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

Xing Zhao [1], Xingliang Xu [2,3,*], Fang Wang [1], Isabel Greenberg [4], Min Liu [1,2], Rongxiao Che [5], Li Zhang [6] and Xiaoyong Cui [1,3,7,*]

1   University of Chinese Academy of Sciences, Beijing 100049, China; zhaoxing@ucas.edu.cn (X.Z.); wangfang13@mails.ucas.edu.cn (F.W.); lium.16s@igsnrr.ac.cn (M.L.)
2   Key Laboratory of Ecosystem Network Observation and Modeling, Institute of Geographic Sciences and Natural Resources Research, Chinese Academy of Sciences, 11A, Datun Road, Chaoyang District, Beijing 100101, China
3   CAS Center for Excellence in Tibetan Plateau Earth Sciences, Chinese Academy of Sciences, Beijing 100101, China
4   Department of Environmental Chemistry, University of Kassel, Nordbahnhofstrasse 1a, 37213 Witzenhausen, Germany; isabel.greenberg@uni-kassel.de
5   Institute of International Rivers and Eco-Security, Yunnan University, Kunming 650500, China; cherongxiao@ynu.edu.cn
6   Ministry of Education Key Laboratory for Biodiversity Science and Ecological Engineering, Coastal Ecosystems Research Station of the Yangtze River Estuary, Shanghai Institute of Eco-Chongming, School of Life Sciences, Fudan University, 2005 Songhu Road, Shanghai 200438, China; zhangl1222@outlook.com
7   Yanshan Eco-Environmental Observatory, Chinese Academy of Sciences, Beijing 101408, China
*   Correspondence: xuxingl@hotmail.com (X.X.); cuixy@ucas.edu.cn (X.C.)

Abstract: Soils δ^{13}C and δ^{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 δ^{13}C and δ^{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 δ^{13}C and δ^{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 δ^{15}N along the transect, while soil δ^{13}C linearly decreased with MAP and MAT. Soil δ^{15}N logarithmically increased with concentrations of SOC, total N and total P. By comparison, soil δ^{13}C linearly decreased with SOC, total N and total P. Soil δ^{15}N logarithmically increased with microbial biomass C and microbial biomass N, while soil δ^{13}C linearly decreased with microbial biomass C and microbial biomass N. Plant belowground biomass linearly increased with soil δ^{15}N but decreased with soil δ^{13}C. Soil δ^{15}N decreased with soil δ^{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 δ^{15}N, whereas climatic and edaphic factors such as MAT and total P have more impact on soil δ^{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 ($\delta^{13}C$) and N ($\delta^{15}N$) has become a powerful tool to explore the stability and dynamics of SOM [12,16–18] and soil development [12,19]. Soils $\delta^{13}C$ and $\delta^{15}N$ 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 ^{13}C and ^{15}N [20]. Generally, older and more microbially-processed SOM is enriched in ^{13}C and ^{15}N compared to less-decomposed substrates [18,21].

Additionally, variation in $\delta^{13}C$ and $\delta^{15}N$ 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 $\delta^{13}C$ and $\delta^{15}N$ 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 $\delta^{13}C$ and $\delta^{15}N$ content of SOM [13,25,26]. As a result, the signature of ^{13}C and ^{15}N 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 $\delta^{13}C$ and $\delta^{15}N$ in SOM, numerous studies have investigated the patterns of soil $\delta^{13}C$ and $\delta^{15}N$ on regional and global scales [9–11,14,31,33,34]. It has been shown that climate controls forest soil $\delta^{13}C$ in the southern Appalachian Mountains [13]. Climate can likewise have an effect on soil $\delta^{15}N$, with values increasing in response to rain events, which enhance the processes that cause the loss of N but discriminate against ^{15}N loss [35]. Further evidence shows that aridity can nonlinearly alter soil $\delta^{15}N$ values in arid and semi-arid grasslands [34]. Consequently, soil $\delta^{15}N$ values along precipitation gradients can reflect the pattern of N losses relative to turnover [36–38]. On a global scale, soil $\delta^{15}N$ 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 $\delta^{13}C$ and $\delta^{15}N$ [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 CO_2 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 $\delta^{15}N$ along an east–west transect in Northern China. Soil $\delta^{15}N$ 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 $\delta^{15}N$ values [34]. Nonetheless, it remains unclear how co-varying climatic, edaphic and biotic factors control soil $\delta^{13}C$ and $\delta^{15}N$ in such temperate grasslands. We hypothesize that distinct factors control the soil $\delta^{13}C$ versus $\delta^{15}N$ 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 δ^{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 × 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 δ^{13}C and δ^{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_2O 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), δ^{13}C and δ^{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: δ^{13}C/δ^{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 δ^{13}C and δ^{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_2SO_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_2SO_4 extracts were measured with a Dimatec-100 TOC/TIC analyzer (Liqui TOCII, Elementar, Germany).

Table 1. Detailed information about climatic, edaphic and biotic factors at 22 sampling sites along the vegetation transect across Inner Mongolian temperate grasslands.

