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Tian Ma and Guohua Dai contributed equally to the paper.

Key Points:

- We present the first large-scale study of root- and shoot-derived carbon components in soils across the Chinese-Mongolian grasslands
- Root- and shoot-derived carbon components have a better preservation in the alpine than temperate grassland soils
- The incorporation efficiency of root components is more sensitive to environmental variations in the alpine than temperate soils

Supporting Information:

- Supporting Information S1
- Data Set S1
- Data Set S2
- Data Set S3

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Distribution and Preservation of Root- and Shoot-Derived Carbon Components in Soils Across the Chinese-Mongolian Grasslands

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Abstract The relative contribution of roots versus shoots to soil organic carbon (SOC) is a long-debated topic related to soil carbon formation and dynamics. However, the variation of root- and shoot-derived carbon in natural soils remains poorly constrained due to limited tools to separate them. Here we overcome the difficulty by analyzing biomarkers for root (suberin) and shoot (cutin) in grassland soils spanning >7,000 km on the Qinghai-Tibetan and Mongolian Plateaus. Coupled with a comprehensive list of climatic, edaphic, and vegetation variables, we assess their inputs, distribution, and preservation patterns. We find that while suberin was a root-specific tracer, cutin biomarkers that occurred only in trace amount in roots also had a minor but nonnegligible contribution from belowground sources due to the massive root mass in the alpine grasslands. Both components had higher SOC-normalized concentrations in the alpine than temperate grasslands, along with a higher preservation percentage against net primary productivity and a lower degradation parameter, suggesting a better preservation of these plant-derived lipid components in the alpine region. Moreover, while cutin and suberin abundances were affected by plant inputs and other SOC constituents, the root mass normalized incorporation efficiency of suberin was only positively correlated to SOC in both regions with a steeper slope in the alpine grasslands and also negatively correlated to root mass in the alpine soils. These results imply that root carbon incorporation is sensitive to environmental variations in the cold region, suggesting potentially more dynamic alterations to root carbon sequestration with environmental changes.

1. Introduction

Approximately 60 Gt of carbon is annually transferred from vegetation into soils globally (Pausch & Kuzyakov, 2018) in the form of aboveground litter and root deposits (including root litter and exudates) as the primary sources for soil organic carbon (SOC; Kögel-Knabner, 2002). Due to their varied chemical composition and decomposition patterns (Hamer et al., 2012; Puget & Drinkwater, 2001; Rasse et al., 2005), the relative input of shoots versus roots is a critical factor affecting the formation and persistence of SOC in terrestrial ecosystems (Kögel-Knabner, 2002). With shifting vegetation and altered carbon allocation under global changes (Jackson et al., 2017; Pausch & Kuzyakov, 2018), it is essential to differentiate the distribution and preservation patterns of root- versus shoot-derived carbon to improve our prediction of future soil carbon dynamics (Kätterer et al., 2011; Rasse et al., 2005).

While root exudates mainly consist of easily degradable organic matter, root litter contains high concentrations of plant structural components (such as lingo-cellulose and suberin) and hard-to-decay compounds such as tannins and shows a much slower turnover rate than plant shoots (Freschet et al., 2013; Sun et al., 2018; Xia et al., 2015). Along with the high volume of belowground relative to aboveground biomass (AGB; Jackson et al., 2017), plant roots are considered to play a more important role than shoots in SOC

accrual (Mendez-Millan et al., 2010; Rasse et al., 2005). For example, Kätterer et al. (2011) found that root-derived carbon was preferentially retained in agricultural soils, contributing 2.3 times more carbon to SOC than shoot residues. A recent review by Jackson et al. (2017) also suggests that ~45% of belowground inputs are stabilized as SOC versus ~8% for aboveground inputs in agricultural soils. Hence, it is particularly important to disentangle environmental influences on the preservation of root-derived carbon in order to understand and maximize soil carbon sequestration potentials.

However, due to limited analytical tools to separate root- versus shoot-derived carbon, the distribution of these components in the soil and its influencing factors remain poorly constrained. Suberin and cutin are polymeric matrices that form protective lipophilic cell wall barriers in plant roots, barks (suberin), and cuticles (cutin; Kögel-Knabner, 2002). In grasslands, suberin- and cutin-derived biomarkers have been used to trace root and shoot components in the soil, respectively, owing to their predominant occurrence in the respective plant tissues (Otto & Simpson, 2006). They hence provide ideal tools to assess the preservation of root- and shoot-derived components in natural soils. Previous studies have observed an accumulation of suberin relative to cutin along soil profiles (Filley et al., 2008; Nierop et al., 2003), mainly attributed to the recalcitrance of suberin as well as its close association with soil mineral matrix (Angst et al., 2016; Lin & Simpson, 2016; Spielvogel et al., 2014). However, the influence of plant (root) distribution on the variation of these biomarkers are poorly studied for the lack of vegetation information. For an accurate assessment on the persistence or preservation of root-derived carbon, plant root mass must be considered. Furthermore, most of previous studies focus on site-level variations in these SOC components, which do not allow a comprehensive assessment of environmental influences on the preservation of root versus shoot carbon in natural soils. For this purpose, large-scale investigations spanning a wide range of environmental gradients are needed.

Here we utilize two well-characterized large-scale transects across the alpine and temperate grasslands in China and Mongolia to examine the distribution of root- and shoot-derived components (suberin and cutin) in the surface soils over continental scales. Our previous study based on the similar grassland transects has shown different mechanisms regulating the distribution of solvent-extractable compounds in the soil, with climate-mediated decomposition processes dominating in the temperate grasslands and plant inputs being more important in the alpine region (Dai et al., 2018). It remains to be investigated whether plant shoot- and root-derived components exhibit varied fate from each other and in the alpine versus temperate grassland soils. Through compiling plant AGB and root mass along with climatic and edaphic variables at each sampling site, we comprehensively evaluate environmental influences on the distribution and preservation of cutin and suberin in the soil. Moreover, utilizing plant wax lipids measured along the similar grassland transects (Dai et al., 2018), we compare the fate of shoot- and root-derived hydrolysable lipids with those of “free” wax lipids in the soil. Considering the better preservation of wax lipids in the alpine grassland soils (Dai et al., 2018), we hypothesize that cutin and suberin have varied distribution patterns in the alpine and temperate grasslands. Moreover, as alpine grasslands are considered to be sensitive to environmental changes related to temperature increases, the preservation of root carbon is hypothesized to be more sensitive to environmental variations in the alpine region.

