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Soil organic matter dynamics under different land-use in grasslands in Inner Mongolia (northern China)

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Abstract

We examined bulk soil properties and molecular biomarker distributions in surface soils from Inner Mongolia grasslands in order to understand the responses of soil organic matter to different land-use. The total of sixteen soils were collected from severely degraded grassland by grassland by grassland without encourter and without encourter and the severely degraded grassland by grassland by

- ⁵ degraded grassland by overgrazing (DG), native grassland without apparent anthropogenic disturbance (NG), groundwater-sustaining grassland (GG) and restored grassland from previous potato cropland (RG). Compared to NG, soil organic carbon content was lower by 50 % in DG, but higher by six-fold in GG and one-fold in RG. The δ^{13} C values of soil organic carbon were $-24.2\pm0.6\%$ in DG, $-24.9\pm0.6\%$ in NG, $-25.1\pm0.1\%$
- ¹⁰ in RG and $-26.2 \pm 0.6 \%$ in GG, reflecting different degradation degrees of soil organic matter or different water use efficiencies. The soils in DG contained the lowest abundance of aliphatic lipids (*n*-alkanes, *n*-alkanols, *n*-alkanoic acids, ω -hydroxylalkanoic acids and α -hydroxylalkanoic acids) and lignin-phenols, suggesting selective removal of these biochemically recalcitrant biomarkers with grassland degradation by microbial
- ¹⁵ respiration or wind erosion. Compared to NG, the soils in GG and RG increased ω hydroxylalkanoic acids by 60–70%, a biomarker for suberin from roots, and increased α -hydroxylalkanoic acids by 10–20%, a biomarker for both cutin and suberin. Our results demonstrate that the groundwater supply and cultivation-restoration practices in Inner Mongolia grasslands not only enhance soil organic carbon sequestration, but
- also change the proportions of shoot vs. root-derived carbon in soils. This finding has important implications for global carbon cycle since root derived aliphatic carbon has a longer residence time than the aboveground tissue-derived carbon in soils.

1 Introduction

Soil organic carbon, representing 80 % of terrestrial active carbon pool, plays a key role in the global carbon cycle (Janzen, 2004; Schlesinger and Andrews, 2000; Watson, 2000). Recently increasing human activities such as stock grazing, cultivation, defor-



estation and plantation substantially change the carbon balance between soil and atmosphere and thereby mitigate or accelerate global climate changes. Guo et al. (2007) reported that soil carbon stock to 100 cm decreased by 20 % after 16 year plantation in a native pasture of Australia, while Islam et al. (2000) found 44 % decline in soil quality

in a tropical forest ecosystem of Bangladesh under cultivation. The change in quality and quantity of soil organic matter inevitably affects various terrestrial ecosystem functions such as the soil fertility, biological diversity and biomass productivity (Kaiser et al., 2010).

Grasslands are important ecological landscapes in China and account for 40% of the national land area (ca. 4000000 km²) (National Statistics Bureau of China, 2002). These grasslands are mainly distributed in the semiarid and semi-humid northern China (e.g., Inner Mongolia) and Tibet Plateau and are highly susceptible ecosystems for changes in climate and land-use (He et al., 2008; Xiao et al., 1995; Zhou et al., 2007). Previous studies have demonstrated that improper land-use can cause grass-

- ¹⁵ land degradation and even desertification in Inner Mongolia (e.g. Cui et al., 2005; Su et al., 2005; Wang et al., 2013; Zhao et al., 2007). However, most of these studies focused on bulk soil properties such as total organic carbon and nitrogen contents. Since SOM consists of heterogeneous mixtures of plant, microbe and animal-derived residues with different environmental stabilities (Baldock and Skjemstad, 2000; Kögel-
- ²⁰ Knabner, 2002), the investigation for different fractions and even molecular compositions of SOM is indispensable for understanding carbon dynamics under different land-use practices (Leifeld and Kögel-Knabner, 2005).

Organic matter biomarkers are compounds specific for certain organisms (e.g., higher plants, fungi and bacteria) or tissues (e.g., leaves and root) (Kögel-Knabner,

25 2000; Otto et al., 2005). Free lipids from plant waxes and biopolymers such as suberin and cutin are the major sources of the aliphatic constituents of SOM, while lignin is an important contributor to aromatic components of SOM (Kögel-Knabner, 2002). Over the past decades, biomarker approaches have been successfully used to estimate SOM responses to different environmental changes such as soil warming (e.g. Feng et al.,



2008), vegetation shifts (e.g. Pisani et al., 2013) and elevated atmospheric CO_2 (e.g. Wiesenberg et al., 2008).

