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Climatic, Edaphic and Biotic Controls over Soil $\delta^{13}C$ and $\delta^{15}N$ in Temperate Grasslands

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Abstract: Soils $\delta^{13}C$ and $\delta^{15}N$ are now regarded as useful indicators of nitrogen (N) status and dynamics of soil organic carbon (SOC). Numerous studies have explored the effects of various factors on soils $\delta^{13}C$ and $\delta^{15}N$ in terrestrial ecosystems on different scales, but it remains unclear how co-varying climatic, edaphic and biotic factors independently contribute to the variation in soil $\delta^{13}C$ and $\delta^{15}N$ in temperate grasslands on a large scale. To answer the above question, a large-scale soil collection was carried out along a vegetation transect across the temperate grasslands of Inner Mongolia. We found that mean annual precipitation (MAP) and mean annual temperature (MAT) do not correlate with soil $\delta^{15}N$ along the transect, while soil $\delta^{13}C$ linearly decreased with MAP and MAT. Soil $\delta^{15}N$ logarithmically increased with concentrations of SOC, total N and total P. By comparison, soil $\delta^{13}C$ linearly decreased with SOC, total N and total P. Soil $\delta^{15}N$ logarithmically increased with microbial biomass C and microbial biomass N, while soil $\delta^{13}C$ linearly decreased with microbial biomass C and microbial biomass N. Plant belowground biomass linearly increased with soil $\delta^{15}N$ but decreased with soil $\delta^{13}C$. Soil $\delta^{15}N$ decreased with soil $\delta^{13}C$ along the transect. Multiple linear regressions showed that biotic and edaphic factors such as microbial biomass C and total N exert more effect on soil $\delta^{15}N$, whereas climatic and edaphic factors such as MAT and total P have more impact on soil $\delta^{13}C$. These findings show that soil C and N cycles in temperate grasslands are, to some extent, decoupled and dominantly controlled by different factors. Further investigations should focus on those ecological processes leading to decoupling of C and N cycles in temperate grassland soils.

Keywords: carbon cycling; natural stable isotope abundance; nitrogen cycling; soil organic matter; temperate grassland
1. Introduction

Soil organic matter (SOM) consists of a heterogeneous mixture of substances in various stages of decay, mainly including plant and animal residues, microbial necromass, and new substances synthesized and released by microbes into the soil [1,2]. The global SOM pool in the surface meter stores approximately 1500 Pg carbon (C) [3] and 95 Pg nitrogen (N) [4] as well as other essential elements for plants and microbes. Therefore, SOM is critical for soil quality and ecosystem dynamics [5,6]. At the same time, SOM plays an important role in global climate change because soils could act as a potential sink for C [7,8]. Therefore, a large number of studies have investigated the effects of various climatic, edaphic and biotic factors on the dynamics of C and N in soils to better understand their turnover and SOM destabilization as well as their role in climate change [9–13].

It is difficult to explore the dynamics of SOM by direct measurement of the change in C and N stocks due to their large size [14]. With the rapid development of isotope ratio mass spectrometry [15], the analysis of stable isotope composition of soil C (δ13C) and N (δ15N) has become a powerful tool to explore the stability and dynamics of SOM [12,16–18] and soil development [12,19]. Soils δ13C and δ15N are ideally suited to provide wider insights into C and N cycles in soil ecosystems because they are primarily based on either an isotopic fractionation during microbial degradation and transformation (e.g., ammonification, nitrification and denitrification) or the preferential decomposition of the substrates depleted in 13C and 15N [20]. Generally, older and more microbially-processed SOM is enriched in 13C and 15N compared to less-decomposed substrates [18,21].

Additionally, variation in δ13C and δ15N content of SOM in natural ecosystems is largely controlled by the input of new plant residues and overall isotopic fractionation during microbial decomposition [18]. The signature of δ13C and δ15N in SOM is closely related to vegetation changes and microbial decomposition as well as anthropogenic N input [22–24]. Moreover, climatic and edaphic factors, including temperature, precipitation, pH, and contents of soil C, N and phosphorus (P) as well as soil texture, greatly impact δ13C and δ15N content of SOM [13,25,26]. As a result, the signature of 13C and 15N in SOM can be used as a valid proxy for SOM dynamics and provide integrated information about the ecosystem N cycling [9,27–32].