Site	Type	Latitude	Longitude	Altitude (m)	MAP (mm)	MAT (°C)	g kg⁻¹			mg kg⁻¹			Biomass (g m⁻²)		‰	
							SOC	Total N	Total P	MBC	MBN	soil pH	Aboveground	Belowground	$\delta^{13}C$	$\delta^{15}N$
1	Temperate meadow steppe	50°12′	119°43′	550	339.1	3.0	36.10 ± 0.79	3.36 ± 0.08	0.9	422.9 ± 89.0	135.7 ± 29.7	6.9	26.9 ± 3.4	785.6 ± 64.8	-25.3 ± 0.1	6.3 ± 0.6
2	Temperate meadow steppe	49°12′	120°22′	590	394.9	3.5	31.30 ± 1.16	2.82 ± 0.13	0.5	506.2 ± 56.8	244.5 ± 115.9	7.3	55.7 ± 15.5	388.2 ± 20.2	-26.8 ± 0.1	5.6 ± 0.2
3	Temperate meadow steppe	49°17′	119°56′	590	337.7	3.2	29.77 ± 0.12	2.76 ± 0.11	0.5	467.8 ± 41.7	154.4 ± 21.1	—	37.4 ± 22.9	537.7 ± 92.9	-25.1 ± 0.2	5.3 ± 0.4
4	Temperate meadow steppe	49°9′	119°50′	500	337.7	3.3	34.64 ± 0.89	2.96 ± 0.09	0.6	384.3 ± 52.9	145.7 ± 20.5	7.1	42.6 ± 52.0	287.3 ± 97.1	-25.7 ± 0.0	5.9 ± 0.2
5	Temperate meadow steppe	49°33′″	117°19′	683	281.7	2.5	17.84 ± 1.55	1.88 ± 0.15	0.5	353.4 ± 47.7	182.5 ± 51.5	8.0	146.6 ± 42.0	439.6 ± 117.8	-24.2 ± 0.3	3.8 ± 0.3
6	Temperate meadow steppe	48°22′	122°17′	450	517.5	4.4	53.36 ± 0.66	4.69 ± 0.10	0.8	660.7 ± 50.2	392.4 ± 63.7	7.2	66.7 ± 14.0	680.7 ± 170.6	-27.1 ± 0.1	3.8 ± 0.6
7	Temperate steppe	43°96′	115°86′	1000	282.6	2.4	12.60 ± 0.32	1.37 ± 0.03	0.4	249.4 ± 57.3	105.8 ± 14.3	7.5	125.9 ± 63.7	268.4 ± 183.3	-23.0 ± 0.4	3.9 ± 0.6
8	Temperate steppe	43°52′	119°22′	644	349.5	3.1	19.57 ± 0.99	1.90 ± 0.11	0.4	273.4 ± 19.6	95.4 ± 14.0	7.3	20.0 ± 0.9	147.6 ± 72.0	-22.7 ± 0.4	2.0 ± 0.5
9	Temperate steppe	43°15′	118°09′	749	377.8	3.0	2.48 ± 0.45	0.27 ± 0.04	0.2	95.9 ± 63.0	68.1 ± 50.4	7.3	70.0 ± 16.0	215.3 ± 168.3	-23.8 ± 0.6	-3.5 ± 3.7
10	Temperate steppe	43°15′	117°11′	1296	399.1	3.3	18.06 ± 1.30	1.85 ± 0.11	0.3	333.7 ± 55.2	180.1 ± 21.7	7.2	62.2 ± 12.5	386.5 ± 71.6	-24.6 ± 0.6	2.1 ± 0.7
11	Temperate steppe	43°43′	112°50′	993	158.8	1.5	3.21 ± 0.19	0.36 ± 0.03	0.5	147.7 ± 10.5	65.4 ± 16.4	8.1	25.4 ± 4.0	143.0 ± 132.0	-21.4 ± 0.7	0.0 ± 2.1
12	Temperate steppe	43°12′	116°09′	1298	335.7	2.8	7.64 ± 0.54	0.72 ± 0.03	0.3	189.4 ± 13.6	79.5 ± 10.6	7.8	31.8 ± 5.1	372.0 ± 62.2	-24.8 ± 0.6	2.6 ± 1.3
13	Temperate steppe	43°19′	119°35′	453	349.5	3.0	6.12 ± 0.09	0.73 ± 0.03	0.1	138.5 ± 35.0	116.5 ± 24.8	8.1	11.0 ± 2.3	345.8 ± 196.8	-21.2 ± 0.3	0.9 ± 0.9
14	Temperate steppe	42°32′	118°53′	794	408.3	3.1	9.23 ± 0.91	1.02 ± 0.09	0.2	157.1 ± 40.1	53.2 ± 12.0	7.9	77.2 ± 46.3	407.1 ± 206.4	-22.9 ± 0.6	1.3 ± 0.7
15	Temperate steppe	41°20′	112°51′	1760	373.7	2.6	10.21 ± 0.50	1.07 ± 0.04	0.7	218.9 ± 54.7	106.7 ± 20.1	8.1	35.5 ± 9.1	324.1 ± 38.5	-24.6 ± 0.3	5.0 ± 1.4
16	Temperate desert	43°21′	111°52′	960	154.5	1.2	1.78 ± 0.12	0.20 ± 0.02	0.3	94.0 ± 26.3	55.4 ± 26.7	8.1	7.8 ± 3.5	78.0 ± 37.9	-22.6 ± 0.7	-1.7 ± 2.4
17	Temperate desert	42°56′	110°50′	1071	200.3	1.3	4.03 ± 0.40	0.55 ± 0.05	0.4	149.4 ± 22.5	71.2 ± 13.5	8.1	6.5 ± 5.7	106.4 ± 95.5	-21.5 ± 0.8	1.9 ± 1.8
18	Temperate desert	42°25′	109°49′	1158	188.2	1.4	3.95 ± 0.58	0.56 ± 0.07	0.3	158.1 ± 10.8	102.0 ± 52.3	8.3	19.6 ± 8.8	118.1 ± 64.8	-22.3 ± 0.4	3.1 ± 1.1
19	Temperate desert	41°54′	108°42′	1533	180.1	1.5	5.65 ± 0.24	0.67 ± 0.04	0.4	166.2 ± 15.4	84.1 ± 13.4	8.0	11.6 ± 3.2	179.0 ± 187.0	-23.6 ± 0.3	3.4 ± 1.5
20	Temperate desert	40°01′	110°03′	1339	347.1	2.8	2.88 ± 0.10	0.26 ± 0.02	0.4	76.2 ± 13.8	46.5 ± 16.0	8.3	31.5 ± 9.2	205.8 ± 92.0	-23.9 ± 0.6	0.4 ± 1.7
21	Temperate desert	39°14′	107°16′	1281	205.8	2.0	5.62 ± 0.55	0.51 ± 0.04	0.3	155.7 ± 14.3	76.8 ± 18.2	8.1	22.6 ± 5.4	58.5 ± 27.3	-22.7 ± 0.2	2.6 ± 0.9
22	Temperate desert	38°44′	107°45′	1345	267.6	2.4	1.97 ± 0.16	0.17 ± 0.02	0.4	71.8 ± 22.6	46.5 ± 24.8	8.7	87.4 ± 85.1	50.9 ± 44.9	-24.1 ± 0.6	0.3 ± 3.4