In this study, we analyzed the composition and distribution of bulk organic matter and molecular biomarkers in grassland soils from Inner Mongolia, northern China (Fig. S1).

- ⁵ These grasslands have developed in the same climate region and have same dominant grass species, but are subject to different levels of human disturbances. The main objectives of our study are to understand the chemical characteristics of SOM at the bulk and molecular levels in temperate grasslands in Inner Mongolia and to evaluate influences of land-use practices on SOM. We also attempt to answer whether certain components of SOM such as aliphatic lipids, cutin/suberin and lignin, usually thought
- components of SOM such as aliphatic lipids, cutin/suberin and lighin, usually thought to be refractory components, are preferentially preserved with changes in grassland uses.

2 Materials and methods

2.1 Study area and sampling

¹⁵ Our study was conducted on the Keshiketengqi Grassland in the Inner Mongolia Autonomous Region (northern China), being 400 km away from Beijing (Fig. S1). The semi-arid climate has a long, cold winter (November to March) and a short summer (June to August). The mean annual temperature is –1.5 °C and the mean annual precipitation is 400 mm (Feng and Zhao, 2011; Tan et al., 2013). The soil is classified as a chestnut soil.

Four experimental fields with the size of $100 \text{ m} \times 100 \text{ m}$ were chosen in our study (Fig. 1), representing severely degraded grassland (DG; $42^{\circ}37' \text{ N}$, $117^{\circ}05' \text{ E}$), native grassland (NG; $42^{\circ}35' \text{ N}$, $117^{\circ}05' \text{ E}$), groundwater-sustaining grassland (GG; $42^{\circ}32' \text{ N}$, $117^{\circ}08' \text{ E}$) and restored grassland from previous cropland (RG; $42^{\circ}33' \text{ N}$, $117^{\circ}13' \text{ E}$).

²⁵ The DG has been subject to intense sheep and cattle grazing and trampling for over 15 years and its vegetation is sparse. At NG, grazing activity is seldom and there was no



apparent anthropogenic disturbance. Pristine grassland at the site RG was converted into a potato cropland in 1990, and the cultivation activities lasted until 2010. Since then, this cropland was restored to the grassland by planting indigenous grasses and banning stock grazing. The GG is in a lowland area and supplied by groundwater in summer (June to September) when rainfall is relatively high. Similar to NG, the grazing activity is seldom at GG. Among four sampling sites, vegetation in GG is the best developed. Since the NG is subject to the least anthropogenic impact and resembles the major native grasslands in the Keshiketenggi Grassland, it is regarded as the ref-

erence site. In May 2012, four soil samples (0-10 cm) were collected from each site by a soil auger. After sampling, the soils were freeze dried at -40° C and passed through a 2 mm sieve to remove large roots and coarse particles. Soil samples were stored at -20° C until further analyses.

2.2 Grain size, elemental and stable isotope analyses

The detailed methods for grain size measurement has been described in Sun ¹⁵ et al. (2011). About 1.0 g dried soils were reacted with excess 1 M hydrochloric acid and then with hydrogen peroxide to remove carbonates and oxidize organic matter, respectively. After that, sodium hexametaphosphate was added, and the solutions were allowed to settle for 24 h. After ultra-sonication for 1 min, the grain size distributions were measured by a Mastersizer 2000 Laser Particle Size Analyzer. The scan range ²⁰ was from 0.02 to 2000 μ m, and categorized into three fractions: sand (20 ~ 2000 μ m), silt (2 ~ 20 μ m) and clay (< 2 μ m).

The procedures for elemental and stable isotopic analyses were modified from Wu et al. (2014). The dried soil samples were treated with 1 M hydrochloric acid to remove inorganic carbon. The residues were analyzed by a CHNOS Element Analyzer (Elementar Germany) for total organic carbon (TOC) and total nitrogen (TN), while the δ^{13} C and δ^{15} N were determined on a Flash EA1112HT coupled with MAT 253 (Thermo Fisher Scientific, Inc). The standard deviations based on the replicate analyses were 0.02 % for TOC, 0.005 % for TN, 0.15 ‰ for δ^{13} C and 0.10 ‰ for δ^{15} N.



2.3 Extractions of free lipids, bound lipids and lignin-phenols

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Figure 2 shows the procedures for biomarkers analyses. About 6 g soil samples were mixed with $2 \mu g$ squalane and extracted with ultrasonication three times, each with 15 mL dichloromethane: methanol (1:1; v/v) for 15 min. The combined solvent extracts were filtered through glass fiber columns, concentrated by rotary evaporation, and dried under N₂ gas in 2 mL glass vials.

