still scarce, and the comprehensive influences of climate and soil were not clear. Therefore, this study focuses on the δ13C and δ15N of plants and soils in grasslands of northern China with various climatic conditions as well as their isotopic differences. We aimed to explore the spatial patterns of δ13C and δ15N in plant–soil system of grasslands in northern China and their driving factors. At the same time, we attempted to answer two questions. First, how do the relationships among ecosystem isotopic values and different climatic soil physico-chemical factors in grasslands of northern China along the climatic gradient? Second, how do climatic factors affect the distribution patterns of ecosystem isotopic values in grasslands of northern China?

Materials and methods

Study area

This study was conducted in the Inner Mongolian Plateau along a precipitation gradient (41.06°–49.48°N, 114.26°–120.94° E) in northern China (Fig. 1). The plateau is characterized by a dry and cold climate and belongs to the continental semi-arid grasslands of the Central Asian steppe ecosystem, with MAT ranging from -0.2 to 9 °C, and MAP ranging from 152 to 502 mm. Over 66% of the plateau is covered by temperate desert steppe (TDS), temperate typical steppe (TTS) and temperate meadow steppe (TMS). The three grassland types are distributed along the precipitation gradient across the plateau. Temperate meadow steppe is located at the wet end of the precipitation gradient, and the dominating species are Stipa baicalensis, Leymus chinensis, Filifolium sibiricum, and Carex pediformis (Shen et al. 2016). Both plant species richness (25 species per square meter) and ANPP (> 200 g m⁻² yr⁻¹) in the TMS are the highest compared to TTS and TDS. Based on our survey, the plant community is mainly composed of gramineae and C₄ species are less than 30% in TMS. By contrast, temperate desert steppe is distributed at the dry end of the precipitation gradient, and consists of Cleistogenes squarrosa, Leymus chinensis, Agropyron mongolicum and C. duriuscula, which has the minimum ANPP (< 60 g m⁻² yr⁻¹) and plant species richness (3 species per square meter). The plant community is mainly composed of forbs and C₄ species are more than 70% in temperate desert steppe. In the middle of the precipitation gradient, temperate typical steppe is dominated by Leymus chinensis, Stipa grandis, S. krylovii and Artemisia sacrorum, which has a medium ANPP and plant species richness. The growing season of vegetation is mainly from May to September, during which the precipitation accounts for about 80% of the whole year. Based on the Chinese soil classification system, the soil types in temperate meadow steppe, temperate typical steppe and temperate desert steppe are Chozem, Kastanozems and Calcisols, respectively.

Field sampling

We conducted three consecutive sampling campaigns during the summer (from June to August) of 2016–2018, and sampled 255 biomass plots and soil profiles from 85 sites covering all major grassland types in northern China. To avoid human disturbance, all sampling sites were located about 1 km away from the major roads. At each site, a 30×30 m quadrat was randomly selected. Soil samples were collected from three pits at a depth of 0–10, 10–20, 20–40, and 40–60 cm. Within each quadrat, aboveground parts of all plants in three 1 × 1 m plots were harvested. All the plant samples were rinsed with pure water (18.2 MΩ cm) to remove dust particles and then oven-dried for 60 h at 65 °C. Soil samples were naturally...
air-dried and then passed through 2 mm mesh sieve after removing big roots and rocks. Both the plant and soil samples were ground into a fine powder for determination of physicochemical properties and isotope values.

Chemical and isotope measurement

Soil pH was measured by immersing an electronic meter in a 1:2.5 mixture of the homogenized soil material and pure water, with a precision of ± 0.5 (Han et al. 2020). Soil samples passed through a 2 mm sieve were used to determine soil particle sizes by Mastersizer 3000 (Malvern Instruments, Malvern, England) and the error was less than 1%. Soil samples were finely ground (< 149 μm), treated with 0.5 mol L⁻¹ HCl solution at room temperature for 24 h and then washed to neutrality, dried and ground for determining soil organic carbon (SOC) content. Although this method can wash lose some labile or soluble carbon. It has less effect on the total SOC content. The values of SOC, soil total nitrogen (STN) and the C and N contents of plant were measured by an elemental analyzer (Elementar analyser vario Max CN, Germany), with a precision of ≤ 0.01%. Quality assurance of methodologies was checked with a standard soil reference sample (GBW07405) and the precision was better than 5%. Actual SOC contents in the original soil samples should be calibrated because the removal of inorganic C reduces sample mass (Liu et al. 2020).