To understand the factors controlling δ13C and δ15N in SOM, numerous studies have investigated the patterns of soil δ13C and δ15N on regional and global scales [9–11,14,31,33,34]. It has been shown that climate controls forest soil δ13C in the southern Appalachian Mountains [13]. Climate can likewise have an effect on soil δ15N, with values increasing in response to rain events, which enhance the processes that cause the loss of N but discriminate against 15N loss [35]. Further evidence shows that aridity can nonlinearly alter soil δ15N values in arid and semi-arid grasslands [34]. Consequently, soil δ15N values along precipitation gradients can reflect the pattern of N losses relative to turnover [36–38]. On a global scale, soil δ15N converges across climate and latitudinal gradients [11]. In addition to climatic factors, substrate age, soil texture and litter input as well as land-use change also can affect soil δ13C and δ15N [25,31,39,40]. Nonetheless, the controls on C and N isotope ratios in soil still remain unclear [27].

Grasslands are an interesting ecosystem to study in this context because they store large amounts of C and N in soil [41,42] and have great potential to affect CO2 concentrations in the atmosphere. Additionally, grasslands are widely distributed over the world and account for 26% of the ice-free land [43]. As a result, grassland soils play an important role in the context of global climate change and regulate biogeochemical cycles [44]. Among the various types, temperate grasslands are widely distributed across the Eurasian continent and form the Eurasian steppe [45]. Recent studies of temperate grasslands showed that climatic variables control approximately 50% of the variation in soil δ15N along an east–west transect in Northern China. Soil δ15N was found to decrease with increasing mean annual precipitation (MAP) and mean annual temperature (MAT) [10]. Further studies demonstrated that the aridity can nonlinearly alter soil δ15N values [34]. Nonetheless, it remains unclear how co-varying climatic, edaphic and biotic factors control soil δ13C and δ15N in such temperate grasslands. We hypothesize that distinct factors control the soil δ13C versus δ15N signature: (1) biotic factors such as microbial biomass C (MBC) and N (MBN) as well as plant belowground biomass could
exert more impact on soil $^{15}$N than edaphic and climatic factors since $^{15}$N fractionation is largely controlled by biological processes [11]; and (2) climatic and edaphic factors have more effects on soil $\delta^{13}$C than biological factors because water can strongly affect $^{13}$C in plant tissues [46]. To test the above hypotheses, we collected soil and plant samples from temperate meadow steppes, temperate steppes and temperate deserts along a vegetation transect in Inner Mongolia.

2. Materials and Methods

2.1. Study Sites

This study was conducted along a 1280 km transect across Inner Mongolia from west to east in northern China (Table 1). The longitude of the transect ranged from 107°15' to 122°17' and the latitude ranged from 38°44' to 50°12'. The region was characterized predominantly by an arid and semi-arid continental climate. MAP ranged from 154 to 517 mm and MAT ranged from 1 to 4°C. The MAP and MAT of each sampling site were calculated from the NMIC (China National Meteorological Information Center). The main vegetation types across this transect were temperate meadow steppe, temperate steppe, and temperate desert. All the soils were classified as chestnut soil, corresponding to Calcicorthic Aridisol according to USDA Soil Taxonomy [47].

In 2014, a field campaign was conducted to collect soil and plant samples along this transect. In total, 22 sampling sites including six temperate meadow steppes, nine temperate steppes and seven temperate deserts were selected. At each site, four plots (1 m x 1 m) were randomly selected for soil and plant sampling. The aboveground plant parts were harvested for the estimation of aboveground net primary production (ANPP). Additionally, five soil cores (2.5 cm diameter, 5 cm depth) were collected randomly using a soil corer from each plot and mixed thoroughly as one composite sample. Living roots were carefully collected from the soil and washed by water and then dried for the estimation of belowground biomass. Soil samples were sieved through a 2.0 mm sieve and then separated into two parts: one was stored in a plastic bag and frozen at -20°C for measurement of soil moisture, MBC and MBN, and the other was air-dried for measurements under natural conditions.