Soil residues after the solvent extraction were subjected to base hydrolysis to cleave bound lipids (Otto et al., 2005). After addition of 1 M methanolic KOH (15 mL) and 20 μ g 5 α -cholestane (internal standard), the air-dried soil residues (~ 3 g) were sealed and heated at 100 °C for 3 h. After cooling, the suspension was acidified to pH 1 by addition of 6 M HCI. The bound lipids were recovered from the water phase by liquid– liquid extraction with 20 mL ethyl acetate three times. Anhydrous Na₂SO₄ was added to remove any remaining water. The ethyl acetate extracts were concentrated by rotary evaporation, transferred to 2 mL glass vials and dried under nitrogen gas.

¹⁵ Subsamples after the base hydrolysis were oxidized with CuO to release ligninderived phenols. Teflon-lined bombs were loaded with 2 g of dry soil, 1 g CuO, 100 mg ammonium iron (II) sulfate hexahydrate and 15 mL of 2 M NaOH. The teflon vessels were purged with nitrogen gas for oxygen exhaustion, sealed and heated for 2.5 h at 170 °C. After reaction, the liquid was decanted into a 50 mL centrifuge tube, and the residue was washed twice, each with 10 mL deionized water. The combined washings were centrifuged for 10 min at 3000 rpm. The supernatant was transferred into another centrifuge tube, acidified to pH 1 by addition of 6 M HCl and kept for 1 h at room temperature in the dark to prevent reactions of cinnamic acids. After centrifugation for 10 min at 3000 rpm again, the supernatant was transferred to a separation funnel and

²⁵ liquid–liquid extracted three times, each with 20 mL ethyl acetate. Anhydrous Na₂SO₄ was added to remove water. The ethyl acetate extracts were concentrated by rotary evaporation, transferred to 2 mL glass vials and dried under nitrogen gas.



2.4 Derivatization of extracts and instrumental analyses

The base hydrolysis extracts were first methylated by reaction of 14 % BF₃ : MeOH for 90 min at 70 °C. The bound lipids were obtained by liquid-liquid extraction with 2 mL hexane $(3\times)$, and dried under N₂ stream. After that, all biomarkers from solvent extracts, base hydrolysis and CuO oxidation were trimethylsilyl (TMS) derivatized by reaction with 50 µL N.O-bis-(trimethylsilyl) trifluoroacetamide (BSTFA) and 10 µL pyridine for 2 h at 60 °C. After cooling, DCM was added to dilute to 1 mL.

The biomarkers were identified by a gas chromatography-mass spectrometry (GC-MS). This instrument was composed of an Agilent 7890A GC coupled to an Agilent 5973N guadruple mass selective detector. The biomarkers were separated by a DB-5 10 capillary column (30 m; 0.25 mm i.d.; 0.25 μm film thickness). A 1 μL of samples was injected in the splitless mode under a constant flow (1.0 mLmin⁻¹). The injector temperature was 300°C. High pure helium (> 99.999 %) was used as carrier gas. The mass spectrometer was operated in the electron impact mode (70 eV). The mass scan range

was 50 to 550 Da. 15

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An Agilent 7890A GC coupled to a flame ionization detector (FID) was used for quantification of biomarkers. The separation was achieved on an HP 5 capillary column (30 m; 0.32 mm i.d.; 0.25 µm film thickness). The injector temperature was 300 °C. A 1 µL volume of sample was injected in splitless mode under a constant flow $(1.5 \,\mathrm{mL\,min^{-1}})$. Helium (purity > 99.999%) was the carrier gas.

Oven programs for GC-MS and GC-FID were set as follows: temperature held at 60 °C for 1 min, increased to 300 °C at a rate of 6 °C min⁻¹ with a final isothermal hold at 300 °C for 20 min. For solvent extracts and base hydrolysis, the biomarkers were quantified by comprising the peak areas of biomarkers to an internal standard (squalane

for free lipids and 5α -cholestane for bound lipids), while the amounts of lignin phenols 25 were determined by the calibration curves of eight lignin-phenol standards.



2.5 Statistical analysis

The program package SPSS 18.0 (Illinois, USA) was used for statistical analyses. Oneway ANOVA analysis was conducted to examine the differences in bulk properties and molecular biomarkers among soils from different grasslands. All analyses were performed with a significance level of P < 0.05.