2. Materials and Methods

2.1. Study Area

This study includes two grassland-dominated transects across the alpine and temperate regions of China and Mongolia (Figure 1; Hou, 1982; United Nations Educational, Scientific and Cultural Organization (UNESCO), 1973). The alpine grassland transect spans >3,200 km across the Qinghai-Tibetan Plateau with elevations of 3,066–5,418 m above the sea level. Mean annual temperature is -0.5 ± 0.4 °C (mean \pm standard error; see supporting information Table S1) with the highest mean monthly temperature occurring in July. Mean annual precipitation (MAP) varies between 147 and 446 mm (WorldClim database; <http://www.worldclim.org>) with >80% occurring in May to September. The dominant vegetation types include alpine meadow (dominated by *Kobresia pygmaea*, *Kobresia humilis*, and *Kobresia tibetica*), alpine steppe (dominated by *Stipa purpurea*, *Carex lanceolata*, and *Stip subsessiliflora*), and alpine desert steppe (dominated by *Oxytropis ochrocephala*, etc.). Soil types vary along the transect with Leptosols, Cambisols, and

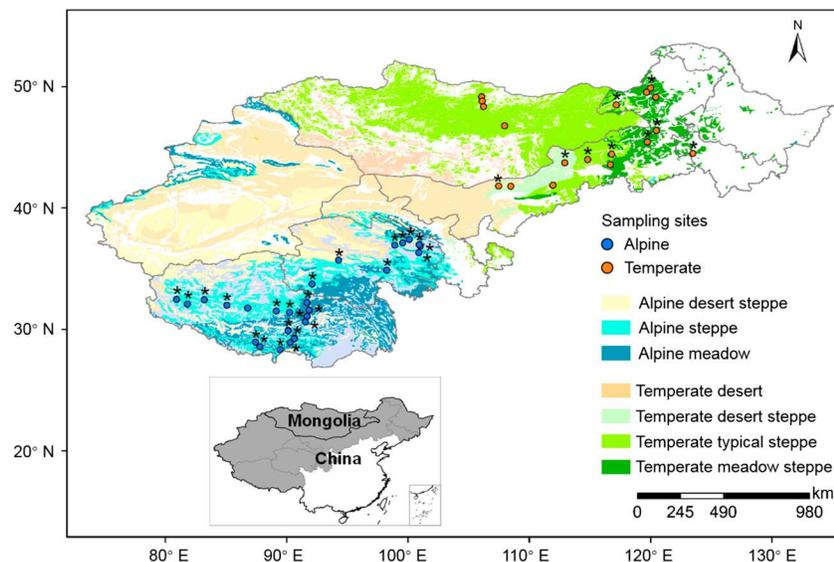


Figure 1. Study area and sampling sites. Soil samples were collected from 48 sampling sites from the alpine (blue) and temperate (orange) grasslands of China and Mongolia. Sites marked with an asterisk are the same as those in Dai et al. (2018). Land cover classification map derived from the Global Land Cover Characteristics Database Version 2.0.

Phaeozems according to the IUSS Working Group World Reference Base (WRB) for Soil Resources (IUSS Working Group WRB, 2015).

The temperate grasslands include a north-to-south and an east-to-west transect across >4,700 km with elevations of 150–1,500 m in the arid and semiarid regions of northern China and Mongolia. Mean annual temperature is 0.6 ± 0.3 °C, significantly higher than that of alpine grasslands ($p < 0.05$; Table S1). MAP ranges from 38 to 436 mm, with 80% occurring in the growing season (May–August). The dominant vegetation types include temperate meadow steppe (dominated by *Stipa baicalensis*, *Leymus chinensis*, and *Carex pediformis*), temperate typical steppe (dominated by *S. grandis*, *L. chinensis*, and *Artemisia sacrorum*) and temperate desert steppe (dominated by *S. klemenzii*, *Agropyron desertorum*, and *Cleistogenes squarrosa*). Soil types include Arenosols, Kastanozems, and Chernozems (WRB, 2015).

2.2. Field Sampling and Measurements

A total of 48 sites with minimal anthropogenic disturbances and grazing activity were selected for soil sampling and biomass survey along the alpine (30 sites) and temperate (18 sites) transects during the summer (July to August) of 2011–2012. The alpine sites match with those in Dai et al. (2018) except one site while the temperate sites vary slightly. Details related to field sampling and bulk soil measurements can be found in Dai et al. (2018). Briefly, AGB was clipped within five random plots (1×1 m) at each site (10×10 m). Roots were sampled by taking five soil cores (diameter of 7 cm) at a depth of 10 cm from each of the five plots. Roots retrieved from the soil cores were immediately placed in a cooler and transported back to the laboratory, where they were soaked in deionized water and cleaned of soil particles using a 0.5-mm sieve. Belowground biomass (BGB) for living plants was separated from dead roots in the temperate grasslands based on color and resilience. Dead roots were not removed from the alpine grassland samples due to the massive volume of dead roots and difficulties to distinguish from living ones. The AGB, BGB (for temperate grasslands), and total root mass (for alpine grasslands) were determined after drying plant tissues at 65 °C to a constant weight. To compare the incorporation efficiency of suberin based on total root mass (see section 3.3), ratios of living-to-total root mass were calculated for different temperate grasslands based on an additional field survey conducted using similar methods along a temperate grassland transect (33 sites) in Inner Mongolia in the summer of 2005 (see Table S2). The average living-to-total root mass ratio was 0.74 for temperate meadow steppe, 0.50 for temperate typical steppe, and 0.45 for temperate desert steppe.

Surface soil samples (0–10 cm) were collected by randomly taking three additional soil cores (diameter of 7 cm) from each of the five plots (1×1 m) at every site. The three cores were mixed in situ as one

composite sample for each plot. After homogenization and removal of stones and visible roots, the soils were passed through a 2-mm sieve and air-dried for subsequent chemical analyses. Soils from three plots were selected for 16 alpine and 10 temperate sites each, while two plots were selected for another two alpine sites. For the remaining 20 sites, soils from three plots were mixed in equal proportions to constitute a single representative sample due to limited sample availability. As a result, a total of 102 surface soils were included for our analysis. Soil pH, texture, bulk density, SOC, nitrogen (N), reactive iron (Fe), and aluminum (Al) contents were analyzed as reported in our previous study (Dai et al., 2018).

