



Soil organic carbon components in inner Mongolian shrub-encroached grasslands

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Abstract

Aims Shrub encroachment has profound influences on regional carbon cycling. However, few studies have examined the changes in soil organic carbon (SOC)

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components at the molecular level along a climate gradient. In this study, we aimed to investigate the effects of biotic and abiotic factors on the patterns of SOC components in the shrub patches and the grassy matrix.

Methods We analyzed the distribution and controlling factors of SOC components (including free lipids, bound lipids, and lignin-derived phenols) in the topsoil of shrub-encroached grasslands along natural climate gradients in Inner Mongolia, China.

Results We found that the concentrations of bound lipids and lignin-derived phenols were significantly higher and the vanillic acid to vanillin ratio ((Ad/Al)_v) was significantly lower in the shrub patches than in the grassy matrix ($p < 0.05$). After excluding variables exhibiting collinearity, redundancy analysis showed that shrub patch cover and soil pH were the most important variables that influenced SOC composition in the shrub patches, while herb characteristics and shrub density were the most important in the grassy matrix. Structural equation modeling showed that shrub characteristics at the plot scale greatly contributed to the variance in all components in the grassy matrix, whereas soil properties were more important in the shrub patches.

Conclusions Our results highlight that although the topsoil carbon content did not change, shrub encroachment altered the SOC components and their drivers in the Inner Mongolian grasslands.

Keywords Biomarkers · Climate change · Plant-soil interaction · Structural equation modeling · Vegetation transition

Introduction

Shrub encroachment, which is the shift in domination from grasses to shrubs, has become one of the most important issues in grasslands throughout the world (Reynolds et al. 2007). Shrub encroachment typically changes soil biogeochemical cycles (Zhou et al. 2018), and leads to the redistribution of soil nutrients (Eldridge et al. 2011). Many previous studies have demonstrated that soil organic carbon (SOC) changes after shrub encroachment vary with climate (Jackson et al. 2002; Knapp et al. 2008), soil properties (Eldridge et al. 2011) and vegetation (Maestre et al. 2009), however, very few studies have evaluated the response of the molecular components of SOC to shrub encroachment (Zhou et al. 2017).

Understanding the molecular components of SOC can provide an insight into soil carbon dynamics (Kögel-Knabner 2002; Pisani et al. 2016; Kögel-Knabner and Rumpel 2018). Soil organic matter is a heterogeneous mixture derived from plants, microbes and animal residues (Kögel-Knabner 2002). Previous studies have revealed that the composition of SOC is controlled by the combination of input characteristics and decomposition processes (Tamura and Tharayil 2014; Li et al. 2017). The precise identification and quantification of the origin and decomposition of SOC are necessary to improve the understanding of C cycling and the response of SOC to changing environmental conditions (Armas-Herrera et al. 2016). The solvent-extractable (“free”, i.e., *n*-alkanols, *n*-alkanes and *n*-alkanoic acids) and hydrolyzable (“bound”, i.e., cutin and suberin) lipids as well as lignin-derived phenols (i.e., vanillyls, syringyls and cinnamyls) play essential roles in the incorporation of residues into the mineral soil. Therefore, these compounds are regarded as important molecular proxies for tracing the origin and degradation of SOC (Dai et al. 2018; Kögel-Knabner and Rumpel 2018; Zhou et al. 2018). Qualitative and quantitative analyses of these molecular components of SOC have been successfully used to examine the response of SOC to climate change (Feng et al. 2010), vegetation shifts (Filley et al. 2008) and land use change (Zhao et al. 2014; Pisani et al. 2016).

Shrub encroachment has widely occurred in the Inner Mongolian grasslands in response to climate change and increased human disturbance, i.e., long-term overgrazing, (Peng et al. 2013; Chen et al. 2015; Fang et al. 2015) and it has strong potential to influence the structure and biogeochemical cycles of this grassland ecosystem (Peng et al. 2013; Zhou et al. 2017; Zhang et al. 2018). Previous studies showed that shrub encroachment did not significantly change the topsoil organic carbon (OC) content in Inner Mongolia at the regional scale, which was partly due to the different responses in the typical grasslands and desert grasslands (Li et al. 2019). This result indicated that the consequences of shrub encroachment were dependent on the climate, vegetation and soil properties (Li et al. 2019). A recent study revealed that shrub encroachment changed the SOC composition by altering the inputs and soil microbial community in the shrub patches and grassy matrix in a Xilinhot grassland (Zhou et al. 2017). However, how shrub encroachment affects the molecular SOC components at the regional scale in Inner Mongolian grasslands remains unclear (Zhou et al. 2017). The SOC composition in different ecosystems was shown to be driven by climate and soil properties (Feng et al. 2010; Pisani et al. 2016; Cai et al. 2017). For example, Feng et al. (2008) found that cutin-derived components increased while other major compounds remained unchanged (or even decreased) after soil warming in a Canadian forest, and Angst et al. (2018) found that free lipids and bound lipids in a forest subsoil accumulated in the sand- and fine-sized aggregates, respectively. However, very few studies have investigated the vegetation, soil, and climate together to assess their effects on SOC composition (Pisani et al. 2016).

This study aimed to quantify SOC components, including free lipids, bound lipids and lignin-derived phenols, in soils of five shrub-encroached grasslands along a climate gradient in Inner Mongolia. The objectives of this study are to (1) explore the effects of shrub encroachment on the chosen components of topsoil OC and (2) investigate the effects of biotic and abiotic factors on the patterns of these components in the shrub patches and the grassy matrix.

Materials and methods

Site description and field sampling

Shrub encroachment forms a mosaic landscape that shrub patches in the grassy matrix, which is significantly different from pure grasslands (Maestre et al. 2009; Chen et al. 2015; Fig. S1). We conducted a field survey along a precipitation gradient from mid-July to September 2013 (Chen et al. 2015). Five study sites distributed in the typical shrub-encroached grassland area in Inner Mongolia were selected. At each study site, we established three 20 × 20 m plots, each containing four 10 × 10 m subplots. The number, size and height of the shrub patches in two randomly selected subplots were measured to assess the shrub characteristics at the plot scale. In each 20 × 20 m plot, we established a pair of 1-m² quadrats, with one quadrat within the shrub patch and one in the neighboring grassy matrix. We recorded the size, cover and height of chosen shrub patch, and then recorded the herb richness in the paired 1-m² quadrats. Then, each 1-m² quadrat was divided into four 0.25 m² subquadrats. We recorded the number and height of each herbaceous species and clipped all aboveground biomass (AGB), including that of shrubs and herbs, at the soil surface in a randomly selected 0.25 m² subquadrat. Herb abundance was calculated as the sum of individual herbs in the subquadrat. Topsoil (0–10 cm) samples and herb belowground biomass (BGB) were also collected from the subquadrats using a stainless-steel corer with a diameter of 5 cm. Each soil core was divided into an air-dried sample for the analysis of nutrient contents, pH and texture, and a frozen sample (−20 °C) for biomarker analysis. The vegetation and soil characteristics were listed in Table 1.