The values are means ± standard errors of 4 replicates. MAP = mean annual precipitation, MAT = mean annual temperature, SOC = soil organic carbon, N = nitrogen, P = phosphorous, 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 $\delta^{15}N$ and $\delta^{13}C$, 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 $\delta^{15}N$ and $\delta^{13}C$ values. Correlations between soil $\delta^{15}N$ and $\delta^{13}C$ 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 $\delta^{15}N$ and $\delta^{13}C$, 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}N and ^{13}C along the vegetation transect across Inner Mongolian temperate grasslands. The asterisks indicate a significant relationship between variables at $P < 0.05$.

	MAP	MAT	SOC	Total N	Total P	pH	MBC	MBN	BB
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 δ^{15}N and δ^{13}C

Soil δ^{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 δ^{15}N values while some temperate steppe and temperate desert sites had negative δ^{15}N values. Soil δ^{15}N in temperate meadow steppes (5.12‰) were higher than those in temperate steppes and temperate deserts ($P < 0.05$). By comparison, soil δ^{13}C cross the grassland transect ranged from −27.1‰ to −21.2‰ (Table 1). Soil δ^{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 δ^{15}N and δ^{13}C

Along the transect, soil δ^{15}N were not correlated with MAP (Figure 1a) and MAT (Figure 1b). However, soil δ^{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).
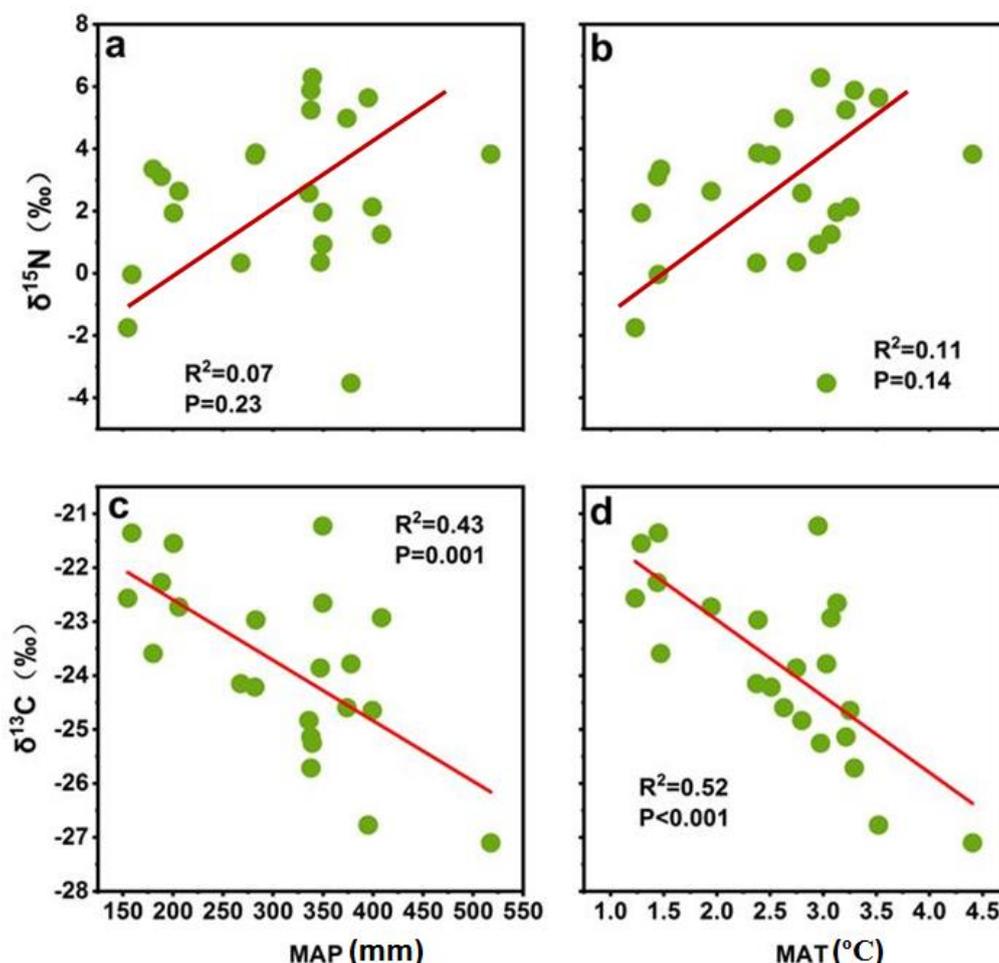
Figure 1. Relationships between soil δ^{15}N and δ^{13}C in the upper 5 cm with mean annual precipitation (MAP, **a**, **c**) and mean annual temperature (MAT, **b**, **d**) at 22 sites along the vegetation transect across Inner Mongolian temperate grasslands.