In addition, whole-plant samples of the dominant vegetation were collected in the summer (July–August) of 2016 to compare biomarker compositions, including five species in the alpine grasslands (*Kobresia pygmaea*, *Kobresia humilis*, *Kobresia tibetica*, *Carex moorcroftii*, and *Oxytropis ochrocephala*) and five species in the temperate grasslands (*Leymus chinensis*, *Stipa grandis*, *Agropyron michnoi*, *Cleistogenes squarrosa*, and *Achnatherum sibiricum*). Although species coverage data were not collected for the studied sites during the original soil sampling campaign, the average coverage of the five dominant species is 10–60% in the alpine grasslands (Liu et al., 2016; X. Wang et al., 2016) and 5–50% in the temperate grasslands (B. Li et al., 1988) based on previous studies in the same region. The aboveground and belowground tissues of fresh plants were separated in situ and kept at 4 °C before returning to the laboratory. After cleaning, the tissues were oven-dried at 65 °C and crushed with a ball mill prior to chemical analysis.

2.3. Analysis of Cutin and Suberin Biomarkers

Cutin and suberin biomarkers were isolated from dried plant tissues and soil samples by alkaline hydrolysis after removing solvent-extractable compounds according to Otto and Simpson (2006). Briefly, dried solvent-extracted residues (~0.1 g plant tissues or ~2 g soil, containing 56 ± 4 mg organic carbon, OC) were heated at 100 °C for 3 hr in Teflon-lined bombs with 20 ml of 1 M potassium hydroxide in a methanol:water solution (4:1, v:v). The soil residues were subsequently sonicated twice with 30 ml dichloromethane:methanol (1:1; v:v) while the extracts were combined, spiked with a standard (C_{19} *n*-alkanoic acid) and acidified to pH < 2 with 12 M hydrochloric acid (HCl). Compounds were recovered by liquid-liquid extraction with dichloromethane three times. The extracts were dried under nitrogen gas and then methylated with methanol:HCl (95:5; v:v) at 70 °C for 14 hr. The methylated products were extracted with hexane:dichloromethane (4:1; v:v) three times and concentrated under nitrogen gas.

Small aliquots of the methylated extracts were derivatized with 50 μ l N,O-bis-(trimethylsilyl)trifluoroacetamide and 10 μ l pyridine for 3 hr at 70 °C. Compounds were quantified on a Trace GC 1310 gas chromatograph coupled to an ISQ mass spectrometer (Thermo Fisher Scientific, United States) using a DB-5MS column (30 m \times 0.25 mm i.d., film thickness, 0.25 μ m). The oven temperature was held at 65 °C for 2 min, increased to 115 °C at a rate of 6 °C/min and then to 300 °C at a rate of 4 °C/min with a final isothermal hold at 300 °C for 15 min. Helium was used as carrier gas (1.2 ml/min). The mass spectrometer was operated in the electron impact mode at 70 eV ionization energy and scanned from 50 to 650 Daltons (Cai et al., 2017). Compounds were identified by interpretation of the mass spectrometric fragmentation patterns. Quantification was achieved by comparison with the standard and the response ratio was assumed to be 1 for different compound classes. Analytical errors were typically <10% based on replicate analysis of the same soil sample. According to our previous experience, the alkaline hydrolysis is quite efficient in removing compounds of interest in that a second alkaline hydrolysis of the hydrolyzed soil residue only yields very low (on a lower order of magnitude) amount of lipid compounds relative to the first hydrolysis. Hence, we regard our extraction as efficient.

2.4. Cutin and Suberin Related Parameters

As cutin- and suberin-specific markers may vary among plant species (Angst et al., 2016; Mueller et al., 2012), we compared the concentrations of generic markers for suberin and cutin in the aboveground (including leaves, sheaths, and stems) and belowground (roots) parts of dominant plants from our studied sites (Otto & Simpson, 2006). As shown in Table S3 (in the supporting information) and Figure 2a, while suberin markers (including C_{20} – C_{30} ω -hydroxyalkanoic acids, C_{20} – C_{30} α , ω -dioic acids, and C_{18} 9,10-epoxy-dioic acid) occurred in significant abundances in the aboveground tissues due to the presence of sheaths and suberized stems of plant aboveground tissues (Kolattukudy, 1980; Mendez-Millan et al., 2011), they were reported to mainly derive from plant roots (Kolattukudy, 1981; Otto & Simpson, 2006) and were