Laboratory analyses

All air-dried soil samples were sieved through a 2-mm sieve, and the plant roots were carefully removed. The soil total carbon (STC) and soil total nitrogen (STN) were measured using an elemental analyzer (Vario El III, Elementar, Germany). The SOC content was calculated as the difference between the STC and the soil inorganic carbon,

which was measured using a carbonate content analyzer (Eijkelkamp 08.53, Netherlands). The soil pH was measured from a soil and water suspension (1:2.5 w/v) using a pH meter. After treatment with H₂O₂ and HCl, the soil texture was measured using a particle size analyzer (Mastersizer 2000, Malvern, UK).

Biomarker extraction

Solvent extraction, base hydrolysis and copper(II) oxide (CuO) oxidation were conducted to separate free lipids, bound lipids and lignin-derived phenols (Feng et al. 2010; Zhou et al. 2017, 2018). Briefly, approximately 6 g of soil samples were extracted using ultrasonication for 15 min and then centrifuged at 3000 rpm for 10 min using 15 mL of chromatography-grade dichloromethane: methanol (1:1; v: v). The combined solvent extracts (free lipids) were concentrated using rotary evaporation.

After solvent extraction, approximately 3 g of soil residue was sealed with 15 mL of 1 M methanolic KOH in Teflon-lined bombs and heated at 100 °C for 3 h. Then, the suspension was acidified to pH 1 with 6 M HCl. The base hydrolysis extracts were recovered using liquid-liquid extraction with 20 mL ethyl acetate. Then, the concentrated products were methylated with 1 mL of 14% BF₃: MeOH (90 min at 70 °C). The bound lipids were obtained using liquid-liquid extraction with 2 mL hexane.

The remaining residues after solvent extraction were sealed with 1 g of CuO, 100 mg of ammonium iron (II) sulfate hexahydrate and 15 mL of 2 M NaOH and heated at 170 °C for 2.5 h after oxygen exhaustion. The mixture was centrifuged at 3000 rpm for 10 min, and the supernatant was then acidified to pH 1 with 6 M HCl. The extracts were kept in the dark for 1 h. After centrifugation (3000 rpm, 10 min), the lignin-derived phenols were recovered using 20 mL ethyl acetate.

All products were converted to trimethylsilyl derivatives using 100 µL *N*, *O*-bis-(trimethylsilyl) trifluoroacetamide and 100 µL pyridine at 60 °C for 2 h. The biomarkers were identified and quantified using an Agilent 7890A gas chromatograph (Agilent Technologies, Palo Alto, CA, USA) coupled to an Agilent 5973 N quadrupole mass selective detector and a flame ionization detector,

Table 1 Location, vegetation and soil types of the five sampling sites in Inner Mongolia, China

Site	Location	Dominant shrub	Dominant herbs	Soil types
Xilinhot	44.5°N, 115.9°E	<i>Caragana microphylla</i> , <i>C. pygmaea</i> , <i>C. stenophylla</i>	<i>Stipa krylovii</i> , <i>Cleistogenes squarrosa</i>	Kastanozems
Zhengxiangbai Banner	42.5°N, 114.9°E	<i>C. microphylla</i>	<i>Agropyron cristatum</i> , <i>Carex korshinskyi</i>	Kastanozems
Bairin Right Banner	43.7°N, 119.0°E	<i>C. microphylla</i>	<i>C. squarrosa</i> , <i>Artemisia</i> <i>frigida</i>	Kastanozems, Arenosols
Sonid Left Banner	43.6°N, 113.4°E	<i>C. microphylla</i> , <i>C. pygmaea</i>	<i>S. klemenzii</i> , <i>A. cristatum</i>	Kastanozems
Sonid Right Banner	42.6°N, 112.5°E	<i>Amygdalus pedunculata</i> , <i>C. stenophylla</i> , <i>C. pygmaea</i> , <i>C. microphylla</i>	<i>C. squarrosa</i> , <i>C. songorica</i> , <i>A. cristatum</i>	Arenosols

respectively. The free lipids and bound lipids were quantified by comparison with the internal standards (squalane and 5 α -cholestane, respectively), whereas the lignin-derived phenols were quantified using the calibration curves of eight lignin-phenol standards (Zhao et al. 2014; Zhou et al. 2017, 2018). All the biomarkers were normalized to the SOC contents.

Statistical analysis

Climate data from 1982 to 2011 were obtained from the China Meteorological Data Sharing Service System (<http://cdc.nmic.cn>). Differences in variables between the shrub patches and grassy matrix were examined using paired *t* tests. Climate, shrub, herb and soil characteristics were expected to explain the variance in SOC components. Details of the explanatory variables were provided in Table 2. The relationships between each biomarker and the explanatory variables were examined using Pearson correlations. Then, the explanatory variables were subjected to redundancy analysis (RDA) to determine the important drivers. To decrease the collinearity, the explanatory variables with the highest variance inflation factor (vif) values were removed one by one until the vifs of all variables in the RDA were lower than 10. Finally, structural equation modeling (SEM) was further conducted to evaluate the relative importance of climate, shrub, herb and soil characteristics in controlling the SOC components. The data of all the environmental variables were Z-score normalized and

included in the principal component analysis (PCA). The first component (PC1) of each explanatory group was used as input in the subsequent SEM analysis. The fit of our model was evaluated using a chi-square test and the root mean square error of approximation (Grace 2006). The statistical analysis was conducted using the R 3.3.1 platform (R Foundation for Statistical Computing, Vienna, AT), and the SEM analysis was conducted in AMOS 17.0 (Amos Development Corporation, Chicago, IL, USA).

Results

Plant community and soil properties

At the plot scale, the density, cover, mean area and height of the shrub patches ranged from 13 to 215 individuals/200 m², 2.8 to 23.1%, 0.2 to 2.9 m² and 18.3 to 41.8 cm, respectively (Table 2). In addition, the patch area, cover, height and AGB of the chosen shrub individuals ranged from 0.7 to 9.2 m², 55.0 to 81.7%, 30.1 to 47.5 cm and 36.9 to 84.7 g/0.25 m², respectively (Table 2).