2.2. Analysis of Soil $\delta^{13}$C and $\delta^{15}$N

Dried soil samples were ground into powder using a ball mill (Retsch MM2; Retsch, Haan, Germany). Approximately 1 g soil was put into 5-ml centrifuge tube. To remove carbonate, 3 ml of 0.5 M HCl was added to the tubes overnight. Afterwards, samples were freeze-dried and washed with H$_2$O until a pH of 7.0 was reached. The soil was weighed into tin capsules for analysis of total N ($N_t$), soil organic C (SOC), $\delta^{13}$C and $\delta^{15}$N by continuous flow gas isotope ratio mass spectrometry (isoprime precisION, Elementar, Germany). The isotope results of soil C or N were calculated as follows: $\delta^{13}$C/$\delta^{15}$N (‰) = (R sample/R standard – 1) * 1000, where R sample and R standard are the ratios of $^{13}$C/$^{12}$C or $^{15}$N/$^{14}$N in the sample and standard. The standards for $\delta^{13}$C and $\delta^{15}$N are Pee Dee Belemnite and atmospheric molecular N, respectively. The standard deviation of repeated measurements of laboratory standards was ±0.15‰ for these isotope analyses.

2.3. Analysis of Soil Properties and Microbial Biomass

Total phosphorus (P$_t$) in the soil was measured using optical emission spectrometry (Optima 5300DV; PerkinElmer, Shelton, USA) after nitric-perchloric acid digestion [48,49]. Soil pH was measured by a dry soil-water ratio of 1:2. Soil MBC and MBN were determined using the chloroform fumigation–extraction method [50,51]. Briefly, 10 g fresh soil was extracted with 24 ml of 0.5 M K$_2$SO$_4$. An additional 10 g soil was fumigated with ethanol-free chloroform for 24 h and then extracted again in the same manner. All extracts were shaken for 1 h and filtered through 5895 paper. Total organic C and N concentrations in the K$_2$SO$_4$ extracts were measured with a Dimatec-100 TOC/TIC analyzer (Liqui TOCII, Elementar, Germany).
| Site Type                      | Latitude | Longitude | Altitude (m) | MAP (mm) | MAT (°C) | g kg⁻¹ | mg kg⁻¹ | Biomass (g m⁻²) | % |
|-------------------------------|----------|-----------|--------------|----------|----------|--------|---------|----------------|---|
| 1 Temperate meadow steppe     | 50°12'   | 119°43'   | 550          | 339.1    | 0.0      | 36.10  | 0.79   | 184.2 ± 29.7    | 6.9 |
| 2 Temperate meadow steppe     | 49°12'   | 120°22'   | 590          | 394.9    | 0.5      | 31.31  | 1.16   | 135.7 ± 29.7    | 6.9 |
| 3 Temperate meadow steppe     | 49°17'   | 119°56'   | 590          | 394.9    | 0.5      | 31.31  | 1.16   | 135.7 ± 29.7    | 6.9 |
| 4 Temperate meadow steppe     | 49°9'    | 119°50'   | 500          | 337.7    | 0.0      | 25.27  | 0.62   | 154.4 ± 21.1    | 6.9 |
| 5 Temperate meadow steppe     | 45°33'   | 117°19'   | 683          | 281.7    | 0.0      | 17.84  | 1.25   | 160.5 ± 20.5    | 6.9 |
| 6 Temperate meadow steppe     | 46°22'   | 122°17'   | 450          | 221.7    | 0.0      | 15.16  | 0.68   | 120.2 ± 16.2    | 6.9 |
| 7 Temperate meadow steppe     | 45°56'   | 119°56'   | 1000         | 228.6    | 0.0      | 12.60  | 0.32   | 129.5 ± 21.7    | 6.9 |
| 8 Temperate meadow steppe     | 43°52'   | 119°22'   | 644          | 340.3    | 0.0      | 19.57  | 0.89   | 147.6 ± 22.0    | 6.9 |
| 9 Temperate meadow steppe     | 43°35'   | 118°09'   | 740          | 377.8    | 0.0      | 2.48   | 0.45   | 20.0 ± 0.9      | 6.9 |
| 10 Temperate meadow steppe    | 43°18'   | 117°11'   | 1296         | 399.1    | 0.0      | 18.06  | 1.30   | 200.3 ± 24.6    | 6.9 |
| 11 Temperate meadow steppe    | 43°43'   | 112°50'   | 993          | 158.8    | 0.0      | 3.21   | 0.16   | 120.2 ± 16.2    | 6.9 |
| 12 Temperate meadow steppe    | 43°12'   | 116°09'   | 1298         | 335.7    | 0.0      | 7.64   | 0.54   | 189.4 ± 21.7    | 6.9 |
| 13 Temperate meadow steppe    | 43°19'   | 119°35'   | 450          | 340.5    | 0.0      | 6.12   | 0.09   | 138.5 ± 21.5    | 6.9 |
| 14 Temperate meadow steppe    | 42°32'   | 118°53'   | 794          | 408.3    | 0.0      | 9.23   | 0.91   | 135.7 ± 22.0    | 6.9 |
| 15 Temperate meadow steppe    | 41°20'   | 112°51'   | 1760         | 373.7    | 0.0      | 10.21  | 0.87   | 157.1 ± 20.4    | 6.9 |
| 16 Temperate desert           | 43°21'   | 111°32'   | 960          | 154.5    | 0.0      | 1.78   | 0.02   | 94.0 ± 26.5     | 6.9 |
| 17 Temperate desert           | 42°56'   | 110°50'   | 1071         | 200.3    | 0.0      | 4.03   | 0.14   | 149.4 ± 21.7    | 6.9 |
| 18 Temperate desert           | 45°25'   | 109°49'   | 1158         | 186.2    | 0.0      | 3.95   | 0.56   | 158.1 ± 21.5    | 6.9 |
| 19 Temperate desert           | 41°54'   | 109°42'   | 1530         | 180.1    | 0.0      | 5.65   | 0.67   | 166.2 ± 21.5    | 6.9 |
| 20 Temperate desert           | 42°01'   | 110°03'   | 1339         | 347.1    | 0.0      | 2.88   | 0.26   | 76.2 ± 13.8     | 6.9 |
| 21 Temperate desert           | 39°14'   | 107°16'   | 1281         | 205.8    | 0.0      | 5.62   | 0.51   | 135.7 ± 22.0    | 6.9 |
| 22 Temperate desert           | 38°44'   | 107°49'   | 1345         | 267.6    | 0.0      | 1.97   | 0.16   | 71.8 ± 22.6     | 6.9 |