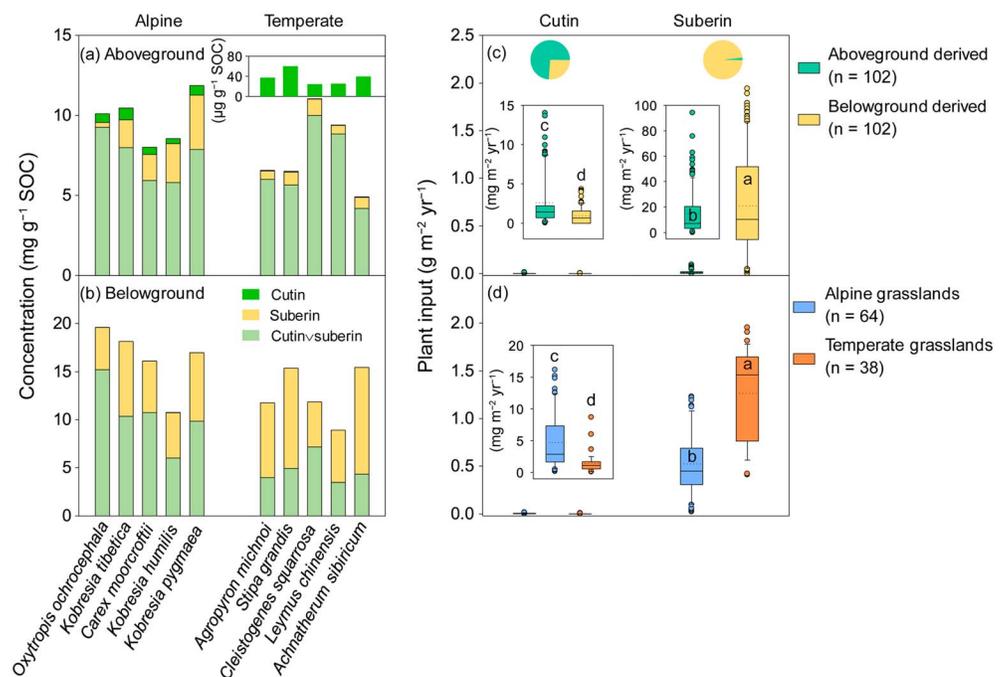


Figure 2. Cutin and suberin abundances in the aboveground (a) and belowground tissues (b) of the dominant grass species, their estimated inputs from aboveground and belowground tissues (c) and their total inputs into the alpine and temperate grassland soils (d). Letters indicate different levels ($p < 0.05$). The pie plot in (c) gives the relative contribution from aboveground and belowground tissues. The inserted graphs in (c) and (d) are magnified versions of the corresponding graphs. Solid and dotted line in the box mark the median and mean of each data set, respectively. The upper and lower ends of boxes denote the 0.25 and 0.75 percentiles, respectively. The upper and lower whisker caps denote the maximum and minimum values, respectively. Dots denote outliers. Biomarkers representing cutin, suberin, and cutin or suberin (cutinvsuberin) are defined in the text. SOC = soil organic carbon.

considered to be predominantly supplied by plant roots into the studied soils due to the high abundance of root mass. Hence, these compounds are used as suberin-specific markers for our samples. By contrast, only two compounds (i.e., C_{14} or C_{15} dihydroxyalkanoic acid and C_{16} hydroxyalkanoic acids) can be used as cutin-specific markers in our studied alpine and temperate grasslands (Table S3), as they were either not detected or occurred in a trace abundance in plant roots (more than one order of magnitude lower than the aboveground tissues). The rest of generic markers were treated as compounds derived from cutin or suberin (cutinvsuberin), including C_{15} hydroxyalkanoic acids, C_{16} monohydroxyalkanoic and dihydroxyalkanoic acids and dioic acid, 9- or 10-hydroxytetradecanoic acid, 7- or 8-hydroxyhexadecane-1,16-dioic acid, x,16-dihydroxyhexadecanoic acid, and x-chloro-x,18-hydroxyoctadecanoic acid (Otto & Simpson, 2006). All the above biomarkers are summarized to represent total cutin and suberin compounds (ΣCS). The degradation of cutin can be evaluated by ratios of $\omega-C_{16}/\Sigma C_{16}$ and $\omega-C_{18}/\Sigma C_{18}$ (i.e., the ratio of C_{16} or C_{18} ω -hydroxyalkanoic acid to the summation of C_{16} or C_{18} ω -hydroxyalkanoic acids, dioic acids, and midchain hydroxy and epoxy acids), which increases with progressing cutin degradation (Feng et al., 2015; Goñi & Hedges, 1990).

Similar to Dai et al. (2018), the following equations are used to estimate annual inputs of cutin and suberin to surface soils (0–10 cm):

$$\text{Input}_a = [A_a \times 1 / (1 + R)] \times \text{NPP} / 1,000, \quad (1)$$

$$\text{Input}_b = [A_b \times R / (1 + R)] \times \text{NPP} / 1,000, \quad (2)$$

where Input is the annual input of cutin- or suberin-specific biomarkers in the units of gram per square meter per year ($\text{g m}^{-2} \text{yr}^{-1}$); A is the OC-normalized concentration (mg/g OC) of biomarkers in the respective tissues of the dominant plants; the subscripts a and b denote aboveground and belowground sources,

respectively; R is the ratio of BGB to AGB at each sampling site; NPP is net primary productivity obtained from the Numerical Terradynamic Simulation Group Data (<http://www.ntsug.umt.edu/data>) with a 1-km resolution ($\text{g C}\cdot\text{m}^{-2}\cdot\text{year}^{-1}$). Total inputs are the summation of Input_a and Input_b at each site. To calculate R , BGB in the alpine sites was estimated using a range of fixed ratios for the living to dead root mass (1.12–1.29) reported for the Chinese alpine meadows and steppes (Wei et al., 2012; Wen et al., 2013). These ratios resulted in similar patterns for Input_a and Input_b for the alpine relative to temperate sites. We hence present the results here using the living to dead root mass of 1.167 that was used for a similar alpine grassland transect previously (Jing et al., 2015).

To compare with plant wax lipids (Dai et al., 2018), we calculated the fraction of NPP preserved as cutin and suberin biomarkers (ΣCS) in the surface soils:

$$\text{Preservation percentage} = \frac{M_{\text{CS}} \times \rho \times h}{\text{NPP} \times t} \times 100\%, \quad (3)$$

where M_{CS} is the concentration of ΣCS in the units of microgram carbon per gram soil ($\mu\text{g C g}^{-1}$ soil) (carbon content of 67%, 72%, and 70% for cutin, suberin, and cutin+suberin biomarkers, respectively); ρ is soil bulk density (g/cm^3); h is soil thickness (10 cm); t is the time (year) for plant OC to accumulate in the surface soil under a steady-state condition, approximated as the turnover time of surface SOC. Based on previous studies, the turnover time is 202 ± 51 years in the alpine grasslands ($n = 10$; L. Wang et al., 2005; Yu et al., 2017) and 112 ± 28 years in the temperate grasslands ($n = 11$; Frank et al., 2012; L. Li et al., 1998; see Figure S1 and Table S4). The preservation percentage was calculated with all reported turnover times for every sample (Table S3) with the mean values estimated for different vegetation types and regions. The standard deviation for the mean was calculated using the following equation:

$$\Delta_{\text{Preservation percentage}} = \sqrt{\sum (\Delta p_i)^2}, \quad (4)$$

where $\Delta_{\text{Preservation percentage}}$ is the standard deviation for different vegetation types or regions and Δp_i is the corresponding standard deviation at every sampling site.