The herb richness in the grassy matrix was significantly higher than that in the shrub patches (7–13 vs. 4–12/m², respectively; *p* = 0.03). However, significant differences in herb abundance (34–164 individuals/m²), height (7.6–19.0 cm), AGB (8.8–42.9 g/0.25 m²) and BGB (129.2–525.5 g/0.05 m³) were not observed

Table 2 Climate, vegetation and soil characteristics of the five locations in Inner Mongolia (mean ± standard error; *n* = 3). MAT, mean annual temperature; MAP, mean annual precipitation; PE, potential evaporation; AGB, aboveground biomass; BGB, belowground biomass; STC, soil total carbon; STN, soil total nitrogen; SOC, soil organic carbon. The *p* values indicate the significance in variables between shrub patches and grassy matrix (paired *t*-test of data from five sites, marked in bold when *p* < 0.05)

Site	Xilimhot		Zhengxiangbai Banner		Bairin Right Banner		Sonid Left Banner		Sonid Right Banner		<i>p</i> values
	Grassy	Shrub	Grassy	Shrub	Grassy	Shrub	Grassy	Shrub	Grassy	Shrub	
Climatic variables											
MAT (°C)	1.4		3.4		5.3		4.0		5.2		
MAP (mm)	258.3		302.4		365.3		189.3		222.9		
PE (mm)	551.5		561.3		605.4		613.5		612.3		
Aridity index	0.5		0.5		0.6		0.3		0.4		
Shrub characteristics at plot scale											
Cover (%)	2.9 ± 1.6		17.9 ± 0.9		23.1 ± 5.1		5.8 ± 1.3		2.8 ± 0.6		
Density (/200m ²)	17 ± 3		13 ± 2		16 ± 2		69 ± 42		215 ± 72		
Mean area (m ²)	0.3 ± 0.1		2.6 ± 0.1		2.9 ± 0.3		0.6 ± 0.2		0.2 ± 0.0		
Mean height (cm)	35.0 ± 5.9		38.6 ± 0.9		41.8 ± 1.1		29.8 ± 1.0		18.3 ± 0.4		
Individual shrub characteristics											
Area (m ²)	0.7 ± 0.2		4.5 ± 3.5		9.2 ± 3.1		1.1 ± 0.2		1.1 ± 0.2		
Cover (%)	81.7 ± 1.7		76.7 ± 6.7		61.7 ± 6.0		55.0 ± 2.9		61.7 ± 6.0		
Height (cm)	41.1 ± 7.3		35.8 ± 3.6		36.9 ± 3.6		47.5 ± 12.2		30.1 ± 4.4		
AGB (g/0.25m ²)	54.6 ± 1		51.8 ± 4.8		42.0 ± 4.2		36.9 ± 1.9		84.7 ± 22.3		
Herb characteristics											
Richness (/m ²)	8 ± 1	5 ± 1	12 ± 0	12 ± 2	13 ± 1	7 ± 1	7 ± 1	4 ± 1	12 ± 2	12 ± 1	0.03
Abundance (/m ²)	44 ± 7	66 ± 4	164 ± 43	130 ± 43	86 ± 10	66 ± 17	50 ± 22	34 ± 12	44 ± 3	58 ± 7	0.68
AGB (g/0.25m ²)	26.3 ± 3.0	26.5 ± 2.4	23.8 ± 7.6	30.2 ± 7.4	36.9 ± 0.6	42.9 ± 18.2	13.2 ± 0.9	8.8 ± 1.7	16.5 ± 2.3	18.5 ± 1.4	0.55
BGB (g/0.05m ³)	206.4 ± 35.7	193.2 ± 28.4	185.4 ± 33.0	270.7 ± 38.4	129.2 ± 42.1	286.0 ± 18.5	516.6 ± 104.0	525.5 ± 244.2	145.9 ± 31.8	188.8 ± 24.6	0.96
Height (cm)	16.3 ± 1.2	17.2 ± 3.6	7.6 ± 1.2	16.3 ± 2.8	12.7 ± 0.5	18.4 ± 2.5	17.0 ± 4.8	19.0 ± 1.5	15.4 ± 2.0	11.2 ± 0.8	0.16
Soil properties											
Clay (%)	27.6	19.5	13.3	11.3	11.2	11.5	12.2	4.7	13.5	6.0	<0.01
Silt (%)	6.8	4.9	9.1	8.5	12.3	13.9	5.6	2.2	13.7	3.9	0.02
Sand (%)	65.6	75.6	77.6	80.2	76.6	74.5	82.3	93.1	72.8	90.1	<0.01
pH	7.4 ± 0.3	8.6 ± 0.2	9.1 ± 0.1	9.1 ± 0.0	9.1 ± 0.1	8.9 ± 0.3	9.5 ± 0.0	9.3 ± 0.1	9.2 ± 0.2	9.2 ± 0.1	0.39
STN (%)	0.08 ± 0.00	0.09 ± 0.01	0.11 ± 0.01	0.12 ± 0.01	0.09 ± 0.01	0.07 ± 0.01	0.02 ± 0.00	0.03 ± 0.00	0.05 ± 0.01	0.04 ± 0.00	0.75
STC (%)	0.70 ± 0.01	0.73 ± 0.05	0.98 ± 0.16	1.24 ± 0.10	0.90 ± 0.11	0.66 ± 0.10	0.19 ± 0.01	0.24 ± 0.05	0.34 ± 0.03	0.38 ± 0.03	0.64
SOC (%)	0.69 ± 0.01	0.72 ± 0.05	0.86 ± 0.07	0.99 ± 0.11	0.73 ± 0.08	0.61 ± 0.10	0.16 ± 0.01	0.22 ± 0.05	0.32 ± 0.03	0.36 ± 0.02	0.44

($p > 0.05$). Similarly, pH (7.4–9.5), STN (0.02–0.12%) and SOC (0.16–0.99%) also did not differ between the shrub patches and the grassy matrix ($p > 0.05$). The soil clay content was significantly higher in the grassy matrix than in the shrub patches (11.3–27.6% vs. 4.7–19.5%, respectively; $p < 0.01$; Table 2).

SOC components

The dominant free lipids from the solvent extraction were C₂₇–C₃₁ *n*-alkanes, C₁₈–C₃₀ *n*-alkanols and C₁₆–C₃₀ *n*-alkanoic acids, with normalized concentrations of 0.44–2.37 mg/g OC, 0.90–14.89 mg/g OC and 0.82–5.02 mg/g OC, respectively (Table 3). The bound lipids from base hydrolysis mainly included C₁₄–C₁₆ alkanolic acids (1.81–3.81 mg/g OC), C₁₆–C₂₆ α , ω -alkanedioic acids (DAs, 0.25–0.89 mg/g OC) and C₁₆–C₂₄ ω -hydroxyalkanoic acids (OH FAs; 0.02–0.83 mg/g OC). Regarding the molecular characteristics of the local plants (Zhou et al. 2017, 2018), we defined the C₁₆ and C₁₈ ω -OH FAs with mid-chain hydroxy or epoxy groups as cutin components, and their concentrations were very low in this study (Table 3). The C₁₆ and C₁₈ ω -OH FAs and DAs were components in both cutin and suberin (Σ CS, 0.13–0.73 mg/g OC), and the long-chain α , ω -DAs and ω -OH FAs were defined as suberin components (0.09–0.80 mg/g OC) in this study. Eight lignin-derived phenols (vanillyls: vanillin, acetovanillone, vanillic acid; syringyls: syringaldehyde, acetosyringone, syringic acid; cinnamoyls: *p*-coumaric acid and ferulic acid) were identified in the CuO products; among them, the vanillyls were the most abundant class of components, with concentrations of 5.67–13.55 mg/g OC (Table 3).