The weight of soil and plant samples used for isotope analysis depends on the C and N contents of the samples. The ¹³C/¹²C and ¹⁵N/¹⁴N ratios were determined by a stable isotope ratio mass spectrometer (MAT-253 plus, USA) coupled to an elemental analyzer (Li et al. 2021; Wu et al. 2019; Yang et al. 2015). The results were normalized based on the measured values of standards (Vienna Pee Dee Belemnite standard for ¹³C/¹²C, atmospheric N₂ standard for ¹⁵N/¹⁴N) and the δ¹³C and δ¹⁵N values were calculated as follows:
\[ \delta^{13}C \, (\text{‰}) = (R_{\text{sample}} / R_{\text{std}} - 1) \times 10^3 \]

\[ \delta^{15}N \, (\text{‰}) = (R_{\text{sample}} / R_{\text{std}} - 1) \times 10^3 \]

where \( R_{\text{sample}} \) is the ratio of \(^{13}\text{C}^{12}\text{C}\) (or \(^{15}\text{N}^{14}\text{N}\)) of the sample and \( R_{\text{std}} \) is the \(^{13}\text{C}^{12}\text{C}\) of Vienna Pee Dee Belemnite standard or the \(^{15}\text{N}^{14}\text{N}\) of atmospheric \(\text{N}_2\) standard. The precision for the \(\delta^{13}C\) and \(\delta^{15}N\) measurements is 0.1‰ and 0.2‰, respectively.

Statistical analyses

One-way ANOVA analysis with least significant difference (LSD) test was performed to determine the significance of \(\delta^{13}C\) and \(\delta^{15}N\) values in different grassland types at the level of \(p < 0.05\) (Tables 1 and 2). The correlations between the \(\delta^{13}C\) and \(\delta^{15}N\) values and different climate factors and soil properties were determined by linear regression analysis (Fig. 2). A general linear model (GLM) was used to evaluate the combined effects of climate factors (MAT and MAP), soil properties (pH, EC, Clay, SOC, STN and the C/N ratio) and plant community composition on \(\delta^{13}C\) and \(\delta^{15}N\) values in vegetation and surface soil (0–10 cm) (Table 3). Meanwhile, the structural equation model (SEM) was used to test direct and indirect effects of soil properties, climate factors and plant community composition on \(\delta^{13}C\) and \(\delta^{15}N\) values in vegetation and surface soil. This statistical method used a multiple regression approach to explain the interactions and non-linearities (Fig. 3).

Results

Soil physicochemical properties

The grasslands of northern China displayed significant differences in soil physicochemical properties (Table 2). Soil pH in temperate meadow steppe was lower than that in temperate typical steppe and temperate desert steppe, and there was no significant difference among various soil layers of each grassland type. However, soil EC in temperate meadow steppe was significantly higher than that in temperate typical steppe and temperate desert steppe, especially in the surface soils (0–10 cm). SOC and TN contents showed similar distribution trends and decreased with soil depth in each grassland type. At the same time, the values of SOC and TN in temperate meadow steppe showed a decreasing trend along the precipitation gradient in the grasslands of northern China, and were highest in the temperate meadow steppe (Table 2). Meanwhile, the C/N ratio showed the opposite trend and slightly increased with increasing precipitation, especially in surface soil. Besides, soil clay contents in temperate meadow were significantly higher than those in temperate typical steppe and temperate desert steppe.