3 Results

5

3.1 Elemental and isotopic compositions

Table 1 shows the grain size distributions, elemental and isotopic compositions of grassland soils. All soils were dominated by sand (64.4 to 87.3%), while clay fractions accounted for only 1.7 to 6.4% in an increasing order of DG, NG, GG and RG. The TOC content ranged from $3.2 \pm 0.2 \text{ gkg}^{-1}$ (DG) to $46.6 \pm 16.7 \text{ gkg}^{-1}$ (GG), while the TN content gradually increased from $0.32 \pm 0.15 \text{ gkg}^{-1}$ (DG) to $3.9 \pm 1.5 \text{ gkg}^{-1}$ (GG). The values of δ^{13} C and δ^{15} N varied from $-24.2 \pm 0.6\%$ (DG) to $-26.2 \pm 0.6\%$ (GG) and $0.36 \pm 0.3\%$ (DG) to $4.8 \pm 1.6\%$ (GG), respectively. The C/N ratio increased from 9.9 to 12.5 in an order of DG, NG, GG and RG. A strong linear correlation was observed between the δ^{13} C and C/N ratio (r = -0.667, n = 16, p < 0.01), suggesting that both parameters are controlled by same factors such as the source and degradation stage of SOM.

3.2 Compositions and distributions of solvent extracts

²⁰ The major components of the solvent extracts were *n*-alkanoic acids, *n*-alkanols, ω -hydroxyalkanoic acids, *n*-alkanes, steroids and sugars (Fig. S2). The concentrations of each type of biomarkers were summarized in Fig. 3. Long-chain (> C₂₀) aliphatic lipids including *n*-alkanes, *n*-alkanols and *n*-alkanoic acid are derived from epicuticular waxes of vascular plants, while short-chain (< C₂₀) aliphatic lipids are of a microbial



origin (Otto et al., 2005; Simoneit, 2005). The soils from NG contained the highest concentrations of free aliphatic lipids $(3.53 \pm 1.18 \text{ mgg}^{-1} \text{ OC})$, followed by those from DG $(2.23 \pm 1.03 \text{ mgg}^{-1} \text{ OC})$, RG $(1.36 \pm 0.73 \text{ mgg}^{-1} \text{ OC})$ and GG $(1.20 \pm 0.65 \text{ mgg}^{-1} \text{ OC})$. Since the biomarker abundances have been normalized to organic carbon, the lowest concentrations of free aliphatic lipids at GG and RG were attributed to a dilution effect of their relatively high TOC contents. The homologues of *n*-alkanoic acids $(C_{14} \text{ to } C_{32})$ were detected at the total concentrations of $1.20 \pm 0.39 \text{ mgg}^{-1} \text{ OC}$ in NG, $0.54 \pm 0.24 \text{ mgg}^{-1} \text{ OC}$ in DG, $0.40 \pm 0.37 \text{ mgg}^{-1} \text{ OC}$ in RG and $0.38 \pm 0.23 \text{ mgg}^{-1} \text{ OC}$ in GG. These *n*-alkanoic acids were characterized by the strong even-over-odd carbon numbered predominance and the C_{max} at C_{16} and C_{24} , suggesting a mixed input of soil microbes and vascular plants. Besides saturated acids, mono- and di-unsaturated C_{16} and C_{18} *n*-alkanoic acids were also detected at the concentrations lower than $0.25 \text{ mgg}^{-1} \text{ OC}$. These short-chain unsaturated alkanoic acids are mainly biosynthesized by bacteria and fungi.

The homologous *n*-alkanes (C₂₂ to C₃₃) displayed a strong odd-over-even predom-15 inance and the maximum abundance (C_{max}) at C₂₉. The soils in NG were composed of the highest abundant *n*-alkanes $(0.33 \pm 0.08 \text{ mgg}^{-1} \text{ OC})$, followed by soils in DG $(0.32 \pm 0.12 \text{ mgg}^{-1} \text{ OC})$, RG $(0.31 \pm 0.20 \text{ mgg}^{-1} \text{ OC})$ and GG $(0.06 \pm 0.02 \text{ mgg}^{-1} \text{ OC})$. Series of *n*-alkanols (C_{18} – C_{32}) presented a strong even-over-odd predominance with the concentration of $0.66 \pm 0.18 \text{ mgg}^{-1} \text{ OC}$ (NG), $0.50 \pm 0.13 \text{ mgg}^{-1} \text{ OC}$ (DG), $0.32 \pm$ 20 $0.19 \text{ mgg}^{-1} \text{ OC}$ (RG) and $0.19 \pm 0.08 \text{ mgg}^{-1} \text{ OC}$ (GG). Five ω -hydroxyalkanoic acids (C₂₂ to C₂₈) were detected with the concentrations of $0.63 \pm 0.24 \text{ mgg}^{-1}$ OC in NG, $0.26 \pm 0.16 \text{ mgg}^{-1} \text{ OC}$ in GG, $0.23 \pm 0.13 \text{ mgg}^{-1} \text{ OC}$ in DG and $0.16 \pm 0.19 \text{ mgg}^{-1} \text{ OC}$ in RG. These compounds were abundant in suberin of roots and bark of higher plants (Otto and Simpson, 2006b; Pisani et al., 2013). Steroids including β -sitosterol, 25 cholesterol, campesterol and stigmasterol were only minor components (< 0.21 ± 0.14 mg g⁻¹ OC). Cholesterol is of a mixture origin from plants, fungi and animals, while other steroids are predominantly derived from higher plants (Otto et al., 2005 and ref-