Factors affecting the SOC components

Vegetation, climate and soil all affect the SOC components in shrub-encroached grasslands. First, we used Pearson correlation to analyze how these factors affect the SOC components. Our data indicated that the *n*-alkanols in the grassy matrix were significantly and negatively correlated with herb richness and AGB ($p < 0.05$; Fig. 1a). The *n*-

alkanes in both the shrub patches and grassy matrix were also significantly and negatively correlated with the soil carbon and nitrogen ($p < 0.05$; Fig. 1).

Suberin showed significant positive correlations with the cover of individual shrub patches and soil clay, carbon and nitrogen and was significantly and negatively correlated with the mean annual temperature (MAT) and potential evaporation (PE) in both the shrub patches and grassy matrix ($p < 0.05$; Fig. 1). Cutin and Σ CS were mainly positively correlated with the soil clay but negatively correlated with the pH in the grassy matrix ($p < 0.05$; Fig. 1a). However, these two components were mainly negatively correlated with the MAT in the shrub patches ($p < 0.05$; Fig. 1b). The lignin-derived phenols and the indicators of the botanical origin of the lignin (cinnamyls/vanillyls (C/V) and syringyls/vanillyls (S/V)) were negatively correlated with the mean annual precipitation (MAP), AGB, and soil carbon and nitrogen and positively correlated with the sand content of the soil in both the shrub patches and grassy matrix (Fig. 1). In addition, the lignin-derived phenols were significantly and positively correlated with the herb BGB in the grassy matrix ($p < 0.05$; Fig. 1a), whereas the indicator of the stage of lignin degradation, vanillic acid to vanillin ratio ((Ad/Al)_v), showed the opposite correlation ($p < 0.05$; Fig. 1). We then conducted RDA to explore the effects of biotic and abiotic variables on the SOC components. Our RDA explained 77.3% of the variance in the SOC composition in the grassy matrix and 67.2% of that in the shrub patches (Fig. 2, S2). Herb AGB, richness, shrub density and herb height were the most important variables explaining the SOC composition in the grassy matrix, with explanatory powers of 29.2%, 14.3%, 12.4% and 12.2%, respectively (Fig. 2a). In addition, the cover of individual shrub patches and pH were the most important variables and explained 21.5% and 14.3% of the variance in the shrub patches, respectively (Fig. 2b).

Furthermore, we applied SEM analysis to quantify the relative contributions of climate and the shrub, herb and soil characteristics to the variations in the SOC components. The models could explain 15–91% of the variance in the SOC

Table 3 Biomarkers and parameters at the sampling locations in Inner Mongolia (mean \pm standard error, $n = 3$). FA Alkanoic acid, Σ CS, components in both cutin and suberin; (Ad/Al)_s, syringic acid/syringaldehyde; (Ad/Al)_v, vanillic acid/vanillin. The p values indicate the significance in variables between shrub patches and grassy matrix (paired t-test of data from five sites, marked in bold when $p < 0.05$)