The values are means ± standard errors of 4 replicates. MAP = mean annual precipitation, MAT = mean annual temperature, SOC = soil organic carbon, N = nitrogen, P = phosphorus, MBC = microbial biomass C, MBN = microbial biomass N.
2.4. Calculations and Statistics

MBC and MBN were calculated as the difference between the total C or total N content in fumigated and non-fumigated soils, divided by a $k_{EC}$ factor of 0.45 [52] and a $k_{EN}$ factor of 0.54, respectively [50,51].

The standard errors of means are presented in figures and tables as a variability parameter. The normality of soil $^{15}\text{N}$ and $^{13}\text{C}$, as well as, other edaphic and biological data were tested. A one-way analysis of variance was performed with SPSS 21.0 (SPSS Inc., Chicago, IL, USA) to evaluate the effects of grassland type on soil $^{15}\text{N}$ and $^{13}\text{C}$ values. Correlations between soil $^{15}\text{N}$ and $^{13}\text{C}$ and climatic (MAP, MAT), edaphic (SOC, Nt, Pt) and biotic factors (MBC, MBN and belowground biomass) were analyzed with SPSS 21.0 (SPSS Inc., Chicago, IL, USA). To identify how all the factors affect soils $^{15}\text{N}$ and $^{13}\text{C}$, we conducted a data analysis in two steps using R version 3.5.2 (R Development Core Team 2019). The first step was to generate a series of all possible multiple linear models based on the information-theoretic method. To avoid overfitting our models, a Pearson correlation test was conducted to identify and remove highly correlated factors (r > 0.6 or < -0.6, Table 2) within one model. The second step was to calculate estimates and the relative importance of predictors considering changes to the models’ Akaike’s information criterion (AIC) changes of less than 2 (model.avg function in MuMIn package) with the model averaging method [53]. Information-theoretic AIC corrected for small samples sizes (AICc), ΔAIC (difference between AICc of one model and the model with the lowest AICc), and AICc weight (wAICc) were calculated for model ranking. All differences were tested for significance ($P < 0.05$).