Soil δ^{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 δ^{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).
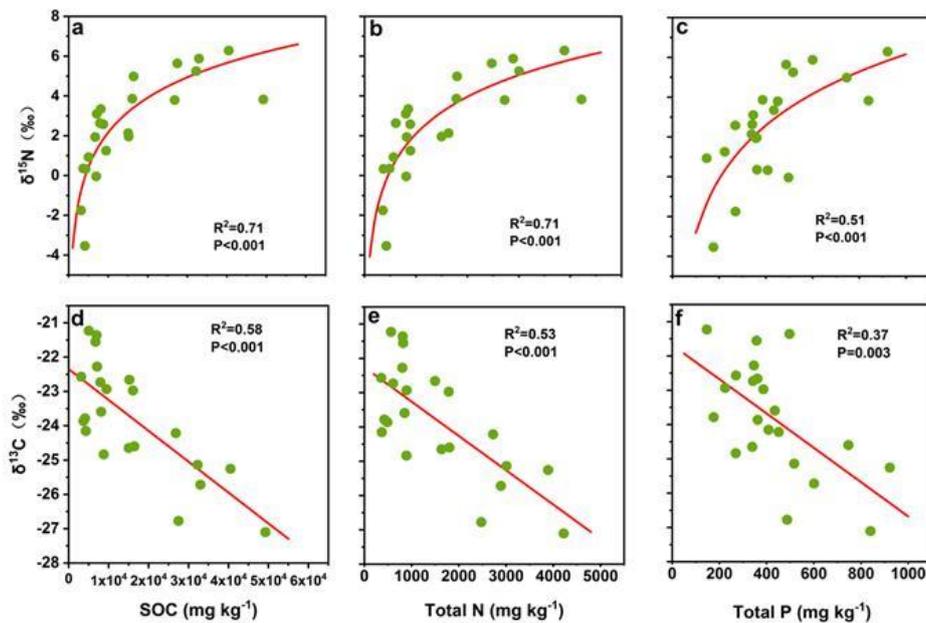
Figure 2. Relationships between soil δ^{15}N and δ^{13}C in the upper 5 cm with soil organic carbon (SOC, **a**, **d**), total nitrogen (N, **b**, **e**) and total phosphorus (P, **c**, **f**) at 22 sites along the vegetation transect across Inner Mongolian temperate grasslands.

Similarly to SOC and N_t, soil δ^{15}N 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 δ^{13}C 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 δ^{15}N ($R^2 = 0.37$, $P = 0.004$, Figure 3e), but decreased with soil δ^{13}C ($R^2 = 0.41$, $P = 0.002$, Figure 3f). Soil δ^{15}N decreased with soil δ^{13}C along the transect ($R^2 = 0.30$, $P = 0.008$, Figure 4).
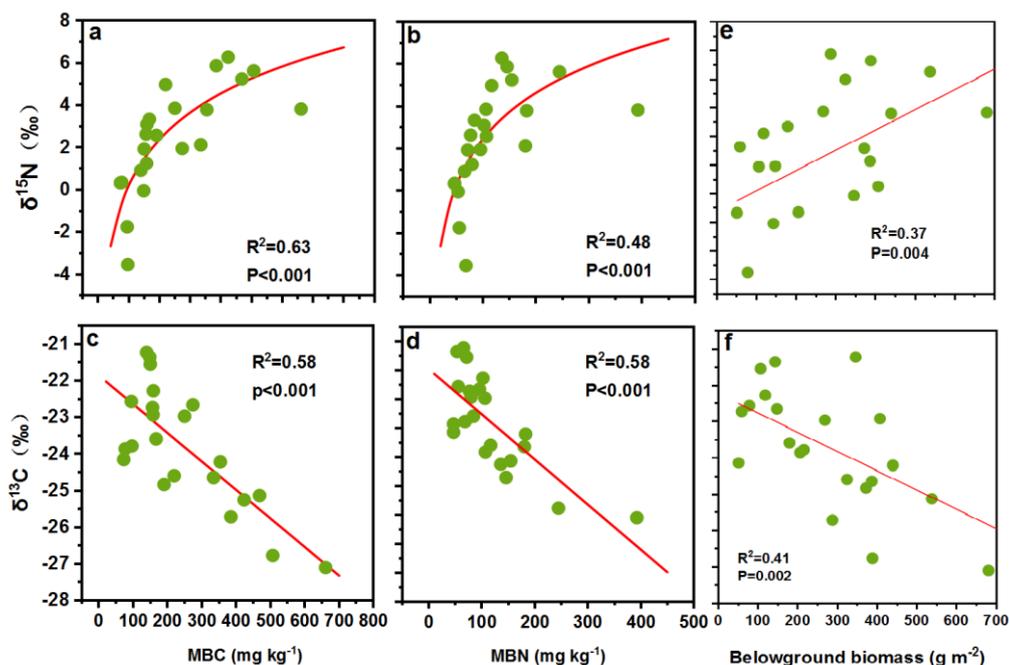
Figure 3. Relationships between soil δ^{15}N and δ^{13}C in the upper 5 cm with microbial biomass carbon (MBC, **a**, **c**) and microbial biomass nitrogen (MBN, **b**, **d**) as well as with plant belowground biomass (**e**, **f**) at 22 sites along the vegetation transect across Inner Mongolian temperate grasslands.
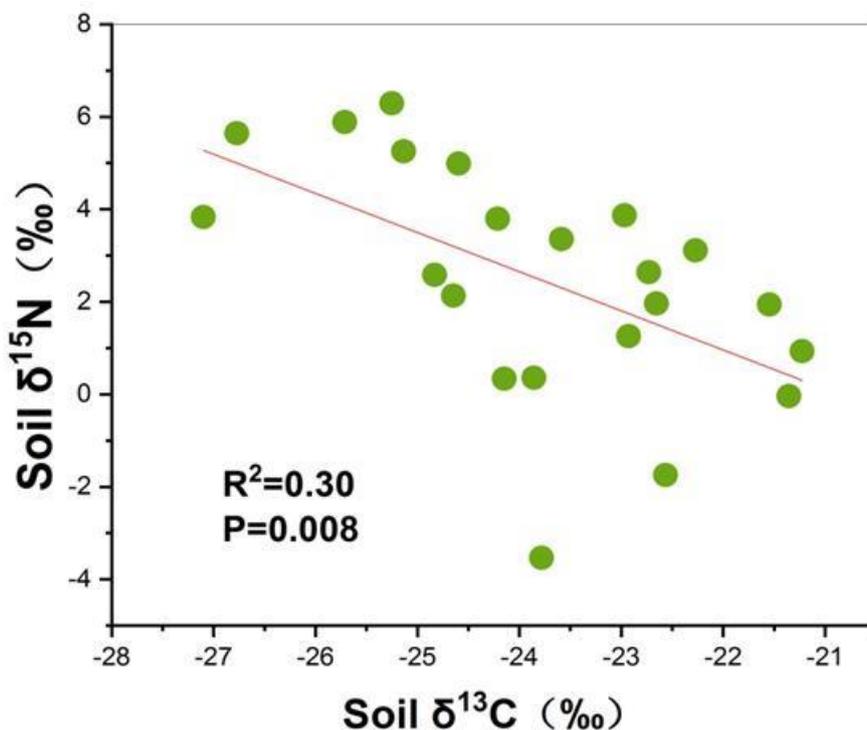
Figure 4. Relationships between soil δ^{15}N and δ^{13}C in the upper 5 cm at 22 sites along a vegetation transect across Inner Mongolian temperate grasslands.