Site	Xilinhot		Zhengxiangbai Banner		Bairin Right Banner		Sonid Left Banner		Sonid Right Banner		p values
	Grassy	Shrub	Grassy	Shrub	Grassy	Shrub	Grassy	Shrub	Grassy	Shrub	
Solvent-extractable compounds (mg/g OC)											
Free lipids	7.57 \pm 4.90	6.50 \pm 1.59	12.90 \pm 5.68	2.62 \pm 0.36	3.81 \pm 0.89	16.35 \pm 5.55	21.41 \pm 6.72	11.35 \pm 2.95	14.58 \pm 8.34	14.07 \pm 5.53	0.61
<i>n</i> -alkanes	0.58 \pm 0.09	1.18 \pm 0.69	0.46 \pm 0.19	0.44 \pm 0.05	1.82 \pm 0.91	0.83 \pm 0.28	2.37 \pm 0.25	1.93 \pm 0.20	1.14 \pm 0.47	1.09 \pm 0.35	0.49
<i>n</i> -alkamols	5.67 \pm 4.68	3.50 \pm 2.61	8.98 \pm 3.68	1.36 \pm 0.33	0.90 \pm 0.11	13.70 \pm 6.15	14.89 \pm 7.56	4.40 \pm 0.85	10.41 \pm 7.56	11.50 \pm 5.77	0.71
<i>n</i> -FAs	1.31 \pm 0.20	1.81 \pm 0.48	3.46 \pm 2.32	0.82 \pm 0.10	1.09 \pm 0.12	1.81 \pm 0.60	4.15 \pm 0.87	5.02 \pm 2.57	3.03 \pm 1.22	1.48 \pm 0.20	0.56
Hydrolysable lipids (mg/g OC)											
Bound lipids	4.53 \pm 0.48	5.00 \pm 0.31	2.54 \pm 0.28	5.01 \pm 0.36	3.06 \pm 0.05	3.22 \pm 0.28	3.49 \pm 0.73	4.76 \pm 0.40	2.51 \pm 0.20	2.76 \pm 0.80	0.02
<i>n</i> -FAs	2.90 \pm 0.14	3.09 \pm 0.40	1.81 \pm 0.19	3.35 \pm 0.35	2.23 \pm 0.11	2.60 \pm 0.24	3.04 \pm 0.69	3.81 \pm 0.39	2.24 \pm 0.17	2.33 \pm 0.55	0.03
Cutin (Σ C)	0.04 \pm 0.02	0.08 \pm 0.01	ns	0.05 \pm 0.03	0.03 \pm 0.01	0.02 \pm 0.01	0.01 \pm 0.01	0.06 \pm 0.03	ns	ns	0.03
Σ CS	0.49 \pm 0.13	0.73 \pm 0.03	0.13 \pm 0.04	0.52 \pm 0.04	0.31 \pm 0.06	0.17 \pm 0.01	0.27 \pm 0.04	0.42 \pm 0.01	0.17 \pm 0.03	0.18 \pm 0.09	0.04
Suberin (Σ S)	0.80 \pm 0.10	0.72 \pm 0.10	0.54 \pm 0.06	0.63 \pm 0.04	0.38 \pm 0.04	0.31 \pm 0.02	0.14 \pm 0.07	0.35 \pm 0.06	0.09 \pm 0.04	0.19 \pm 0.10	0.29
Lignin-derived phenols (mg/g OC)											
Lignin	13.79 \pm 0.64	15.3 \pm 0.31	11.91 \pm 0.53	15.82 \pm 0.91	11.73 \pm 0.47	14.31 \pm 1.38	30.62 \pm 1.05	31.67 \pm 1.14	15.64 \pm 0.53	19.14 \pm 1.93	<0.01
Cinnamyls (C)	2.75 \pm 0.10	2.95 \pm 0.06	2.41 \pm 0.11	2.81 \pm 0.27	2.41 \pm 0.05	2.89 \pm 0.37	6.89 \pm 0.44	6.34 \pm 0.69	3.80 \pm 0.14	3.95 \pm 0.21	0.56
Syringyls (S)	3.84 \pm 0.14	4.62 \pm 0.09	3.55 \pm 0.11	5.44 \pm 0.33	3.65 \pm 0.18	4.99 \pm 0.54	11.19 \pm 0.37	11.78 \pm 0.64	4.86 \pm 0.14	6.42 \pm 0.82	<0.01
Vanillyls (V)	7.20 \pm 0.44	7.73 \pm 0.23	5.94 \pm 0.32	7.57 \pm 0.46	5.67 \pm 0.26	6.43 \pm 0.47	12.53 \pm 0.26	13.55 \pm 0.6	6.98 \pm 0.39	8.77 \pm 1.00	<0.01
Parameters											
Suberin/Cutin	2.68 \pm 0.50	1.81 \pm 0.14	5.58 \pm 1.08	2.05 \pm 0.07	2.14 \pm 0.33	2.52 \pm 0.10	1.43 \pm 0.22	1.60 \pm 0.03	1.68 \pm 0.41	1.99 \pm 0.05	0.15
ω -C ₁₆ / Σ C ₁₆	0.55 \pm 0.05	0.57 \pm 0.08	0.23 \pm 0.15	0.51 \pm 0.05	0.40 \pm 0.10	0.11 \pm 0.04	ns	0.03 \pm 0.03	ns	0.05 \pm 0.05	0.77
C/V	0.38 \pm 0.02	0.38 \pm 0.01	0.40 \pm 0.00	0.37 \pm 0.04	0.43 \pm 0.02	0.45 \pm 0.03	0.55 \pm 0.03	0.47 \pm 0.05	0.54 \pm 0.03	0.46 \pm 0.04	0.12
S/V	0.54 \pm 0.01	0.60 \pm 0.02	0.60 \pm 0.02	0.72 \pm 0.03	0.64 \pm 0.01	0.77 \pm 0.03	0.89 \pm 0.01	0.87 \pm 0.03	0.70 \pm 0.04	0.73 \pm 0.01	<0.01
(Ad/Al) _s	0.66 \pm 0.06	0.61 \pm 0.04	0.50 \pm 0.04	0.66 \pm 0.06	0.50 \pm 0.01	0.52 \pm 0.02	0.56 \pm 0.01	0.52 \pm 0.02	0.58 \pm 0.02	0.59 \pm 0.03	0.40
(Ad/Al) _v	1.32 \pm 0.09	1.25 \pm 0.01	1.37 \pm 0.05	1.00 \pm 0.14	1.11 \pm 0.03	0.90 \pm 0.10	0.70 \pm 0.03	0.61 \pm 0.03	0.92 \pm 0.10	0.96 \pm 0.01	0.03

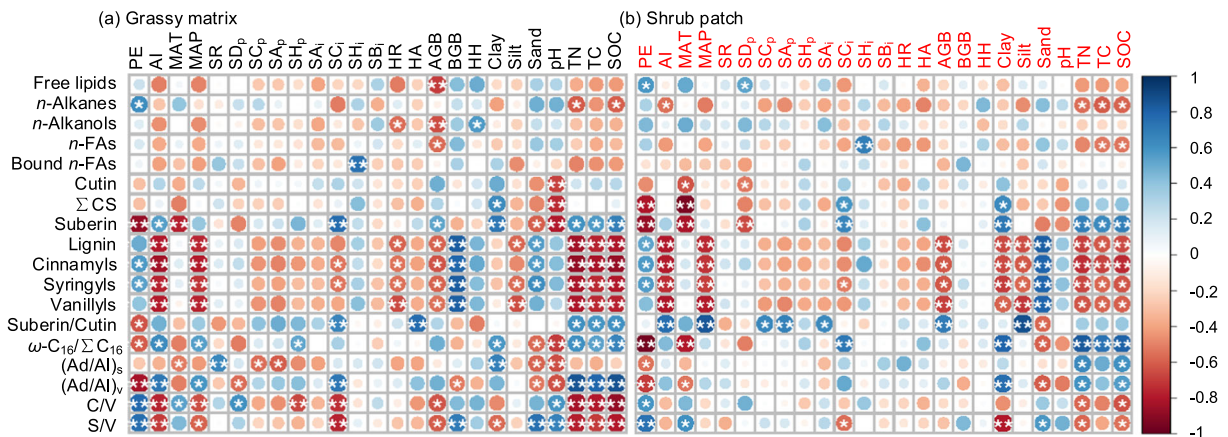


Fig. 1 Pearson correlations between different soil organic carbon biomarkers and explanatory variables in the grassy matrix (a) and shrub patches (b). Different colors represent different coefficients. Significance is indicated by asterisks: *, $p < 0.05$; **, $p < 0.01$. FA, alkanolic acid; Σ CS, components in both cutin and suberin; C/V, cinnamyls/vanillyls; S/V, syringyls/vanillyls; (Ad/Al)_s, syringic acid/syringaldehyde; (Ad/Al)_v, vanillic acid/vanillin; PE, potential evaporation; AI, aridity index; MAT, mean annual temperature;

SR, shrub richness; SD_p, SC_p, SA_p and SH_p represent shrub density, cover, area and height at the plot scale, respectively; SA_i, SC_i, SH_i and SB_i represent the size, cover, height and biomass of the individual shrub patches, respectively; HR, herb richness; HA, herb abundance; HH, herb height; AGB, aboveground biomass; BGB, belowground biomass; clay, silt and sand represent the particles of soil; TN, TC and SOC represent soil total nitrogen, total carbon and organic carbon contents, respectively

components (Fig. 3). In the grassy matrix, the herb and soil characteristics had direct and negative effects on the lignin-derived phenols (Fig. 3c), whereas climate had significant direct and negative effects on the bound lipids and lignin-derived phenols in the shrub patches (Fig. 3bc).