To further assess root mass normalized incorporation of root components into soils, we estimated the incorporation efficiency of suberin:

$$\text{Incorporation efficiency} = \frac{M_s \times \rho \times h}{\text{RM}} \times 100\%, \quad (5)$$

where M_s is the concentrations of suberin biomarkers in the soil ($\mu\text{g}/\text{g}$ soil); RM is the amount of root mass (g/m^2) in the surface soil; ρ and h are defined in equation (3). Assuming that suberin is transferred from roots into the soil within the same timeframe as root decomposes, the incorporation efficiency reflects root mass normalized abundance of suberin in the soil.

2.5. Statistics Analyses

Differences in the biomarker concentrations and parameters between the alpine and temperate grassland soils were examined using Mann-Whitney U-test, followed by post hoc analysis (Kruskal-Wallis H test) among different vegetation types (IBM SPSS Statistics 20.0, Chicago, IL). All data (except soil pH) were transformed (square-root transformation for biomarker abundances and ln transformation for all other parameters) to achieve normal distribution (confirmed by Shapiro-Wilk test) and examined by Pearson's correlations to assess environmental effects on biomarker concentrations and the incorporation efficiency of suberin. To confirm the results, original untransformed data were also examined by Spearman's correlations. Mean values (\pm standard error) were used for sites with spatial replicates. Differences and correlations were considered significant at a level of $p < 0.05$. Environmental variables showing significant correlations with the investigated parameter were selected for multiple stepwise regression to further elucidate the most important factors or mechanisms. Among the selected variables, soil N and Al contents were excluded due to their high correlations (i.e., $|r| > 0.7$) with other variables from the same category (i.e., SOC and Fe, respectively).

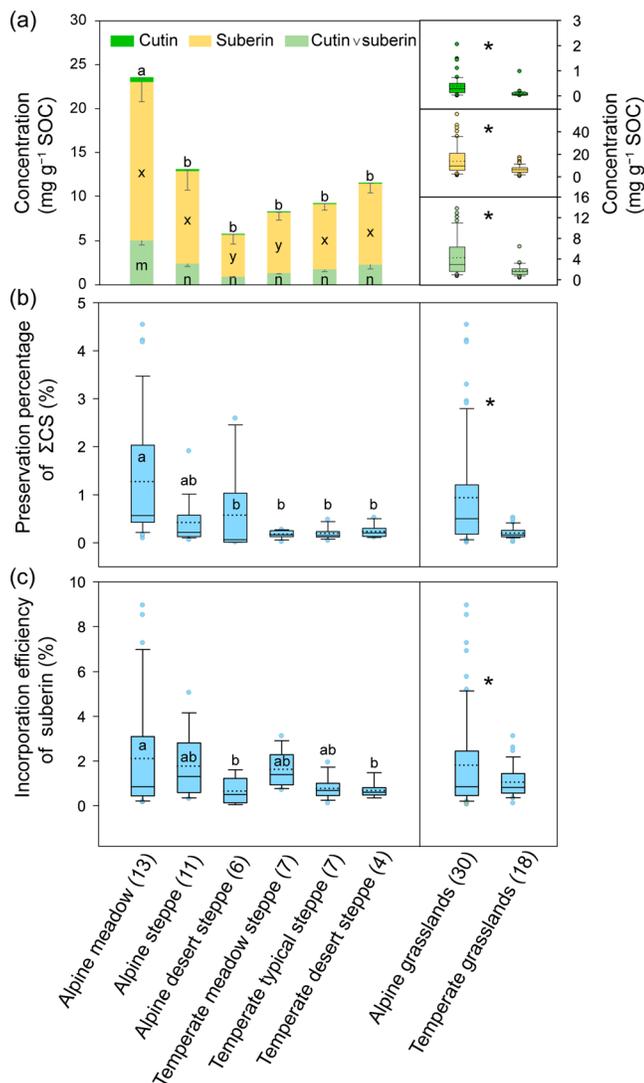


Figure 3. Comparison of biomarker concentrations (a), preservation percentage (b), and incorporation efficiency of suberin (c) in the surface soils of alpine and temperate grasslands. Letters indicate different levels among different vegetation types ($p < 0.05$): a–b, x–y, and m–n for suberin, cutin, and cutin or suberin in (a), respectively. Numbers in brackets indicate the number of samples. Asterisk denotes significant difference between alpine and temperate grassland soils ($p < 0.05$). Solid and dotted line in the box mark the median and mean of each data set, respectively. The upper and lower end of boxes denote the 0.25 and 0.75 percentiles, respectively. The upper and lower whisker caps denote the maximum and minimum values, respectively. Dots denote outliers. SOC = soil organic carbon; Σ CS = summation of biomarkers from cutin, suberin, and cutin or suberin (cutin vs suberin).

3. Results

3.1. Cutin and Suberin in Plants and Inputs to Soils

Cutin biomarkers had higher OC-normalized concentrations in the aboveground tissues of the alpine than temperate plants ($p < 0.05$; Figure 2a). Suberin had comparable concentrations in the belowground tissues from both regions ($p > 0.05$; Figure 2b). The concentration of cutin vs suberin biomarkers was similar in the aboveground tissues between the two regions ($p > 0.05$) but higher in the belowground tissues of alpine than temperate plants ($p < 0.05$; Figures 2a–2b). In the absence of species coverage data, we estimated the upper and lower ranges for the plant inputs of cutin and suberin at every sampling site, assuming a 100% coverage by the dominant plant species typical for each vegetation type. Approximately 78.8–100% ($97.5 \pm 0.33\%$; $n = 102$; see Table S5) of suberin was supplied by roots. The $\text{Input}_b/\text{Input}_a$ ratio was higher in the alpine (102 ± 22 ; $n = 63$) than temperate grasslands (84 ± 8 ; $n = 37$; $p < 0.05$). Cutin was solely contributed by plant aboveground tissues in the temperate grassland soils. By contrast, despite the trace abundance of cutin in the belowground tissues of alpine plants, a minor but nonnegligible fraction ($26.4 \pm 2.0\%$; $n = 102$) of cutin was supplied by grass roots into the soil due to the high volume of root mass relative to AGB at the alpine sites (Figure 2c). The resulting $\text{Input}_b/\text{Input}_a$ ratios for cutin was 0.35 ± 0.1 ($n = 63$) in the alpine region. Overall, cutin inputs were higher in the alpine than temperate grasslands while suberin inputs were higher in the temperate than alpine grasslands ($p < 0.05$; Figure 2d).