The result of model averaging approach after model selection showed that MBC, total N, total P and SOC significantly positive related with soil δ^{15}N. Microbial C, total P, and MAT were three most important factors which significantly negatively affected soil δ^{13}C (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 ^{15}N and ^{13}C values. Values were derived through a model averaging approach. Factors with parameter values highlighted in bold significantly affected soil ^{15}N and ^{13}C ($P < 0.05$).

	MBC	Total N	MAP	Total P	SOC	MAT
Soil ^{15}N	**0.52** (0.012)	**0.21** (1.398)	0.20 (−0.007)	**0.16** (8.559)	**0.12** (0.119)	
Soil ^{13}C		**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 δ^{13}C and δ^{15}N 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 δ^{13}C and δ^{15}N 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 δ^{15}N whereas climatic and edaphic factors such as MAT and total P have more impacts on soil δ^{13}C.

Other studies have found that variations in soil δ^{15}N 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 δ^{13}C and δ^{15}N signatures. In the current study, soil ^{15}N values across the transect had positive values, with the exception of two sites. Higher soil δ^{15}N values in drier ecosystems reflects 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 δ^{15}N in soil [10,11,34,57]. In contrast to previous studies, the current study did not demonstrate a clear relationship between δ^{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 δ^{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 δ^{15}N across our transect. Previous studies demonstrated that soil δ^{15}N increased or decreased with N_t contents [10,11], but we observed a significant positive logarithmic relationship between soil δ^{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 δ^{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 δ^{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 δ^{13}C [13,62]. Additionally, a previous study also showed that the spatial variation of soil δ^{13}C was related to soil texture in a subtropical lowland woodland [25]. Considering that δ^{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 ^{13}C values. Therefore, our results confirm our second hypothesis that climatic and edaphic factors have a higher effect on soil δ^{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, 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 δ^{13}C or δ^{15}N signature. Along the transect, soil δ^{15}N was not correlated with MAP and MAT, while soil δ^{13}C linearly decreased with MAP and MAT. Soil δ^{15}N logarithmically increased with concentrations of SOC, N_t and P_t, but soil δ^{13}C linearly decreased with concentrations of SOC, N_t and P_t. Similarly, soil δ^{15}N logarithmically increased with MBC and MBN, while soil δ^{13}C linearly decreased with MBC and MBN. Soil δ^{15}N linearly increased with plant belowground biomass, but soil δ^{13}C decreased with plant belowground biomass. Multiple linear regressions showed that MBC especially, but also N_t to a lesser extent, affect soil δ^{15}N, while MAT and P_t have more impact on soil δ^{13}C. Thus, biotic factors controlled soil ^{15}N signature most dominantly while climatic and edaphic factors exerted greater control on soil δ^{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].

References

1. Miltner, A.; Bombach, P.; Schmidt-Bruecken, B.; Kaestner, M. SOM genesis: Microbial biomass as a significant source. *Biogeochemistry* **2012**, *111*, 41–55. [CrossRef]

2. Schnitzer, M. A lifetime perspective on the chemistry of soil organic matter. *Adv. Agron.* **1999**, *68*, 1–58. [CrossRef]

3. Batjes, N.H. Total carbon and nitrogen in the soils of the world. *Eur. J. Soil Sci.* **1996**, *47*, 151–163. [CrossRef]

4. Post, W.M.; Pastor, J.; Zinke, P.J.; Stangenberger, A.G. Global patterns of soil nitrogen storage. *Nature* **1985**, *317*, 613–616. [CrossRef]