Discussion

Differences in SOC components between the shrub patches and grassy matrix

Relative to the Inner Mongolian grasslands that have been subject to different land uses, the shrub-encroached grasslands in this region were characterized by low bound lipid concentrations and extremely high lignin-derived phenol concentrations (Zhao et al. 2014), indicating that shrub encroachment has altered the SOC composition in the grasslands. Our results showed that the main components that differed were also the bound lipids and lignin-derived phenols (i.e., bound *n*-alkanoic acids, Σ CS, syringyls and vanillyls; $p < 0.05$; Table 3), which were more abundant in the shrub patches than in the grassy matrix. In

contrast, the lignin degradation parameter (Ad/Al)_v was much lower in the shrub patches than that in the grassy matrix ($p < 0.05$; Table 3; Fig. S3). These results suggested the occurrence of more inputs and a low degradation state in the shrub patches (Otto and Simpson 2006; Angst et al. 2016). In our study, the concentration of cutin (a tracer of aboveground inputs) significantly differed between the shrub patches and grassy matrix (Table 3; Fig. S3), whereas a difference in suberin (the tracer of belowground inputs) was not observed, indicating that aboveground inputs made greater contributions in the shrub patches than in the grassy matrix (Kögel-Knabner 2002; Feng et al. 2010; Angst et al. 2018). This result agreed with findings regarding the accumulation of cutin under the tree canopy after 5 years of cultivation in a grassland in New Zealand (Huang et al. 2011). These results could reasonably be explained by the following mechanisms: (1) shrubs produce more litter than herbaceous species (Table 2; Knapp et al. 2008; Eldridge et al. 2011); (2) leguminous shrubs increase microbial decomposition in the grassy matrix by providing additional available resources (i.e., a low C: N ratio) (Zhang et al. 2018). A previous study in an Inner Mongolian

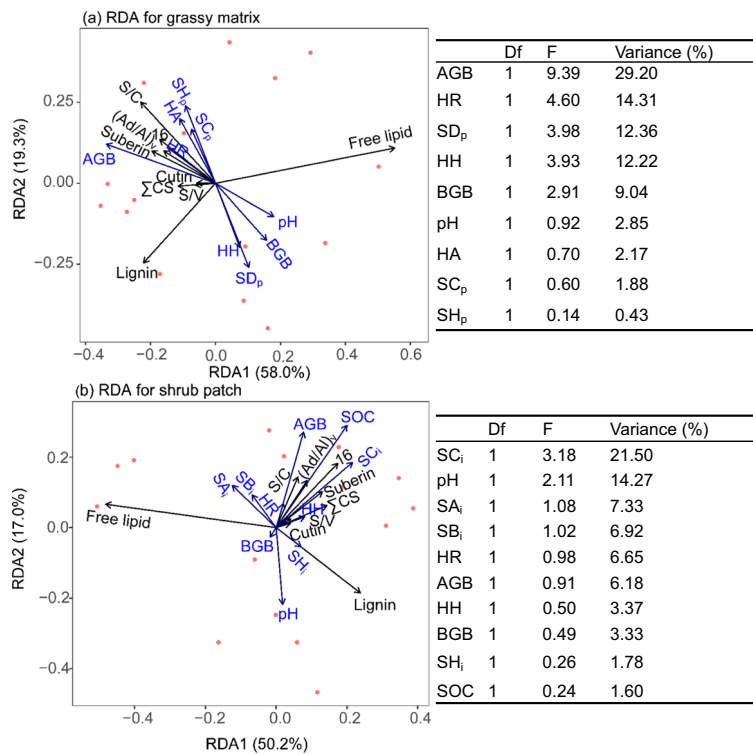


Fig. 2 The results of redundancy analysis based on the biomarkers and the explanatory variables in the grassy matrix (a) and shrub patches (b) in Inner Mongolia. S/C, suberin/cutin; Σ CS, components in both cutin and suberin; S/V, syringyls/vanillyls; (Ad/Al)_v, vanillic acid/vanillin; 16, ω -C₁₆/ Σ C₁₆; AGB, above-ground biomass; BGB, belowground biomass; HR, herb richness;

HH, herb height; HA, herb abundance; SD_p, SC_p and SH_p represent shrub density, cover and height at the plot scale, respectively. SC_i, SA_i, SB_i and SH_i represent the cover, size, biomass and height of the individual shrub patches, respectively. SOC represent the soil organic carbon

shrub-encroached grassland showed that the SOC in shrub patches was mainly derived from above-ground inputs, whereas that in the grassy matrix originated from both aboveground and below-ground inputs (Zhou et al. 2017). Aboveground inputs are incorporated into the organic layer or mineral soil via decomposition by microbes over time (Six et al. 2004; Wardle et al. 2004; Wilkinson et al. 2009), whereas belowground inputs, such as rhizodeposition, released by living roots, can be rapidly consumed by soil microorganisms in situ (Kögel-Knabner 2002; Angst et al. 2016). In addition, leguminous shrubs can transfer nitrogen to the soils under the grassy matrix via their horizontal root system (Temperton et al. 2007; Maestre et al. 2009; Zhang et al. 2018; Zhou et al. 2018), which can partly explain the increased degradation of the lignin-derived phenols in the grassy matrix. The effects of shrubs on the grassy matrix have been reported in many studies

(Maestre et al. 2009; Rascher et al. 2012; Rasmussen et al. 2013). Rascher et al. (2012) found that the area influenced by nitrogen fixers could be at least 3.5-fold greater than the area of their canopy in a sand dune ecosystem. In Mongolian grasslands, the distance over which nitrogen was transferred by shrub species was even found to reach 5 m (Zhang et al. 2016).

The influences of vegetation, climate, and soil on SOC components

Plants are believed to be the critical factor that controls the SOC dynamics and composition (Kögel-Knabner 2002; Kögel-Knabner and Rumpel 2018). We found that the free lipids were strongly correlated with herb richness and AGB in the grassy matrix ($p < 0.05$; Fig. 1a), while the free lipids, cutin and suberin were significantly correlated with the shrub characteristics in the shrub