Changes of \(\delta^{13}C\) in different grasslands

As shown in Table 1, mean plant \(\delta^{13}C\) values increased with the decrease of precipitation and increased from \(-27.55\text{‰}\) in temperate meadow steppe to \(-23.52\text{‰}\) in temperate desert steppe. In each grassland ecosystem, there were no significant difference about soil \(\delta^{13}C\) among various soil

### Table 1  Plant C and N contents and their isotope composition in different grasslands

| Grassland type | C content \(\text{(mg g}^{-1}\) | N content \(\text{(mg g}^{-1}\) | C/N ratio | \(\delta^{13}C\) \(\text{(‰)}\) | \(\delta^{15}N\) \(\text{(‰)}\) |
|---------------|-----------------|-----------------|-----------|-----------------|-----------------|
| TMS \(n=9\)   | \(438 \pm 6^a\) | \(11.7 \pm 0.8^a\) | \(38.2 \pm 2.5^a\) | \(-27.55 \pm 0.49^a\) | \(3.81 \pm 1.32^a\) |
| TTS \(n=56\)  | \(441 \pm 2^b\) | \(20.9 \pm 0.8^b\) | \(23.1 \pm 1.1^b\) | \(-26.39 \pm 0.31^a\) | \(-0.78 \pm 0.34^b\) |
| TDS \(n=20\)  | \(425 \pm 5^b\) | \(24.2 \pm 2.0^b\) | \(19.0 \pm 1.4^b\) | \(-23.52 \pm 0.96^b\) | \(1.22 \pm 0.85^a\) |

\(TMS\) temperate meadow steppe, \(TTS\) temperate typical steppe, \(TDS\) temperate desert steppe. Different letters indicate significant difference among the three grassland types (LSD tests, \(p < 0.05\))
layers. However, there were significant difference in the same layer between different grassland ecosystems ($p < 0.05$) (Table 2). Soil $\delta^{13}$C was lowest in temperate meadow steppe (from $-25.60 \pm 0.66^{\%}$ to $-25.04 \pm 0.56^{\%}$) (Table 2). Compared to temperate meadow steppe, soil $\delta^{13}$C increased significantly by $5.2\%$ and $11.7\%$ in temperate typical steppe and temperate desert steppe in 0–10 cm layer, respectively. Based on GLMs and linear regression analysis (Table 3 and Fig. 2), MAT had significant positive effects on soil $\delta^{13}$C. SOC had significant negative effects on soil $\delta^{13}$C.

Changes of $\delta^{15}$N in different grasslands

There was significant difference in plant $\delta^{15}$N among various grasslands in northern China. Plant $\delta^{15}$N was highest in temperate meadow steppe (mean $3.81^{\%}$), followed by temperate desert (1.22%) and temperate typical steppe ($-0.78^{\%}$) (Table 1). For each grassland, soil $\delta^{15}$N slightly increased with soil depth. In surface soil, soil $\delta^{15}$N showed similar pattern trend with plant $\delta^{15}$N. Compared to temperate meadow steppe, soil $\delta^{15}$N significantly decreased by 13.6% and 27.3% in temperate desert steppe and temperate typical steppe in 0–10 cm layer, respectively (Table 2). Climatic factors, soil texture, and their interactions significantly affected soil $\delta^{15}$N (Fig. 2). The interactions between MAT and MAP on soil $\delta^{15}$N were significant (Table 3).

Relative effects of various environmental factors on ecosystem isotope values

As shown in Fig. 3, SEM analyses indicated that plant C content had positive effects on the plant $\delta^{13}$C values, and MAT, plant $\delta^{13}$C values and the first principal component of SOC and TN had direct effects on soil $\delta^{13}$C values compared to other factors. In addition, MAP exerted a overall effect on soil $\delta^{13}$C values via a negative effect on plant N content. In contrast, MAT and MAP had negative effects on plant $\delta^{15}$N values. MAP, MAT and plant $\delta^{15}$N values had direct effects on soil $\delta^{15}$N values. In addition, MAP could also indirectly affect soil $\delta^{15}$N values via its effects on the first principal component of SOC and TN.