Table 2. Pearson’s correlation matrix for raw input variables in explaining change in soil $^{15}\text{N}$ and $^{13}\text{C}$ along the vegetation transect across Inner Mongolian temperate grasslands. The asterisks indicate a significant relationship between variables at $P < 0.05$.

| MAP   | 1.000 |
|-------|-------|
| MAT   | 0.959 * 1.000 |
| SOC   | 0.629 * 0.742 * 1.000 |
| Total N | 0.627 * 0.732 * 0.996 * 1.000 |
| Total P | 0.273 0.320 0.721 * 0.716 * 1.000 |
| pH    | -0.584 * -0.676 * -0.780 * -0.788 -0.402 1.000 |
| MBC   | 0.580 * 0.688 * 0.966 * 0.973 * 0.677 * -0.742 * 1.000 |
| MBN   | 0.626 * 0.675 * 0.867 * 0.870 * 0.575 * -0.579 * 0.919 * 1.000 |
| BB    | 0.677 * 0.697 * 0.781 * 0.802 * 0.602 * -0.670 * 0.771 * 0.677 * 1.000 |

MAP = mean annual precipitation, MAT = mean annual temperature, SOC = soil organic carbon, N = nitrogen, P = phosphorous, MBC = microbial biomass C, MBN = microbial biomass N, BB = belowground biomass.

3. Results

3.1. Climatic, Edaphic and Biotic Factors along the Transect

Along the transect, MAT ranged from 1.2 to 4.5 °C with a mean of 2.6 °C and MAP ranged from 155 to 518 mm with a mean of 308 mm (Table 1). SOC across 22 sites varied from 1.97 to 53.36 g kg$^{-1}$, with the lowest value in temperate desert and the highest value in the temperate meadow steppe (Table 1). N$_t$ ranged from 0.17 to 4.69 g kg$^{-1}$, while P$_t$ varied from 0.1 to 0.9 g kg$^{-1}$. Soil C:N ratios were between 9.0 and 13.0. Soil pH varied from 7.1 to 8.3 (Table 1). MBC ranged from 71.8 to 660.5 mg kg$^{-1}$ and MBN varied from 46.5 to 392.4 mg kg$^{-1}$ (Table 1). Aboveground biomass was in the range of 11.6 to 146.6 g dry weight (d.w.) m$^{-2}$, while belowground biomass varied from 50.9 to 785.6 g d.w. m$^{-2}$ (Table 1). All observed parameters decreased from temperate meadow steppe to temperate steppe to temperate desert along the transect.
3.2. Soil $\delta^{15}$N and $\delta^{13}$C

Soil $\delta^{15}$N cross 22 sites along this transect varied from -3.53‰ to 5.88‰ (Table 1). All seven temperate meadow steppe sites had positive $\delta^{15}$N values while some temperate steppe and temperate desert sites had negative $\delta^{15}$N values. Soil $\delta^{15}$N in temperate meadow steppes (5.12‰) were higher than those in temperate steppes and temperate deserts ($P < 0.05$). By comparison, soil $\delta^{13}$C cross the grassland transect ranged from -27.1‰ to -21.2‰ (Table 1). Soil $\delta^{13}$C values in temperate meadow steppes (-25.7‰) were lower than those in temperate steppes and temperate deserts ($P < 0.05$).

3.3. Correlation Climatic, Edaphic and Biotic Factors with Soil $\delta^{15}$N and $\delta^{13}$C

Along the transect, soil $\delta^{15}$N were not correlated with MAP (Figure 1a) and MAT (Figure 1b). However, soil $\delta^{13}$C linearly decreased with MAP ($R^2 = 0.43$, $P < 0.001$, Figure 1c) and MAT ($R^2 = 0.52$, $P < 0.001$, Figure 1d).