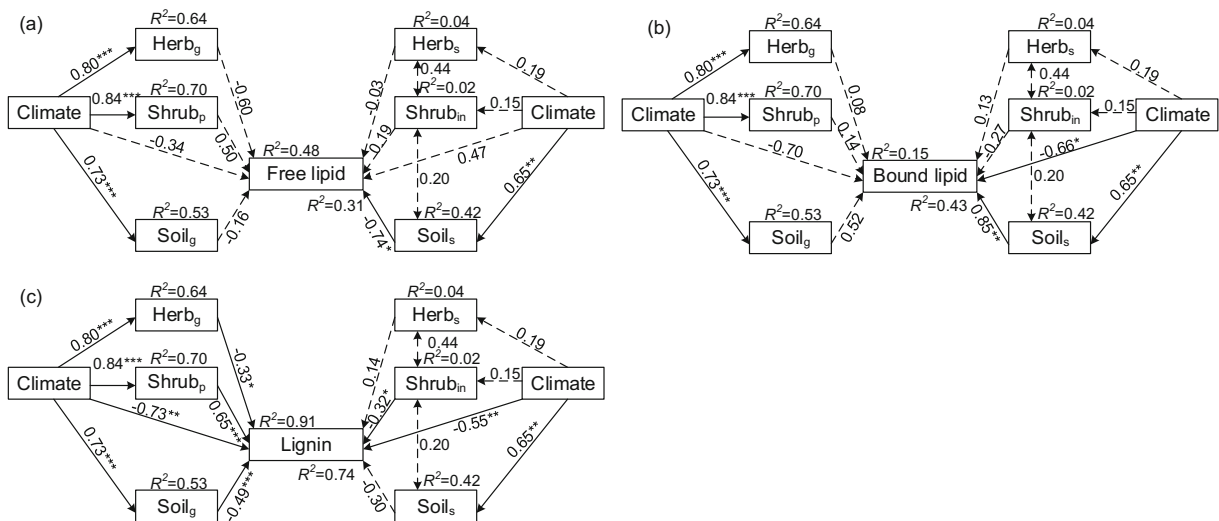


Fig. 3 Structure equation modeling examining the multivariate effects of climate, shrub, herb and soil characteristics on the free lipids (a), bound lipids (b) and lignin-derived phenols (c) of the grassy matrix (left) and shrub patches (right) in Inner Mongolia. Single-headed arrows indicate the hypothesized direction of causation between the related variables. Double-headed arrows indicate covariance between the related variables. The numbers adjacent to the arrows are the standard path coefficients. Herb_g,

Herb_s, Soil_g, and Soil_s represent the herb community and soil characteristics in the grassy matrix and shrub patches, respectively. Shrub_p and Shrub_{in} represent the shrub characteristics at the plot scale and the characteristics of the individual shrub patches, respectively. R², the proportion of variance explained. All models adequately fit the data (p -values of χ^2 test ≥ 0.60 , RMSEA = 0.00 in all cases)

patches ($p < 0.05$; Fig. 1b), indicating the different contributions of vegetation in this landscape (Zhou et al. 2017). Shrubs can influence the bound lipids in the grassy matrix via root inputs, evidenced by the significant correlations of shrub characteristics (i.e., individual shrub patch cover) with suberin compounds and the suberin/cutin ratio in the grassy matrix ($p < 0.05$; Fig. 1a; Angst et al. 2016). In the grassy matrix, lignin-derived phenols were positively correlated with herb BGB ($p < 0.05$; Fig. 1a), but negatively correlated with the shrub characteristics ($p < 0.05$; Fig. 1a), whereas lignin degradation parameter (Ad/AI)_v showed the opposite correlations with these variables (Fig. 1a). These results support our previous findings that the lignin-derived phenols in the grassy matrix are mainly derived from herbaceous roots and shrubs can mediate the process of decomposition in the grassy matrix via their horizontal root system (Zhang et al. 2016; Zhou et al. 2018). In our study area, the dominant shrub *Caragana* has a complex root system that can extend several meters horizontally (Maestre et al. 2009; Zhang et al. 2016).

Cutin and suberin were significantly and negatively correlated with the MAT, especially in the shrub patches (Fig. 1). This result contrasted with the increase in cutin components observed in Duke Forest after 14 months of soil warming (Feng et al. 2008), this difference may be attributed to the water limitations in our study area, as rising temperatures can be expected to lead to increased water stress (Bai et al. 2007). In both the shrub patches and grassy matrix, the MAP and aridity index (AI) had strongly negative effects on the lignin-derived phenols but opposite effects on the (Ad/AI)_v (Fig. 1). This finding was consistent with the patterns of lignin-derived phenols observed in Inner Mongolian pure grasslands (Ma et al. 2018). Previous studies showed that precipitation influenced the patterns of lignin-derived phenols indirectly by changing the soil microbial activities, soil moisture and vegetation rather than via direct control (Filley et al. 2008; Pisani et al. 2016). Li et al. (2017) found that the biomass of fungi (the major decomposers of lignin-derived phenols) increased with increasing precipitation in Inner Mongolian shrub-encroached grasslands. We inferred

that water was the main factor driving degradation and that increased precipitation enhanced lignin-phenol degradation by facilitating microbial activity (Ma et al. 2018).

Our results demonstrated that the bound lipids (i.e., suberin and Σ CS) were positively correlated with the soil clay content ($p < 0.05$; Fig. 1), suggesting that these compounds were physically protected by the clay fractions. Similarly, Lin and Simpson (2016) found that cutin and suberin were physically protected in grassland, forest and agricultural soils, and Angst et al. (2018) found similar results, showing that bound lipids accumulated in the clay fractions of forest subsoil. These results may have occurred because clay fractions have a greater capacity than coarse particles to adsorb alkyl-C onto their surface (Feng et al. 2005). Consistent with other findings in pure Inner Mongolian grasslands, the lignin-derived phenols were negatively correlated with the soil nutrients and positively correlated with the sand fractions ($p < 0.05$; Fig. 1); however, the degradation parameter $(Ad/Al)_v$ showed the opposite correlations with these soil properties (Ma et al. 2018). These results indicated that lignin-derived phenols decrease with increasing degradation under nutrient-rich conditions (Otto and Simpson 2006; Feng et al. 2010; Ma et al. 2018). These results further suggested that the lignin-phenols were not selectively preserved in the clay fraction, which was consistent with the findings from grasslands in North America (Amelung et al. 1999).