erences therein). Several free carbohydrates including glucose, mannose and sucrose were detected and their abundance was lower than $0.30 \pm 0.46 \text{ mgg}^{-1} \text{ OC}$.

3.3 Compositions and distributions of bound lipids

Homologous aliphatic lipids (alkanoic acids, alkanols, alkanedioic acids, hydroxyalka-5 noic acids) and steroids were major constituents of the base hydrolysis products (Figs. 3 and S2). These bound aliphatic lipids are mainly derived from the biomacromolecular suberin and cutin of vascular plants (Kögel-Knabner, 2002; Nierop et al., 2003; Otto et al., 2005). Among them, *n*-alkanoic acids ranging from C_{14} to C_{32} and with C_{max} at C_{16} , mono- and di-unsaturated $n-C_{16}$ and $n-C_{18}$ acids are dominant with the total concentrations of $7.1 \pm 0.9 \text{ mgg}^{-1}$ OC (RG) to $13.4 \pm 2.1 \text{ mgg}^{-1}$ OC 10 (NG). Branched alkanoic acids including iso-C₁₄, iso-C₁₅, iso-C₁₆, iso-C₁₇ and iso-C₁₈ were also detected and varied in concentrations from $1.05 \pm 0.23 \text{ mgg}^{-1}$ OC (GG) to 2.28 ± 0.37 mgg⁻¹ OC (NG). Branched alkanoic acids, along with short-chain *n*-alkanoic acids $(< C_{20})$ reflect inputs from soil microbes since these compounds are usually not biosynthesized by higher plants (e.g., Otto et al., 2005). After *n*-alkanoic acids, ω -15 hydroxyalkanoic acids from C₁₆ to C₂₆ and with C_{max} at C₂₀ or C₂₂ were the secondarily most abundant type of biomarkers with the concentration of $1.85 \pm 1.80 \text{ mg g}^{-1}\text{ OC}$ (DG) to $5.05 \pm 2.07 \text{ mgg}^{-1}$ OC (GG). The α, ω -alkanedioic acids including C₈, C₉, C₁₀, C_{11} and long-chain even numbered acids from C_{16} to C_{28} varied from 1.68 ± 0.51 to $2.38 \pm 0.38 \text{ mgg}^{-1}$ OC. The homologues of C₁₆–C₂₈ α -hydroxyalkanoic acids and *n*-20 alkanols ($C_{16}-C_{30}$) were minor components of bound lipids (< 2.0 mg g⁻¹ OC).

3.4 Lignin degradation products after CuO oxidation

Eight lignin derived phenols were identified in the CuO oxidation extracts of the soil residues after base hydrolysis, including three vanillyl (V; vanillin, acetovanillone, vanillic acid), three syringyl (S; syringaldehyde, acetosyringone, syringic acid) and two cin-

²⁵ lic acid), three syringyl (S; syringaldehyde, acetosyringone, syringic acid) and two cinnamyl (C; *p*-coumaric acid and ferulic acid) groups (Fig. S2). The concentrations of



total lignin phenols (V + S + C) were $9.5 \pm 2.1 \text{ mgg}^{-1}$ OC in GG, $12.9 \pm 2.7 \text{ mgg}^{-1}$ OC in RG, $13.3 \pm 5.1 \text{ mgg}^{-1}$ OC in NG, and $22.2 \pm 7.7 \text{ mgg}^{-1}$ OC in DG (Fig. 3). The ratios of C: V and S: V were calculated to estimate the source of lignins since S-unit is absent in gymnosperms, while C-unit is specific for non-woody tissues (Hedges and Mann, 1979). In addition, the ratios of acids over corresponding aldehydes (Ad/Al) are 5 indicative of degradation stage of SOM since aldehydes are oxidized into corresponding acids with lignin degradation by white-rot and brown-rot fungi (e.g. Hedges et al., 1988). Non-woody tissues such as grasses and leaves have been reported to have higher Ad/Al values (0.2–1.6) than angiosperm and conifer wood (0.1–0.5) (Hedges et al., 1988; Otto and Simpson, 2006a). In our study, all soils showed similar S/V 10 values ranging from 0.67 to 0.79 (Table 2), in agreement with the same vegetation cover in all grasslands. Significantly differences of $(Ad/AI)_{V}$ (0.35 ~ 1.47 in an increasing order of NG < RG < DG < GG) and $(Ad/Al)_{S}$ (0.58 ~ 2.26 in an increasing order of NG < DG < RG < GG) were observed (Table 2), reflecting different degradation degrees

15 of lignin.