Soil $\delta^{15}$N logarithmically increased with concentrations of SOC ($R^2 = 0.71$, $P < 0.001$, Figure 2a), $N_t$ ($R^2 = 0.71$, $P < 0.001$, Figure 2b) and $P_t$ ($R^2 = 0.51$, $P < 0.001$, Figure 2c). By comparison, soil $\delta^{13}$C linearly decreased with SOC ($R^2 = 0.58$, $P < 0.001$, Figure 2d), $N_t$ ($R^2 = 0.53$, $P < 0.001$, Figure 2e) and $P_t$ ($R^2 = 0.37$, $P < 0.003$, Figure 2f).
Similarly to SOC and N, soil δ¹⁵N logarithmically increased with concentrations of MBC ($R^2 = 0.63$, $P < 0.001$, Figure 3a) and MBN ($R^2 = 0.48$, $P < 0.001$, Figure 3b). Soil δ¹³C linearly decreased with MBC ($R^2 = 0.58$, $P < 0.001$, Figure 3c) and MBN ($R^2 = 0.58$, $P < 0.001$, Figure 3d). Plant belowground biomass linearly increased with Soil δ¹⁵N ($R^2 = 0.37$, $P = 0.004$, Figure 3e), but decreased with soil δ¹³C ($R^2 = 0.41$, $P = 0.002$, Figure 3f). Soil δ¹⁵N decreased with soil δ¹³C along the transect ($R^2 = 0.30$, $P = 0.008$, Figure 4).

Figure 2. Relationships between soil δ¹⁵N and δ¹³C in the upper 5 cm with soil organic carbon (SOC, a, d), total nitrogen (N, b, e) and total phosphorus (P, c, f) at 22 sites along the vegetation transect across Inner Mongolian temperate grasslands.

Figure 3. Relationships between soil δ¹⁵N and δ¹³C in the upper 5 cm with microbial biomass carbon (MBC, a, c) and microbial biomass nitrogen (MBN, b, d) as well as with plant belowground biomass (e, f) at 22 sites along the vegetation transect across Inner Mongolian temperate grasslands.
To answer the above question, a large-scale soil collection across temperate grasslands was carried out across forests. Higher soil δ15N and δ13C values in drier ecosystems reflect a larger loss of mineral N through strongly varying climatic, edaphic and biotic factors control soil δ13C and δ15N in temperate grasslands on a large scale. However, it is still unclear how co-varying climatic, edaphic and biotic factors control soils δ13C and δ15N in temperate grasslands on a large scale. To answer the above question, a large-scale soil collection across temperate grasslands was carried out along a vegetation transect in Inner Mongolia. We found that biological and edaphic factors such as MBC and total N exert more effects on soil δ15N whereas climatic and edaphic factors such as MAT and total P have more impacts on soil δ13C.

The result of model averaging approach after model selection showed that MBC, total N, total P and SOC significantly positively related with soil δ15N. Microbial C, total P, and MAT were three most important factors which significantly negatively affected soil δ13C (Table 3).

Table 3. Relative importance and regression coefficients (given in parenthesis) of microbial biomass carbon (MBC), total nitrogen (N), mean annual precipitation (MAP), total phosphorous (P), soil organic carbon (SOC), and mean annual temperature (MAT) for determining soil δ15N and δ13C values. Values were derived through a model averaging approach. Factors with parameter values highlighted in bold significantly affected soil δ15N and δ13C (P < 0.05).

Table:<br />| Soil δ15N | MBC | Total N | MAP | Total P | SOC | MAT |
|----------|-----|---------|-----|---------|-----|------|
| 0.52 (0.012) | 0.21 (1.398) | 0.20 (-0.007) | 0.16 (8.559) | 0.12 (0.119) |
| Soil δ13C | 0.29 (-0.006) | 0.29 (-0.005) | 0.71 (-3.420) | 0.71 (-1.152) |

4. Discussion

Numerous studies have explored the effects of various factors on soils δ13C and δ15N in terrestrial ecosystems on different scales [9,11,18,27,29,54]. However, it is still unclear how co-varying climatic, edaphic and biotic factors control soils δ13C and δ15N in temperate grasslands on a large scale. To answer the above question, a large-scale soil collection across temperate grasslands was carried out along a vegetation transect in Inner Mongolia. We found that biological and edaphic factors such as MBC and total N exert more effects on soil δ15N whereas climatic and edaphic factors such as MAT and total P have more impacts on soil δ13C.