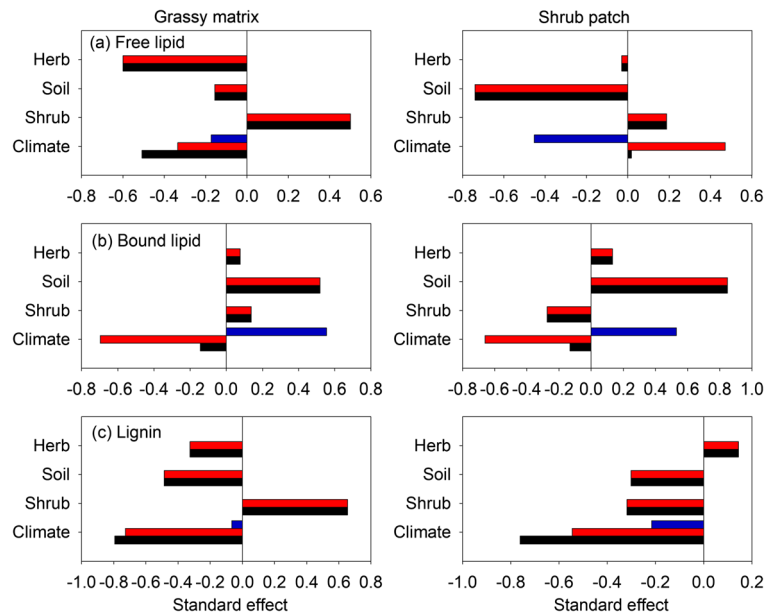
The relative importance of environmental factors in driving SOC components

We conducted RDA to examine the effects of the chosen variables on the SOC composition (Fig. 2). The shrub characteristics at the plot scale, which represented the degree of shrub encroachment, had higher explanatory power than the characteristics of the individual shrub patches in explaining the variation in SOC components in the grassy matrix (Fig. 2, S2; 77.3% vs. 61.5%, respectively). The highest explanatory power of herb AGB suggested herb productivity was an important resource in the grassy matrix (29.2%; Fig. 2a). The critical role of herb richness was ascribed to increasing herb productivity via complementary resource use (Chen

et al. 2018). Moreover, the shrub density, which is an important indicator of shrub encroachment, also explained 12.4% of the variance in the grassy matrix, which highlights the effects of shrub encroachment (Eldridge et al. 2011). By comparison, individual shrub patch cover and soil pH were the most important variables explaining 21.5% and 14.3% of the variance in SOC components in the shrub patches, respectively (Fig. 2b). High shrub patch cover facilitates the addition of shrub litter inputs into the soil (Table 1; Knapp et al. 2008), and high shrub patch cover is usually associated with complex root systems, which indicates a heterogeneous microenvironment under shrub canopies (Maestre et al. 2009; Soliveres and Eldridge 2014). Positive or negative effects of soil pH on free lipids (i.e., *n*-alkanoic acids), hydrolysable lipids and lignin-derived phenols have been reported in grasslands, shrublands and forests; and can likely be ascribed to mediation of the soil fauna (Bull et al. 2000; Nierop et al. 2003; Cai et al. 2017; Ma et al. 2018).

The SEM in this study was well established, as indicated by the nonsignificant chi-square test result ($p > 0.05$) and low RMSEA (< 0.05) (Fig. 3; Table S1; Delgado-Baquerizo et al. 2017). The standard effects analyses based on SEM showed that all group variables collectively influenced the SOC composition of the grassy matrix (Fig. 4). Among these variables, free lipids were mostly controlled by herb characteristics (-0.60 ; Fig. 4a), bound lipids were mainly controlled by the soil characteristics (0.52 ; Fig. 4b), and lignin-derived phenols were mainly controlled by the climate (-0.79) and shrub characteristics (0.65 ; Fig. 4c). By comparison, soil properties had the greatest standard effects on the free lipids and bound lipids in the shrub patches (-0.74 and 0.85 ; Fig. 4ab), while the climate was the most important factor driving the lignin-derived phenols (-0.76 ; Fig. 4c). Conversely, herb characteristics had little effect on the SOC components in the shrub patches. These results further confirm that shrub encroachment exerts important influences on the SOC composition in the grasslands, including the grassy matrix (Fig. 4; Eldridge et al. 2011; Zhou et al. 2018). Previous studies have demonstrated that SOC composition is mainly controlled by vegetation (Kohl et al. 2017). However, our results indicate that the

Fig. 4 Structural equation modeling showing the standard total effects (black), direct effects (red) and indirect effects (blue) of climate, shrub, herb and soil characteristics on the free lipids (a), bound lipids (b) and lignin-derived phenols (c) in grassy matrix (left) and shrub patches (right) in Inner Mongolia



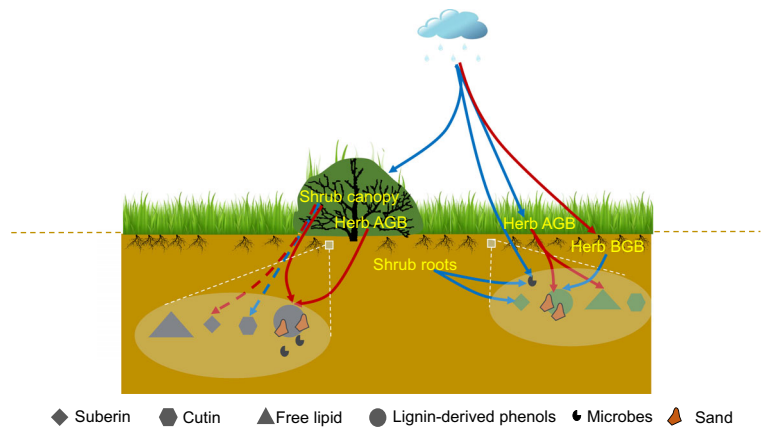
soil properties were also important drivers (Fig. 4). The unexplained variance in SOC composition might be ascribed to variables that were not included in our models, such as topography (Wu and Archer 2005) and the microbial community (Li et al. 2017).

Conclusions

Our field investigation along a climate gradient represents the first assessment of the relative contributions of the biotic and abiotic factors that drive the SOC components in Inner Mongolian

shrub-encroached grasslands (Fig. 5). Our study highlights that although shrub encroachment did not significantly change the topsoil OC contents, it could still increase the soil heterogeneity by changing the SOC components, especially the bound lipids and lignin-derived phenols. In addition, leguminous shrubs could influence the degradation of lignin-derived phenols by transferring nitrogen to the grassy matrix via their horizontal root system. The Pearson correlations showed that the bound lipids and lignin-derived phenols were strongly influenced by temperature and precipitation, respectively. The herb BGB made a great contribution to the SOC of the grassy matrix. In

Fig. 5 Schematic showing how the abiotic and biotic factors influence the soil organic carbon components in the shrub patches and grassy matrix in the shrub-encroached grasslands in Inner Mongolia. Blue arrows represent the positive correlations, while red arrows represent the negative correlations



addition, the bound lipids were protected by the clay fractions, while the physical protection of lignin-derived phenols was not observed in our study area. The herb characteristics and shrub density were the most important variables that controlled the SOC components in the grassy matrix, while the individual patch cover and soil pH were the most important variables in the shrub patches. Furthermore, our SEM analysis revealed that the climate, vegetation and soil characteristics collectively influenced the SOC compounds in the grassy matrix, while the soil properties had this effect in the shrub patches. Our findings provide new insights into the response and relevant mechanisms of soil carbon to vegetation shifts. Our results will also be helpful in the development of carbon models and guiding grassland management.

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