**Discussion**

Factors controlling plant and soil $\delta^{13}$C in grasslands of northern China

Previous studies have revealed strong impacts of land use change, especially grassland degradation,
on the plant and soil δ\textsuperscript{13}C (Li et al. 2021; Yang et al. 2015). However, the patterns and controls of plant and soil δ\textsuperscript{13}C in grasslands of northern China are less well studied. In this study, the lack of response of plant δ\textsuperscript{13}C values to MAP (Table 3) are consistent with previous studies in the humid climate areas (Bai et al. 2012; Feng et al. 2020; Peri et al. 2012). These insensitive responses could be explained by the fact that plant species under aridity condition tend to close their stomata during photosynthesis, which leads to high plant δ\textsuperscript{13}C (Cooper 1988; Wang et al. 2013). The plant δ\textsuperscript{13}C could also be affected by different photosynthetic pathways, where plants with the C\textsubscript{4} photosynthetic pathway commonly have relatively lower δ\textsuperscript{13}C values than those that use the C\textsubscript{3} pathway (Li et al. 2021; Dong et al. 2018). C\textsubscript{4} plants have higher water use efficiency than C\textsubscript{3} plants and are commonly the most dominant species at sites with strong water stress (Feng et al. 2020). Compared to the temperate meadow steppe, more C\textsubscript{4} species (e.g., Artemisia dracunculus and A. anethifolia) were in the temperate typical steppe and temperate desert steppe due to the low precipitation, and due to their higher relative abundance in the community, this may lead to the increase of plant δ\textsuperscript{13}C values in arid regions, especially in temperate desert steppe. Therefore, the declining trend of plant δ\textsuperscript{13}C values at community-level with MAP in our study area mainly results from the shift in the dominant plant functional group from C\textsubscript{4} to C\textsubscript{3}.

Plant δ\textsuperscript{13}C values are also different among various plant species (Zheng and Shangguan 2007). Previous studies indicated that the δ\textsuperscript{13}C values of forbs were significantly higher than those of sedges and herbs (Li et al. 2021; Zheng and Shangguan 2007). Some forbs species are competitive due to their higher water use efficiency under limited soil water availability and the decrease in stomatal conductance (Gebauer et al. 2002). Meanwhile, insufficient CO\textsubscript{2} supply due to the reduction in stomatal conductance leads to higher δ\textsuperscript{13}C values in forbs. Therefore, the decreased percentage of graminoids and sedges along with the decrease water availability from temperate meadow steppe to temperate desert steppe might be the other causes for the increase of plant δ\textsuperscript{13}C values in temperate desert steppe.

In natural ecosystems, soil δ\textsuperscript{13}C values are generally determined by plant litter δ\textsuperscript{13}C signature, which
is further supported by the positive relationship between soil and plant community level δ13C values in our study area (Fig. 2). The variation in vegetation δ13C and soil δ13C was largely explained by the aboveground different plant community composition, suggesting that changes of the plant community composition are important to ecosystem C cycling among different grasslands in northern China. Besides, consistent with results of the Zoige Plateau in China (Zhao et al. 2019), there was a distinct negative relationship between soil δ13C and MAP in our study. Besides, microbial activity would increase with precipitation (MAP), soil pH, EC, SOC, TN, the soil C/N ratio, clay, plant C content and plant N content on plant and soil δ13C and δ15N (at 0–10 cm depth).

Table 3 Summary of the results of the general linear models (GLMs) for temperate grassland of northern China, showing the effects of mean annual temperature (MAT), mean annual precipitation (MAP), soil pH, EC, SOC, TN, the soil C/N ratio, clay, plant C content and plant N content on plant and soil δ13C and δ15N (at 0–10 cm depth)