3.2. Cutin and Suberin in Soils

Both cutin, suberin, and cutin vs suberin biomarkers exhibited higher abundances in the alpine than temperate grassland soils ($p < 0.05$; Figure 3a). Among different vegetation types, both cutin and cutin vs suberin biomarkers were most abundant in the soils of alpine meadows, while suberin was less abundant in the alpine desert steppe and temperate meadow steppe than the other vegetation types ($p < 0.05$; Figure 3c). The degradation indicator of $\omega\text{-C}_{16}/\Sigma\text{C}_{16}$ ratio showed little variation among different sites ($p = 0.22$). By comparison, the $\omega\text{-C}_{18}/\Sigma\text{C}_{18}$ ratio was higher in the soils of temperate (0.63 ± 0.02 ; $n = 38$) than alpine grasslands (0.56 ± 0.02 ; $n = 64$; $p < 0.05$). The preservation percentage of ΣCS was significantly higher in the alpine ($0.86 \pm 0.24\%$; $n = 30$) than temperate grassland soils ($0.20 \pm 0.04\%$; $n = 18$; $p < 0.05$; Figure 3b). Soils of the alpine meadows showed a higher preservation percentage than other vegetation types except alpine desert steppe ($p < 0.05$). Meanwhile, the incorporation efficiency of suberin was also higher in the alpine ($1.81 \pm 0.27\%$) than temperate grassland soils ($1.05 \pm 0.12\%$; $p < 0.05$) with variations among vegetation types (Figure 3c).

3.3. Environmental Influences on the Distribution and Incorporation Efficiency

The effects of environmental variables on biomarker concentrations are shown for the transformed data (Figure 4) and confirmed by Spearman's correlation with the original data (see Table S6). For comparison, plant wax lipids isolated from similar grassland transects (Dai et al., 2018) are also summarized, including long-chain ($>\text{C}_{19}$) even-numbered n -alkanoic acids and n -alkanols as well as cyclic lipids other than ergosterol and cholesterol. In the alpine grasslands, root mass, soil pH, SOC (and N), and Fe (and Al) contents

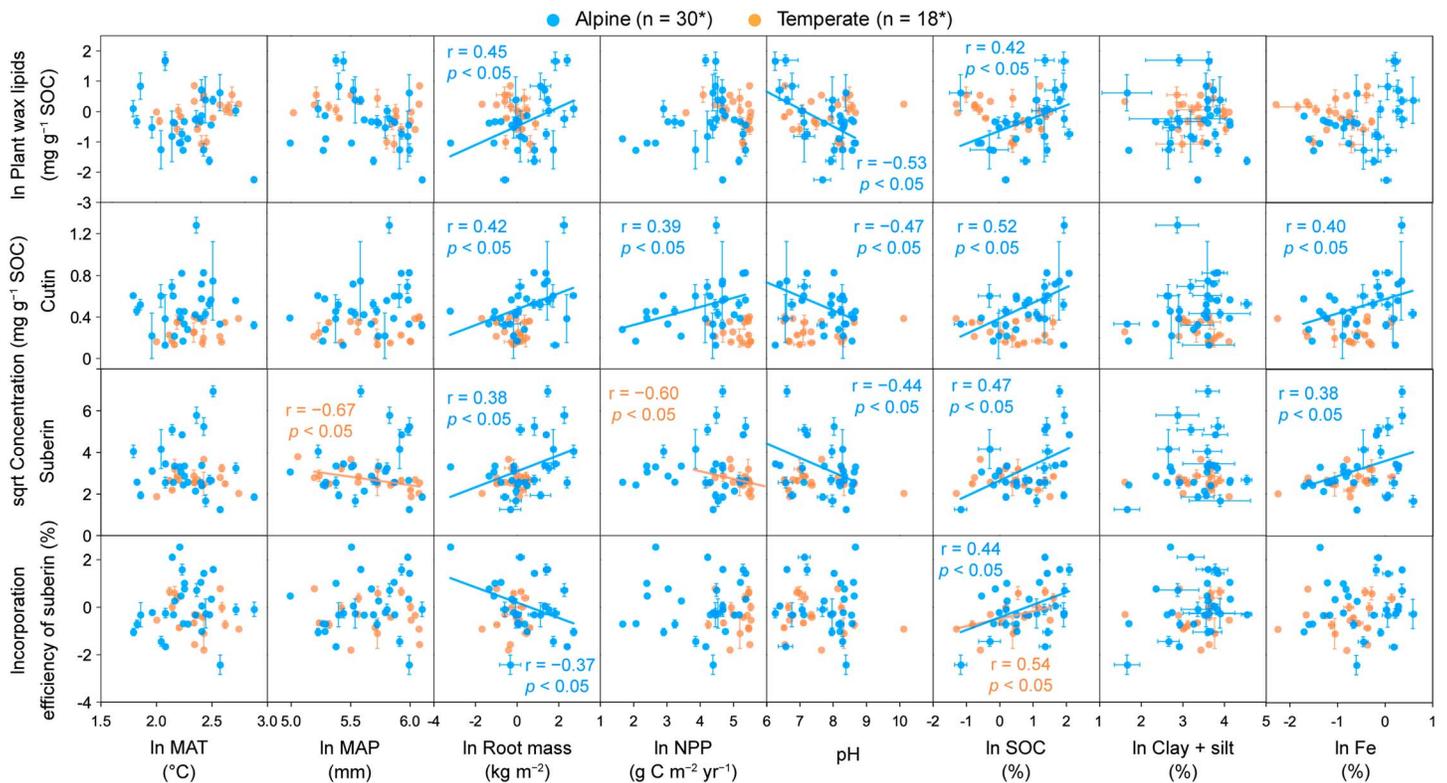


Figure 4. Pearson's correlations for the SOC-normalized concentrations of plant wax lipids, cutin, suberin, and the incorporation efficiency of suberin with environmental variables in the alpine and temperate grasslands. Data are ln- or square-root (sqrt) transformed except soil pH. Blue and orange lines indicate correlations for the alpine and temperate grasslands, respectively. MAT = mean annual temperature; MAP = mean annual precipitation; NPP = net primary productivity; clay + silt, soil clay, and silt contents; Fe, dithionite-extractable iron. *For plant wax lipids, original data are derived from Dai et al. (2018) with n of 24 and 25 for the alpine and temperate grasslands, respectively. Error bars represent standard error of mean for sites with spatial replicates. SOC = soil organic carbon.

have a significant influence on both cutin and suberin, with NPP exerting an additional positive effect on cutin abundances. By comparison, in the temperate grasslands, MAP and NPP both negatively affect suberin abundances, while no significant correlation was found between the investigated environmental factors and cutin abundances. These patterns are similar for plant wax lipids in the alpine (except with Fe) but not temperate grasslands. Using multiple stepwise regression analyses, we further show that SOC play a dominant and positive effect on both cutin and suberin abundances in the alpine grasslands (Table 1). By comparison, in the temperate grasslands, MAP exert a strong, negative effect on suberin abundances.