4 Discussion

4.1 Changes in bulk soil organic matter properties under different land-use

Over recent decades, the land use such as cultivation and grazing have caused considerable reduction of vegetation cover, destruction of topsoil structure and compaction of soil in the Inner Mongolia grasslands in northern China (Cui et al., 2005; Wang et al., 2013). Consequently, degradation and even desertification have become a common phenomenon in the Inner Mongolia grasslands (Su et al., 2005). A series of strategies including grazing exclusion, planting indigenous plants, and returning farmlands to grasslands have been implemented to protect the regional environments (Jiang et al.,

²⁵ 2006). However, the effects of these strategies on the quality and quantity of SOM are not fully understood.



In this study, a strong correlation was observed between TOC and TN (r = 0.999, n = 16, p < 0.01), suggesting that nitrogen is primarily associated with SOM. There are significant differences in soil organic carbon and nitrogen contents among four sampling sites. A six-fold increase of soil organic carbon content in GG compared to NG

- ⁵ suggests that enhanced water supply can be an effective strategy for the sequestration of organic carbon in soils. This is not surprising because the Inner Mongolia grasslands are mainly distributed in semiarid and arid regions where water is one of limiting factors for vegetation growth. In addition, increasing water content in soils can reduce oxygen diffusion rate and thereby decreases the decomposition of SOM. Relative to NG, soil
- ¹⁰ organic carbon and nitrogen contents in RG is about one-fold higher, likely owing to two factors: (1) potato cultivation and fertilization for two decades (1990–2010) resulted in the accumulation of organic carbon and nitrogen in soils; and (2) banning grazing for 2 years (2010–2012) increased organic carbon inputs to soil. Among four field sites, RG is characterized by the highest δ^{15} N values (4.5 ± 0.4‰; Table 1), suggesting an important effect of previous cultivation activities. In the Inner Mongolia, stock manures
- were commonly used as fertilizers, which has remarkably higher δ^{15} N values (10 to 20‰) than from plants (close to 0‰) (Bateman et al., 2005; Kohl et al., 1973).

In contrast to GG and RG, about 50 % reduction of soil organic carbon and nitrogen contents was observed for DG, suggesting that overgrazing caused sparse vegetation

- ²⁰ cover and thereby decreased organic matter inputs to soils. In addition, with the lack of vegetation, the strong northwestern wind during Asian winter monsoon can accelerate soil erosion particularly for fine particles (e.g., Cui et al., 2005; Su et al., 2005; Zhao et al., 2007). This wind erosion effect is reflected by significantly lower clay fraction in DG (1.7±0.9%) than NG (4.6±1.6%; Table 1). Since SOM is enriched in fine particles
 ²⁵ (Anderson et al., 1981; Nichols, 1984), the loss of clay fractions further reduced SOM
- (Anderson et al., 1981; Nichols, 1984), the loss of clay fractions further reduced SOM levels in DG.

The δ^{13} C value is a useful tracer for organic matter sources (e.g., C3 and C4 plants) degradation stage (Farquhar et al., 1989; O'Leary, 1981) and water use efficiency (Wang et al., 2008). In our study, the dominant grass species, *Leymus chinensis* and



Stipa grandis, are C3 plants, and thus the effect of C3/C4 plant shift on the δ¹³C value was not considered. The degradation of soil organic matter can cause δ¹³C values to positively shift by several per mil due to the preferential utilization of light carbon isotope by soil microbes (Feng, 2002; Natelhoffer and Fry, 1988), while the δ¹³C values of C3
⁵ plant leaves decreases with increasing rainfall by a coefficient of ca. -0.4‰/100mm (Wang et al., 2008). Among four sampling sites, GG presented significant difference in δ¹³C values of SOM (-26.2±0.6‰) from other three sites (-24.2 to -25.1‰; Table 1), reflecting the largest inputs of fresh, undegraded organic matter due to high primary productivity or the change of water use efficiency with groundwater supply.