5. Janzen, H.H.; Campbell, C.A.; Ellert, B.H.; Bremer, E. Soil organic matter dynamics and their relationship to soil quality. *Dev. Soil Sci.* **1997**, *25*, 277–291. [CrossRef]

6. Schmidt, M.W.I.; Torn, M.S.; Abiven, S.; Dittmar, T.; Guggenberger, G.; Janssens, I.A.; Kleber, M.; Kögel-Knabner, I.; Lehmann, J.; Manning, D.A.C.; et al. Persistence of soil organic matter as an ecosystem property. *Nature* **2011**, *478*, 49–56. [CrossRef]

7. Goh, K.M. Carbon sequestration and stabilization in soils: Implications for soil productivity and climate change. *Soil Sci. Plant Nutr.* **2004**, *50*, 467–476. [CrossRef]

8. Lal, R. Managing soils and ecosystems for mitigating anthropogenic carbon emissions and advancing global food Security. *Bioscience* **2010**, *60*, 708–721. [CrossRef]

9. Amundson, R.; Austin, A.T.; Schuur, E.A.G.; Yoo, K.; Matzek, V.; Kendall, C.; Uebersax, A.; Brenner, D.L.; Baisden, W.T. Global patterns of the isotopic composition of soil and plant nitrogen. *Glob. Biogeochem. Cycles* **2003**, *17*, 1031. [CrossRef]

10. Cheng, W.X.; Chen, Q.S.; Xu, Y.Q.; Han, X.G.; Li, L.H. Climate and ecosystem ^{15}N natural abundance along a transect of Inner Mongolian grasslands: Contrasting regional patterns and global patterns. *Glob. Biogeochem. Cycles* **2009**, *23*, GB2005. [CrossRef]

11. Craine, J.M.; Elmore, A.J.; Wang, L.; Augusto, L.; Baisden, W.T.; Brookshire, E.N.J.; Cramer, M.D.; Hasselquist, N.J.; Hobbie, E.A.; Kahmen, A.; et al. Convergence of soil nitrogen isotopes across global climate gradients. *Sci. Rep.* **2015**, *5*, 8280. [CrossRef]

12. Ehleringer, J.R.; Buchmann, N.; Flanagan, L.B. Carbon isotope ratios in belowground carbon cycle processes. *Ecol. Appl.* **2000**, *10*, 412–422. [CrossRef]

13. Garten, C.T.; Cooper, L.W.; Post, W.M.; Hanson, P.J. Climate controls on forest soil C isotope ratios in the southern appalachian mountains. *Ecology* **2000**, *81*, 1108–1119. [CrossRef]

14. Wang, C.; Houlton, B.Z.; Liu, D.; Hou, J.; Cheng, W.; Bai, E. Stable isotopic constraints on global soil organic carbon turnover. *Biogeosciences* **2018**, *15*, 987–995. [CrossRef]

15. *Stable Isotopes in Ecology and Environmental Science*, 2nd ed.; Michener, R.H.; Lajtha, K. (Eds.) Blackwell Publishing Ltd.: Oxford, UK, 2007; ISBN 978-1-4051-2680-9.

16. Bernoux, M.; Cerri, C.C.; Neill, C.; de Moraes, J.F.L. The use of stable carbon isotopes for estimating soil organic matter turnover rates. *Geoderma* **1998**, *82*, 43–58. [CrossRef]

17. Conen, F.; Zimmermann, M.; Leifeld, J.; Seth, B.; Alewell, C. Relative stability of soil carbon revealed by shifts in δ^{15}N and C:N ratio. *Biogeosciences* **2008**, *5*, 123–128. [CrossRef]

18. Natelhoffer, K.J.; Fry, B. Controls on natural ^{15}N and ^{13}C abundances in forest soil organic matter. *Soil Sci. Soc. Am. J.* **1988**, *52*, 1633–1640. [CrossRef]

19. Bai, E.; Boutton, T.W.; Liu, F.; Wu, X.B.; Archer, S.R. Spatial patterns of soil δ^{13}C reveal grassland-to-woodland successional processes. *Org. Geochem.* **2012**, *42*, 1512–1518. [CrossRef]

20. Balesdent, J.; Mariotti, A. Measurement of soil organic matter turnover using ^{13}C natural abundance. In *Mass Spectrometry of Soil*; Boutton, T.W., Yamasaki, S.I., Eds.; Marcel Dekker, Inc.: New York, NY, USA, 1996; pp. 83–111. ISBN 0-8247-9699-3.

21. Kramer, M.G.; Sollins, P.; Sletten, R.S.; Swart, P.K. N isotope fractionation and measures of organic matter alteration during decomposition. *Ecology* **2003**, *84*, 2021–2025. [CrossRef]

22. Biedenbender, S.H.; McClaran, M.P.; Quade, J.; Weltz, M.A. Landscape patterns of vegetation change indicated by soil carbon isotope composition. *Geoderma* **2004**, *119*, 69–83. [CrossRef]

23. Mayor, J.R.; Wright, S.J.; Schuur, E.A.; Brooks, M.E.; Turner, B.L. Stable nitrogen isotope patterns of trees and soils altered by long-term nitrogen and phosphorus addition to a lowland tropical rainforest. *Biogeochemistry* **2014**, *119*, 293–306. [CrossRef]

24. Stevenson, B.A.; Parfitt, R.L.; Schipper, L.A.; Baisden, W.T.; Mudge, P. Relationship between soil δ^{15}N, C/N and N losses across land uses in New Zealand. *Agric. Ecosyst. Environ.* **2010**, *139*, 736–741. [CrossRef]

25. Bai, E.; Boutton, T.W.; Liu, F.; Ben Wu, X.; Hallmark, C.T.; Archer, S.R. Spatial variation of soil δ^{13}C and its relation to carbon input and soil texture in a subtropical lowland woodland. *Soil Biol. Biochem.* **2012**, *44*, 102–112. [CrossRef]