The incorporation efficiency of suberin is positively correlated with SOC in both regions ($p < 0.05$; Figure 4) with root mass exerting an additional negative effect in the alpine grasslands ($p < 0.05$). Correlations with

Table 1

Standardized Regression Coefficient of the Multiple Stepwise Regression for Cutin and Suberin Abundances and the Incorporation Efficiency of Suberin With Environmental Variables in the Alpine ($n = 30$) and Temperate ($n = 18$) Grasslands

Biomarker	Alpine grasslands					Temperate grasslands					
	Variables				R^2	p	Variables				
Root mass	SOC	pH	Fe	MAP			NPP	SOC	R^2	p	
Cutin	ns	0.52	ns	ns	0.27	<0.01	na	na	na	na	na
Suberin	ns	0.47	ns	ns	0.22	<0.01	-0.67	ns	na	0.45	<0.01
Incorporation efficiency	-0.81	0.96	na	na	0.54	<0.01	ns	ns	0.54	0.30	<0.05

Note. All data are transformed (square-root or ln) except pH, showing consistent results of the original data (see Table S6). Abbreviations are defined in the footnotes of Figure 4; ns = not significant; na = not applicable.

SOC show a slightly steeper slope in the alpine than temperate grasslands for the incorporation efficiency. Multiple stepwise regression further shows an almost equally important influence of root mass and SOC on the incorporation efficiency of suberin in the alpine grasslands.

4. Discussion

4.1. Cutin and Suberin as Shoot and Root Tracers in Grasslands

Based on the concentration of compounds measured in the aboveground versus belowground tissues of plants, previous studies have often found that even-numbered C_{20} – C_{32} ω -hydroxyalkanoic acids, C_{20} – C_{32} α,ω -dicarboxylic acids, and C_{18} 9,10-epoxy-dicarboxylic acid mainly originate from suberin, whereas C_{14} , C_{15} , C_{17} midchain hydroxyalkanoic acids, C_{16} monohydroxyalkanoic and dihydroxyalkanoic acids, and dicarboxylic acids mainly derive from cutin (Mendez-Millan et al., 2011; Otto & Simpson, 2006; Rasse et al., 2005). As the classification of cutin and suberin markers may vary for different plants (Angst et al., 2016; Hamer et al., 2012; Mueller et al., 2012), we develop our cutin markers based on the abundances of the corresponding compounds in the plant tissues from the alpine and temperate grasslands. Our cutin- and suberin-specific compounds show a predominant occurrence (in terms of concentration) in the aboveground and belowground tissues of the ten dominant grass species, respectively. However, as the aboveground tissues also include sheaths and stems and suberin may be present in the suberized organs of aboveground tissues (such as bundle sheaths of leaves and stems; Kolattukudy, 1980; Mendez-Millan et al., 2011), they are also detected in the other part of the plants (Figure 2). For cutin biomarkers, despite their predominant occurrence in aboveground tissues, we demonstrate that belowground sources contribute a minor but nonnegligible fraction (average of 26%) of total cutin marker inputs into soils due to the high volume of root mass in the alpine grasslands (Table S5). For the same reason, although suberin is detected in a sizable concentration in grasses' aboveground tissues in our study, belowground parts remain the predominant source of suberin, accounting for $97.5 \pm 0.3\%$ of total suberin inputs into soils. Hence, our results highlight that future work on cutin- and suberin-specific biomarkers does not only need to include accompanied analysis of aboveground and belowground tissues of the local vegetation but also need to take into account the mass ratio of aboveground versus belowground tissues.

4.2. Distribution of Cutin and Suberin in Grassland Soils

Cutin and suberin are major lipid components of plants along with plant waxes. To examine whether these plant lipid constituents show varied fate in grassland soils, we compare the distribution of cutin and suberin as well as plant wax lipids isolated from the same batch of soils (excluding Mongolia) in our previous study (Dai et al., 2018). In line with our first hypothesis and similar to plant wax lipids (Dai et al., 2018), both cutin and suberin exhibit higher abundances in the surface soils of the alpine than temperate grasslands (Figure 3a) despite similar or lower inputs from plants (Figures 2c–2d). In addition, the degradation parameter of ω - $C_{18}/\Sigma C_{18}$ ratio (Feng et al., 2015; Goñi & Hedges, 1990) is lower while the preservation percentage of ΣCS is higher in the alpine than temperate grassland soils, confirming a better preservation of these plant-derived lipid components in the cold region. It is worth noting that if we use the same assumed turnover time for surface SOC as in Dai et al. (2018), that is, 64 years in the alpine grasslands (Yu et al., 2017) and 30 years in the temperate grasslands (L. Li et al., 1998), the preservation percentage of ΣCS ($1.13 \pm 0.17\%$ and $0.28 \pm 0.03\%$ for alpine and temperate grasslands, respectively) was significantly higher than that of plant wax lipids ($0.09 \pm 0.01\%$ and $0.03 \pm 0.003\%$ for alpine and temperate grasslands, respectively; $p < 0.05$). This result is consistent with the higher recalcitrance of cutin and suberin compared with plant waxes due to the complex, chemically bonded structures of cutin and suberin macromolecules (Nierop et al., 2003; Rasse et al., 2005).