| Variables | Plant δ13C | Soil δ13C | Plant δ15N | Soil δ15N |
|-----------|------------|-----------|------------|-----------|
|           | MS | SS(%) | p value | MS | SS(%) | p value | MS | SS(%) | p value |
| MAT       | 5.13 | 1.09 | 0.42 | 48.37 | 29.29 | <0.01 | 76.68 | 17.33 | <0.01 | 84.58 | 32.72 | <0.01 |
| MAP       | 18.73 | 3.99 | 0.13 | 4.17 | 2.52 | 0.12 | 113.68 | 25.70 | <0.01 | 41.83 | 16.18 | <0.01 |
| pH        | /   | /   | /   | 1.63 | 0.99 | 0.32 | 13.00 | 2.94 | 0.10 | 3.91 | 1.51 | 0.23 |
| EC        | 8.19 | 1.74 | 0.31 | /   | /   | /   | 1.89 | 0.43 | 0.52 | 0.55 | 0.21 | 0.65 |
| SOC       | /   | /   | /   | 15.88 | 9.62 | <0.01 | /   | /   | /   | 8.17 | 3.16 | 0.08 |
| TN        | 6.97 | 1.49 | 0.35 | 1.68 | 1.02 | 0.32 | /   | /   | /   | /   | /   | /   |
| C/N ratio | 9.75 | 2.08 | 0.27 | 0.27 | 0.16 | 0.69 | 0.45 | 0.10 | 0.75 | 0.43 | 0.17 | 0.69 |
| Clay      | 10.99 | 2.34 | 0.24 | 0.73 | 0.44 | 0.51 | 2.47 | 0.56 | 0.46 | 0.27 | 0.10 | 0.75 |
| Plant C   | 10.50 | 2.24 | 0.25 | /   | /   | /   | 19.44 | 4.40 | 0.04 | /   | /   | /   |
| Plant N   | 5.41 | 1.15 | 0.41 | /   | /   | /   | 0.90 | 0.20 | 0.66 | /   | /   | /   |

Bold values are statistically significant (p < 0.05)
Abbreviations: MS mean squares, SS the proportion of the total variance explained by the variable

Fig. 3 Structural equation model (SEM) of the effects of environmental variables on ecosystem δ13C and δ15N values in temperate grassland of northern China. Note: high P-values associated with χ2 tests indicate good model fit to data, i.e., no significant discrepancies. Solid red and dashed blue lines indicate positive and negative relationships, respectively. Numbers adjacent to lines are the effect size of the relationships (*p < 0.05; **p < 0.01; ***p < 0.001). The priori model of SEM is explained in Figure S1

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increased soil moisture and thus this would result in greater loss of $^{12}$C and result in $^{13}$C enrichment of the remaining SOC (An and Li 2015; Peri et al. 2012). Thus, the accumulation of old SOC with low turnover rates can result in a relatively high soil $\delta^{13}$C value.

The GLM analysis showed that MAP and SOC explained 29.3% and 9.6% of the soil $\delta^{13}$C variance, respectively (Table 3), indicating that MAP is an important factor controlling the soil $\delta^{13}$C variation in grasslands of northern China. Meanwhile, our results indicated that MAP was indirectly influencing soil $\delta^{13}$C values via influencing plant N contents (Fig. 3). This is mainly due to the fact that MAP can regulate the amount of nitrogen absorbed by plants, and then affect the plant C/N ratios, and finally affect soil $\delta^{13}$C values via decomposing $^{12}$C so that the remaining SOC would be enriched in $^{13}$C (Feng et al. 2020). Besides, SEM analyses indicated MAT, plant $\delta^{13}$C values and the first principal component of SOC and TN also had direct effects on soil $\delta^{13}$C values (Fig. 3). These were mainly due to the increasing microbial decomposition rate with the increase of MAT (Li et al. 2021). At the same time, microbes prefer decomposing $^{12}$C so that the remaining SOC would be enriched in $^{13}$C (Feng et al. 2020). More importantly, it demonstrates that soil $\delta^{13}$C values were also controlled by plant inputs and soil C/N ratios (Dong et al. 2018; Shan et al. 2019; Wu et al. 2019).

Factors controlling plant and soil $\delta^{15}$N in grasslands of northern China

Compared to C isotope, the discriminations of N isotope in responded to various climatic factors are more complex (Luo et al. 2018; Feng et al. 2020). Soil $\delta^{15}$N composition typically ranges from -6‰ to 16‰, providing important information about N dynamics in different natural grassland ecosystems (Kahmen and Buchmann 2008; Shan et al. 2019). In our study, soil $\delta^{15}$N overall increased with depth, which was consistent with previous studies (Dong et al. 2018). This phenomenon was mainly attributed to the biogeochemical processes such as mineralization, denitrification, plant uptake and microbial immobilization, etc., which usually preferred lighter isotope ($^{14}$N) and made $^{15}$N enrichment with soil depth in N cycling (An and Li 2015; Li et al. 2021).