10 4.2 Molecular compositions of SOM in different grasslands

Compared to NG, DG contained significantly lower concentrations of total free lipids, cutin/suberin-monomers and lignin-phenols (Fig. 3). Such difference can be explained by two mechanisms. First, aliphatic and lignin biomarkers are preferentially degraded constituents of bulk SOM. This explanation is consistent with previous results that the turnover of lignin is faster than bulk SOM (e.g., Dignac et al., 2005; Gleixner 15 et al., 2002; Thevenot et al., 2010) as well as soil fungi and bacteria synthesized compounds (e.g., Amelung et al., 2008). In contrast, carbohydrates such as glucose, mannose and sucrose, usually thought to be biochemically labile compounds (Lorenz et al., 2007; Schmidt et al., 2011), were significantly higher abundance in DG $(304.0 \pm 460.5 \mu g g^{-1} OC)$ than NG $(56.1 \pm 21.9 \mu g g^{-1} OC)$; Fig. 3). The enrichment of 20 free carbohydrates in DG is indicative of higher proportions of microbial carbon in degraded soils, supported by their heaviest δ^{13} C values of SOM (Table 1). Our results support the hypothesis that molecular structure does not control long-term decomposition of organic matter in mineral soils (Schmidt et al., 2011 and references therein). The

²⁵ persistence of SOM is actually dependent on complex interactions between organic matter and its environment such as compound chemistry, climate, water availability, soil pH and soil microbial community (Schmidt et al., 2011; Thevenot et al., 2010).



Another explanation for the low concentration of aliphatic lipids and lignin in DG is physical removal of fine fractions. It has been reported that lignin and aliphatic components in soils are enriched in fine particles (Quenea et al., 2004; Thevenot et al., 2010). In the arid and semiarid Inner Mongolia, strong wind erosion can disproportion-

- ⁵ ally remove clay contents of grasslands (e.g., 1.7 % in DG vs. 4.6 % in NG; Table 1), and thereby cause a decrease in proportions of aliphatic and lignin biomarkers in SOM. The investigation for the different size fractions of soils is great needed in future study in order to answer which mechanism is more important for observed change patterns of different biomarkers.
- ¹⁰ With land-use changes such as cultivation activities, planting indigenous grasses, banning grazing and groundwater supply, the abundance of ω -OH alkanoic acids and α -OH alkanoic acids in soils increased in RG and GG compared to NG, whereas the abundance of lignins and free lipids significantly decreased (Fig. 3). In the grassland ecosystems, long-chain ω -OH alkanoic acids are predominantly derived from suberin
- ¹⁵ of roots (Kolattukudy and Espelie, 1989; Otto and Simpson, 2006b), whereas α -OH alkanoic acids are characteristic of both cutin of leaf waxes and suberin of roots (Deleeuw et al., 1995; Otto and Simpson, 2006a). In order to estimate response of biomarkers to land-use changes, we defined an enrichment factor ($E_{\rm C}$) which is a ratio of the biomarker concentration in the disturbed grasslands over the native grasslands.
- ²⁰ The mean values of $E_{\rm C}$ were 1.69 (GG) and 1.66 (RG) for ω -OH alkanoic acids, 1.24 (GG) and 1.18 (RG) for α -OH alkanoic acids, but only 0.64 (GG) and 0.87 (RG) for lignin-phenols (Table 3), suggesting that more suberin-derived carbon have been sequestered in soils.

5 Conclusions

By analyzing bulk and biomarker characteristics of grassland soils in the Inner Mongolia (China), we confirm that land-use changes alter soil organic carbon not only in quantity but also in molecular compositions. Over 10 year overgrazing has caused



about 50% reduction of soil organic carbon and nitrogen, whereas the groundwater supply and potato cultivation-restoration activities are beneficial for the accumulation of soil organic carbon and nitrogen. With land-use changes, the root-derived aliphatic carbon (e.g., suberin) are preferentially preserved in soils compared to aboveground-

- ⁵ derived carbon. This finding is important for prediction of global carbon cycling under climate and land-use changes since root-derived carbon (particularly aliphatic suberin) has longer resident time than aboveground carbon (Rasse et al., 2005) and other type organic carbon (e.g., lignin) in soils (Feng and Simpson, 2007; Lorenz et al., 2007). Future studies will be investigation for other types of inert carbon such as charcoal to understand the response of different cell organic matter to land use changes.
- ¹⁰ understand the response of different soil organic matter to land-use changes.

Supplementary material related to this article is available online at http://www.biogeosciences-discuss.net/11/5613/2014/ bgd-11-5613-2014-supplement.pdf.