26. Wynn, J.G.; Bird, M.I. Environmental controls on the stable carbon isotopic composition of soil organic carbon: Implications for modelling the distribution of C$_3$ and C$_4$ plants, Australia. *Tellus* **2008**, *60*, 604–621. [CrossRef]

27. Craine, J.M.; Brookshire, E.N.J.; Cramer, M.D.; Hasselquist, N.J.; Koba, K.; Marin-Spiotta, E.; Wang, L. Ecological interpretations of nitrogen isotope ratios of terrestrial plants and soils. *Plant Soil* **2015**, *396*, 1–26. [CrossRef]

28. Dawson, T.E.; Mambelli, S.; Plamboeck, A.H.; Templer, P.H.; Tu, K.P. Stable isotopes in plant ecology. *Annu. Rev. Ecol. Syst.* **2002**, *33*, 507–559. [CrossRef]

29. Högberg, P. ^{15}N natural abundance in soil-plant systems. *New Phytol.* **1997**, *137*, 179–203. [CrossRef]

30. Makarov, M.I. The nitrogen isotopic composition in soils and plants: Its use in environmental studies (a review). *Eurasian Soil Sci.* **2009**, *42*, 1335–1347. [CrossRef]

31. Pardo, L.H.; Nadelhoffer, K.J. Using nitrogen isotope ratios to assess terrestrial ecosystems at regional and global scales. In *Isoscapes: Understanding Movement, Pattern, and Process on Earth through Isotope Mapping*; West, J.B., Bowen, G.J., Dawson, T.E., Tu, K.P., Eds.; Springer: New York, NY, USA, 2010; pp. 221–249. ISBN 978-90-481-3353-6.

32. Robinson, D. δ^{15}N as an integrator of the nitrogen cycle. *Trends Ecol. Evol.* **2001**, *16*, 153–162. [CrossRef]

33. Powers, J.S.; Veldkamp, E. Regional variation in soil carbon and δ^{13}C in forests and pastures of northeastern Costa Rica. *Biogeochemistry* **2005**, *72*, 315–336. [CrossRef]

34. Wang, C.; Wang, X.; Liu, D.; Wu, H.; Lu, X.; Fang, Y.; Cheng, W.; Luo, W.; Jiang, P.; Shi, J.; et al. Aridity threshold in controlling ecosystem nitrogen cycling in arid and semi-arid grasslands. *Nat. Commun.* **2014**, *5*, 4799. [CrossRef]

35. Handley, L.L.; Raven, J.A. The use of natural abundance of nitrogen isotopes in plant physiology and ecology. *Plant Cell Environ.* **1992**, *15*, 965–985. [CrossRef]

36. Aranibar, J.N.; Otter, L.; Macko, S.A.; Feral, C.J.W.; Epstein, H.E.; Dowty, P.R.; Eckardt, F.; Shugart, H.H.; Swap, R.J. Nitrogen cycling in the soil-plant system along a precipitation gradient in the Kalahari sands. *Glob. Chang. Biol.* **2004**, *10*, 359–373. [CrossRef]

37. Austin, A.T.; Vitousek, P.M. Nutrient dynamics on a precipitation gradient in Hawai'i. *Oecologia* **1998**, *113*, 519–529. [CrossRef]

38. Handley, L.L.; Austin, A.T.; Robinson, D.; Scrimgeour, C.M.; Raven, J.A.; Heaton, T.H.E.; Schmidt, S.; Stewart, G.R. The ^{15}N natural abundance (δ^{15}N) of ecosystem samples reflects measures of water availability. *Aust. J. Plant Physiol.* **1999**, *26*, 185–199. [CrossRef]

39. Qiao, N.; Xu, X.; Cao, G.; Ouyang, H.; Kuzyakov, Y. Land use change decreases soil carbon stocks in Tibetan grasslands. *Plant Soil* **2015**, *395*, 231–241. [CrossRef]

40. Wang, L.; D'Odorico, P.; Ries, L.; Macko, S.A. Patterns and implications of plant-soil δ^{13}C and δ^{15}N values in African savanna ecosystems. *Quat. Res.* **2010**, *73*, 77–83. [CrossRef]

41. Conant, R.T.; Cerri, C.E.P.; Osborne, B.B.; Paustian, K. Grassland management impacts on soil carbon stocks: A new synthesis. *Ecol. Appl.* **2017**, *27*, 662–668. [CrossRef]

42. Ramankutty, N.; Evan, A.T.; Monfreda, C.; Foley, J.A. Farming the planet: 1. Geographic distribution of global agricultural lands in the year 2000. *Glob. Biogeochem. Cycles* **2008**, *22*, GB1003. [CrossRef]

43. Foley, J.A.; Ramankutty, N.; Brauman, K.A.; Cassidy, E.S.; Gerber, J.S.; Johnston, M.; Mueller, N.D.; O'Connell, C.; Ray, D.K.; West, P.C.; et al. Solutions for a cultivated planet. *Nature* **2011**, *478*, 337–342. [CrossRef]

44. Climate Change 2014: Synthesis Report. *Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*; Core Writing Team, Pachauri, R.K., Meyer, L.A., Eds.; IPCC: Geneva, Switzerland, 2014; ISBN 978-92-9169-143-2.