Generally, $\delta^{15}$N values for $C_4$ plants are higher than those of $C_3$ plants due to their different mycorrhizal type, preferred N forms and root depth (Feng et al. 2020). Similar to those in global ecosystems (Craine et al. 2010), plant $\delta^{15}$N values in this study were negatively correlated with MAP (Fig. 2), which was mainly attributed to the shift of dominant functional group from $C_4$ to $C_3$ leading to the decline in plant $\delta^{15}$N values as MAP increases. Besides, plant $\delta^{15}$N values in this study were positively correlated with soil $\delta^{15}$N values (Fig. 2), which demonstrated that soil N was dominant N source of plant community in northern China. The decreasing trend of soil $\delta^{15}$N values with increasing MAP is mainly to the result of a relatively more ‘open’ N biogeochemical cycle (that is, both N inputs and outputs are large relative to internal N cycling) in arid environments (Chen et al. 2021; Cheng et al. 2009; Yang et al. 2013). Previous study has indicated that high MAT can accelerate $^{14}$N loss by increasing organic N mineralization and NH$_3$ volatilization, which would result in higher soil $\delta^{15}$N values (Wu et al. 2018). However, there was a significant negative correlation between soil $\delta^{15}$N and MAT in this study (Figs. 2 and 3). The main reason for this phenomenon is that the regions with higher MAT in this study usually have lower MAP and the soil is mainly composed of sand. This will lead to a low mineralization rate of soil organic matter in this area, resulting in the negative correlation between soil $\delta^{15}$N and MAT. Therefore, these various N cycle processes in different grasslands of northern China have systematic effects on soil $\delta^{15}$N composition. At the same time, the GLM analysis showed that MAT and MAP explained 32.7% and 16.2% of the soil $\delta^{15}$N variance, respectively (Table 3), which further indicates that climatic factors are the major factors controlling the soil $\delta^{15}$N variation in grasslands of northern China.

Implications for C and N biogeochemical cycles

Our results have two important implications for understanding the C and N biogeochemical cycles in temperate grasslands. Firstly, plant community composition influences C cycling via changes in the relative abundance of different functional groups (An and Li 2015; Dong et al. 2018). The appearance of more forbs and $C_4$ species in the temperate desert steppe could increase plant and soil $\delta^{13}$C, plant N content and C/N ratio (Chen et al. 2021; Nel et al. 2018; Xia et al. 2021). All of these processes could influence on soil C and N turnover, via changing N mineralization...
rate and soil organic matter decomposition rate. Secondly, our results suggest a relatively more ‘open’ N biogeochemical cycle (that is, both N inputs and outputs are large relative to internal N cycling) in arid environments (Chen et al. 2021; Cheng et al. 2009; Yang et al. 2013), and where plant and soil δ15N values in grasslands of northern China were mainly controlled by MAT and MAP. Taken together, the C and N isotope patterns in our study area provide insights into the effects of plant community composition and climate on C and N biogeochemical cycles in the grasslands of northern China.

Conclusions

Grasslands in northern China play a crucial role in the terrestrial biogeochemical cycles of C and N. In this study, different grassland ecosystems had significant different patterns of plant and soil δ13C and δ15N values. Plant and soil δ13C values increased with the decrease of MAP and the increase of MAT. However, plant and soil δ15N decreased with the increase of MAT and MAP. All of these results were mainly attributed to the microbial decomposition and the shift in plant community composition. Besides, MAT, plant δ13C values and the first principal component of SOC and TN had direct effects on soil δ13C values. MAT and MAP had negative effects on plant δ15N values. MAP, MAT and plant δ15N values had direct effects on soil δ15N values. All of these indicate that climatic factors (MAT and MAP) play important role in regulating the δ13C and δ15N compositions. The results will provide scientific references for future research on C and N biogeochemical cycles in temperate grasslands of northern China.

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