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	(%)	(%)	(%)	(gkg^{-1})	(gkg^{-1})	(‰)	(‰)		Cor
DG	1.7 ± 0.9^{2} c	11.0 ± 0.8b	87.3±5.6a	$3.2 \pm 0.2d$	0.32 ± 0.15d	-24.2 ± 0.6a	4.14 ± 0.50a		
NG	4.6 ± 1.6b	21.6 ± 7.8a	$73.8 \pm 9.4b$	$6.6 \pm 0.25c$	$0.65 \pm 0.25c$	-24.9 ± 0.6a	$3.31 \pm 0.45b$		
GG	5.3 ± 0.7a, b	26.0 ± 4.5a	$68.8 \pm 5.1b$	46.6 ± 16.7a	3.9 ± 1.5a	$-26.2 \pm 0.6b$	$2.77 \pm 0.27b$	SCL	
RG	6.4 ± 1.0a	29.2 ± 5.6a	$64.4 \pm 6.5b$	15.9 ± 3.4b	$1.3 \pm 0.03b$	–25.1 ± 0.1a	4.51 ± 0.37a	S	

 1 Clay (< 2 μm), silt (2 $\sim 200\,\mu m$) and sand (200 $\sim 2000\,\mu m$).

² All values are expressed as means of four replicates \pm standard errors. * Different letters (a, b, c, d) in column indicate significant difference (P < 0.05).





Table 2. Source and degradation parameters of lignin phenols in grassland soils in Inner Mongolia. DG: degraded grassland by overgrazing; NG: native grassland without apparent anthropogenic disturbance; GG: groundwater-sustaining grassland; RG: restored grassland from previous cultivated land.

Fields	C/V	S/V	$(Ad/AI)_V$	$(Ad/AI)_{S}$
DG	0.26 ± 0.04 b	0.75 ± 0.29a	1.43 ± 0.55a	$0.59 \pm 0.49c$
NG	$0.24 \pm 0.02b$	0.67 ± 0.05a	$0.58 \pm 0.07b$	$0.35 \pm 0.03c$
GG	0.47 ± 0.16a	0.79 ± 0.4a	2.26 ± 0.99a	1.47 ± 0.32a
RG	0.28 ± 0.04 b	0.71 ± 0.09a	0.98 ± 0.13a	$0.76 \pm 0.09b$

* Values are expressed as mean \pm SD of four determinations. Different letters (a, b, c, d) indicate significant difference (P < 0.05).

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Table 3. Enrichment factors (Ec) of biomarkers in the grassland soils (Inner Mongolia) under different land use practices. DG: degraded grassland by overgrazing; NG: native grassland without apparent anthropogenic disturbance; GG: groundwater-sustaining grassland; RG: restored grassland from previous cultivated land. FA = fatty acids.

Free lipids	<i>ω</i> -FA	α-FA	Lignin
0.63 ± 0.29b	$0.62 \pm 0.60c$	0.31 ± 0.18b	$0.90 \pm 0.03b$
1.00 ± 0.33a	$1.00 \pm 1.52b$	1.00 ± 0.53a	$1.00 \pm 0.06a$
$0.34 \pm 0.18c$	1.69 ± 0.69a	1.24 ± 0.43a	$0.64 \pm 0.14c$
$0.39 \pm 0.21c$	1.66 ± 0.58a	1.18±0.37a	$0.87 \pm 0.18b$
	Free lipids $0.63 \pm 0.29b$ $1.00 \pm 0.33a$ $0.34 \pm 0.18c$ $0.39 \pm 0.21c$	Free lipids ω -FA $0.63 \pm 0.29b$ $0.62 \pm 0.60c$ $1.00 \pm 0.33a$ $1.00 \pm 1.52b$ $0.34 \pm 0.18c$ $1.69 \pm 0.69a$ $0.39 \pm 0.21c$ $1.66 \pm 0.58a$	Free lipids ω -FA α -FA $0.63 \pm 0.29b$ $0.62 \pm 0.60c$ $0.31 \pm 0.18b$ $1.00 \pm 0.33a$ $1.00 \pm 1.52b$ $1.00 \pm 0.53a$ $0.34 \pm 0.18c$ $1.69 \pm 0.69a$ $1.24 \pm 0.43a$ $0.39 \pm 0.21c$ $1.66 \pm 0.58a$ $1.18 \pm 0.37a$

* Values are expressed as mean \pm SD of four determinations. Different letters (a, b, c, d) indicate significant difference (P < 0.05).



Fig. 1. Pictures for Inner Mongolia grasslands, DG: degraded grassland by overgrazing; NG: native grassland without apparent anthropogenic disturbance; GG: groundwater-sustaining grassland; RG: restored grassland from potato cropland.



Interactive Discussion



Fig. 2. Flow chart of bulk parameters and molecular biomarker analyses for grassland soils, Inner Mongolia. FA = fatty acids.