45. Bredenkamp, G.J.; Spada, F.; Kazmierczak, E. On the origin of northern and southern hemisphere grasslands. *Plant Ecol.* **2002**, *163*, 209–229. [CrossRef]

46. Farquhar, G.D.; Richards, R.A. Isotopic composition of plant carbon correlates with water use efficiency of wheat genotypes. *Aust. J. Plant Physiol.* **1984**, *11*, 539–552. [CrossRef]

47. Soil Survey Staff. *Keys to Soil Taxonomy*, 9th ed.; USDA-Natural Resources Conservation Service: Washington, DC, USA, 2003.

48. Grimshaw, H.M. The determination of total phosphorus in soils by acid digestion. In *Chemical Analysis in Environmental Research*; Rowland, A.P., Ed.; NERC/ITE: Abbots Ripton, UK, 1987; pp. 92–95. ISBN 0-904282-98-8.

49. Parkinson, J.A.; Allen, S.E. A wet oxidation procedure suitable for the determination of nitrogen and mineral nutrients in biological material. *Commun. Soil Sci. Plant Anal.* **1975**, *6*, 1–11. [CrossRef]

50. Brookes, P.C.; Landman, A.; Pruden, G.; Jenkinson, D.S. Chloroform fumigation and the release of soil nitrogen: A rapid direct extraction method to measure microbial biomass nitrogen in soil. *Soil Biol. Biochem.* **1985**, *17*, 837–842. [CrossRef]

51. Vance, E.D.; Brookes, P.C.; Jenkinson, D.S. An extraction method for measuring soil microbial biomass C. *Soil Biol. Biochem.* **1987**, *19*, 703–707. [CrossRef]

52. Wu, J.S.; Joergensen, R.G.; Pommerening, B.; Chaussod, R.; Brookes, P.C. Measurement of soil microbial biomass C by fumigation extraction—An automated procedure. *Soil Biol. Biochem.* **1990**, *22*, 1167–1169. [CrossRef]

53. Burnham, K.P.; Anderson, D.R.; Huyvaert, K.P. AIC model selection and multimodel inference in behavioral ecology: Some background, observations, and comparisons. *Behav. Ecol. Sociobiol.* **2011**, *65*, 23–35. [CrossRef]

54. Cheng, S.L.; Fang, H.J.; Yu, G.R.; Zhu, T.H.; Zheng, J.J. Foliar and soil ^{15}N natural abundances provide field evidence on nitrogen dynamics in temperate and boreal forest ecosystems. *Plant Soil* **2010**, *337*, 285–297. [CrossRef]

55. Xu, Y.; He, J.; Cheng, W.; Xing, X.; Li, L. Natural ^{15}N abundance in soils and plants in relation to N cycling in a rangeland in Inner Mongolia. *J. Plant Ecol.* **2010**, *3*, 201–207. [CrossRef]

56. Kahmen, A.; Wanek, W.; Buchmann, N. Foliar δ^{15}N values characterize soil N cycling and reflect nitrate or ammonium preference of plants along a temperate grassland gradient. *Oecologia* **2008**, *156*, 861–870. [CrossRef]

57. Weintraub, S.R.; Cole, R.J.; Schmitt, C.G.; All, J.D. Climatic controls on the isotopic composition and availability of soil nitrogen across mountainous tropical forest. *Ecosphere* **2016**, *7*, e01412. [CrossRef]

58. Shan, Y.; Huang, M.; Suo, L.; Zhao, X.; Wu, L. Composition and variation of soil δ^{15}N stable isotope in natural ecosystems. *Catena* **2019**, *183*, 104236. [CrossRef]

59. Nissenbaum, A.; Schallinger, K.M. The distribution of stable carbon isotope (^{13}C/^{12}C) in fractions of soil organic matter. *Geoderma* **1974**, *11*, 137–145. [CrossRef]

60. Vankessel, C.; Farrell, R.E.; Pennock, D.J. ^{13}C and ^{15}N natural abundance in crop residues and soil organic matter. *Soil Sci. Soc. Am. J.* **1994**, *58*, 382–389. [CrossRef]

61. Santruckova, H.; Bird, M.I.; Lloyd, J. Microbial processes and carbon-isotope fractionation in tropical and temperate grassland soils. *Funct. Ecol.* **2000**, *14*, 108–114. [CrossRef]

62. Du, B.; Liu, C.; Kang, H.; Zhu, P.; Yin, S.; Shen, G.; Hou, J.; Ilvesniemi, H. Climatic control on plant and soil delta C-13 along an altitudinal transect of Lushan mountain in subtropical China: Characteristics and interpretation of soil carbon dynamics. *PLoS ONE* **2014**, *9*, e86440. [CrossRef]

63. Guo, Q.; Hu, Z.; Li, S.; Li, X.; Sun, X.; Yu, G. Spatial variations in aboveground net primary productivity along a climate gradient in Eurasian temperate grassland: Effects of mean annual precipitation and its seasonal distribution. *Glob. Chang. Biol.* **2012**, *18*, 3624–3631. [CrossRef]

64. Jiao, C.; Yu, G.; He, N.; Ma, A.; Ge, J.; Hu, Z. Spatial pattern of grassland aboveground biomass and its environmental controls in the Eurasian steppe. *J. Geogr. Sci.* **2017**, *27*, 3–22. [CrossRef]

65. Bai, X.; Cheng, J.; Zheng, S.; Zhan, S.; Bai, Y. Ecophysiological responses of *Leymus chinensis* to nitrogen and phosphorus additions in a typical steppe. *Chin. J. Plant Ecol.* **2014**, *38*, 103–115. [CrossRef]

66. Dong, C.; Wang, W.; Liu, H.; Xu, X.; Zeng, H. Temperate grassland shifted from nitrogen to phosphorus limitation induced by degradation and nitrogen deposition: Evidence from soil extracellular enzyme stoichiometry. *Ecol. Indic.* **2019**, *101*, 453–464. [CrossRef]