Other studies have found that variations in soil δ15N values largely depend on isotopic signatures of inputs and outputs, the input–output balance, N transformation and their specific isotope effects [29]. The factors affecting the above-mentioned processes can impact soil δ13C and δ15N signatures. In the current study, soil δ15N values across the transect had positive values, with the exception of two sites. Higher soil δ15N values in drier ecosystems reflect a larger loss of mineral N through strongly
$^{15}$N-discriminating processes, e.g., higher gaseous N losses caused by N-cycling microbes [34,55] and increased N mineralization and nitrification [56]. Numerous studies have suggested that climatic factors control $\delta^{15}$N in soil [10,11,34,57]. In contrast to previous studies, the current study did not demonstrate a clear relationship between $\delta^{15}$N and MAT or MAP [10,11,34]. These different findings could be ascribed to two reasons: (1) The length of our transect (i.e., the scale) was much short than those in both previous studies in the same region [10,34]. (2) As an indicator that integrates many processes affecting the N cycle, controls on soil $\delta^{15}$N are very complicated, including various climatic, edaphic and biological factors [29,32]. Consistently with a previous study on the Chinese Loess Plateau [58], edaphic factors such as SOC, $N_t$ and $P_t$ strongly influenced soil $\delta^{15}$N across our transect. Previous studies demonstrated that soil $\delta^{15}$N increased or decreased with $N_t$ contents [10,11], but we observed a significant positive logarithmic relationship between soil $\delta^{15}$N and $N_t$ along the transect. Among the various factors, MBC, i.e., a biotic factor, played a more important role in controlling the soil $^{15}$N signature than climatic and edaphic factors (Table 2). This reflects that microbial processes are responsible for soil $^{15}$N dynamics across the investigated temperate grassland, supporting our first hypothesis.

Previous studies have shown that soil $^{13}$C signature corresponds similarly to biotic factors, such as plant residue input from litterfall and rhizodeposition, including root mortality and root exudation [59,60]. Over time, dynamics of soil $^{13}$C are therefore largely controlled by C inputs from vegetation and subsequent microbial decomposition [13,18,61]. However, we found that climate and edaphic properties exerted greater control on soil $^{13}$C in the investigated temperate grasslands (Table 2), which is consistent with previous studies demonstrating the importance of climate on soil $^{13}$C [13,62]. Additionally, a previous study also showed that the spatial variation of soil $^{13}$C was related to soil texture in a subtropical lowland woodland [25]. Considering that $^{13}$C can be regarded as an indicator of SOC dynamics, our results suggest that SOC dynamics in temperate grasslands are largely controlled by climatic and edaphic factors since MAP and $P_t$ most dominantly affected soil $^{15}$C values. Therefore, our results confirm our second hypothesis that climatic and edaphic factors have a higher effect on soil $^{13}$C than biological factors. This could be because water is a critical factor limiting growth of plants and microorganisms in these arid and semi-arid temperate grasslands [63,64]. Additionally, $P$ is a key nutrient in temperate grasslands and, together with $N_t$, co-limits plant net primary production and microbial activities [65,66]. Therefore, both precipitation and $P_t$ affect soil $^{13}$C values by altering C input and microbial decomposition. These findings indicate that climatic and edaphic factors should be taken into account in order to better understand SOC dynamics, especially focusing on their roles in the microbial decomposition of plant residues and SOC.

5. Conclusions

In summary, a large-scale soil collection was carried out in temperate grasslands along a vegetation transect in Inner Mongolia to examine how climatic, edaphic and biological factors affect the soil $^{13}$C or $^{15}$N signature. Along the transect, soil $^{15}$N was not correlated with MAP and MAT, while soil $^{13}$C linearly decreased with MAP and MAT. Soil $^{15}$N logarithmically increased with concentrations of SOC, $N_t$ and $P_t$, but soil $^{13}$C linearly decreased with concentrations of SOC, $N_t$ and $P_t$. Similarly, soil $^{15}$N logarithmically increased with MBC and MBN, while soil $^{13}$C linearly decreased with MBC and MBN. Soil $^{15}$N linearly increased with plant belowground biomass, but soil $^{13}$C decreased with plant belowground biomass. Multiple linear regressions showed that MBC especially, but also $N_t$ to a lesser extent, affect soil $^{15}$N, while MAT and $P_t$ have more impact on soil $^{13}$C. Thus, biotic factors controlled soil $^{15}$N signature most dominantly while climatic and edaphic factors exerted greater control on soil $^{13}$C signature. These results indicate that soil C and N cycles are to some extent decoupled in these temperate grasslands. Further investigations should focus on those ecological processes leading to decoupling of C and N cycles in temperate grassland soils for a better understanding of SOC dynamics [12].
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