Furthermore, consistent with our first hypothesis, the distribution of cutin and suberin is mediated by different variables in the alpine versus temperate grasslands. In the alpine grasslands, both cutin and suberin abundances are mainly related to SOC content (Table 1), suggesting a coupled accumulation of cutin and suberin with bulk SOC in the cold region. It may also imply that cutin and suberin are an important contributor to SOC (Bull et al., 2000; Filley et al., 2008; Mueller et al., 2012). In addition, similar to plant wax lipids (Dai et al., 2018), soil pH also have a significant negative effect on cutin and suberin concentrations in the alpine soils (Figure 4), suggesting an enhanced preservation of lipids at lower pHs likely due to decreased enzyme activities (Nierop et al., 2003; Sinsabaugh, 2010) and/or stronger sorption of lipids to mineral

surfaces at lower pHs (Feng et al., 2005). Reactive Fe also has a positive effect on cutin and suberin in the alpine grassland soils (Figure 4), indicating enhanced preservation of soil lipids via interaction with mineral matrix (Angst et al., 2018; Cai et al., 2017; Lin & Simpson, 2016). The positive effect of NPP on cutin abundances in the alpine grassland soils corroborates the key role of plant inputs on cutin distribution in this region. It should be also mentioned that soil N and Al are likely to have a positive effect on the accumulation of cutin and suberin in the alpine grasslands given their high correlations with SOC ($r = 0.94$, $p < 0.01$) and Fe ($r = 0.93$, $p < 0.01$), respectively.

By comparison, in the temperate grassland soils, climate (i.e., MAP) rather than SOC has a dominant negative effect on suberin abundances (Table 1). This relationship likely reflects an enhanced microbial decomposition relative to plant inputs with increasing precipitation (Ma et al., 2018; Yang et al., 2010) in these arid and semiarid grasslands (Bai et al., 2004; He et al., 2012). Alternatively, elevated accrual of other SOC constituents (such as microbial carbon) with increasing moisture (Ma et al., 2018) may dilute suberin in the soil. As suberin are considered to be relatively hard to decay (Barré et al., 2018; Feng et al., 2008; Mendez-Millan et al., 2010), the latter explanation (dilution effect) may be more reasonable. No environmental variable is found to significantly influence cutin distribution in the temperate grassland soils, suggesting that other factors may have a more important effect on the plant aboveground-derived carbon component in the arid and semiarid grasslands. For instance, J. Wang et al. (2015) emphasized the role of ultraviolet radiation in the degradation of aboveground litter in these arid and semiarid ecosystems. The moisture-mediated dilution effect does not work for plant wax lipids, cutin, or suberin in the alpine region, presumably due to a relatively low contribution of microbial carbon to SOC in the temperature constrained cold region (Iqbal et al., 2010).

4.3. Incorporation of Root Components Into Grassland Soils

As plant inputs strongly impact biomarker concentrations in the soil, we calculate the incorporation efficiency of suberin as root mass normalized abundance of this root tracer to assess the preservation of root components in soils. This parameter is neither related to plant input nor complicated by other SOC components. It hence provides a simple assessment on the efficiency for suberin to accumulate in the soil which is only affected by decomposition or preservation processes. The calculation reveals several interesting trends (Figure 4). First, the incorporation efficiency is higher in the alpine than temperate grasslands despite similar suberin concentrations in the alpine and temperate grass roots (Figure 3), corroborating the better preservation of root components in the alpine region.

Second, SOC is the dominant factor that positively correlates with the incorporation efficiency of suberin in both regions, suggesting an enhanced preservation of root-derived lipid components in SOC-rich soils. It also suggests a coupled accumulation of SOC and suberin in grasslands (Hamer et al., 2012; Puget & Drinkwater, 2001), adding large-scale evidence for the importance of root inputs in soil carbon stabilization. Moreover, opposite to suberin concentrations in the soil, the incorporation efficiency of suberin is negatively correlated to root mass in the alpine grasslands. Root fragmentation due to microbial degradation of labile root structures (such as cellulose and hemicellulose; Xia et al., 2015) is hypothetically elevated in soils with a low root mass, hence facilitating the transfer and incorporation of the remaining recalcitrant compounds (such as suberin) into soils. It is also interesting to observe that unlike suberin concentrations, the incorporation efficiency of suberin is not related to soil minerals (i.e., clay + silt, Fe, and Al) and soil pH in the alpine soils or MAP and NPP in the temperate soils. Again, these results highlight different environmental effects on the concentration versus incorporation efficiency of root-derived carbon components when the effect of plant inputs is controlled for.

Third, correlations between the incorporation efficiency of suberin and SOC show a slightly steeper slope in the alpine than temperate grasslands, implying a higher sensitivity of root carbon preservation in response to SOC variations in the cold regions. It also implies that root carbon (suberin) plays a more important role in SOC accumulation (Kätterer et al., 2011; Rasse et al., 2005) in the alpine grasslands. This result somewhat supports our second hypothesis and suggests that the preservation of root structural components in alpine soils is more sensitive to environmental changes (such as root mass and SOC changes). Hence, it will be interesting to speculate how root carbon preservation would change, should global changes induce alterations to root mass in the alpine grasslands. The high sensitivity of suberin incorporation efficiency to SOC variations also suggests that root carbon sequestration is subject to more dynamic alterations to in the alpine grasslands under global changes, which warrants further investigation in the future.

5. Conclusions

In summary, this study utilizes suberin and cutin biomarkers and present the first continental-scale investigation on the distribution patterns of root- and shoot-derived components in soils across the Chinese-Mongolian grasslands. We show that while cutin and suberin biomarkers are good tracers for shoot- and root-derived carbon components in the studied grasslands, the mass ratio of aboveground versus belowground tissues of plants needs to be considered to determine the total input of these markers into soils. Both plant-derived lipid components are better preserved in the alpine than temperate soils, reflected by higher concentrations, higher preservation percentages against NPP, and lower $\omega\text{-C}_{18}/\Sigma\text{C}_{18}$ ratios. Most importantly, using the root mass normalized incorporation efficiency of suberin, we demonstrate that root carbon preservation is consistently higher in SOC-rich grassland soils and shows a higher sensitivity to SOC and root mass variations in the alpine grasslands. In the context of shifting vegetation and altered carbon allocation under global changes, it is important to extend this calculation to other ecosystems to compare the efficiency of root carbon accumulation in soils under varied land uses (i.e., forest, grassland vs farmland) and climate zones to better understand mechanisms influencing root carbon preservation and to predict influences of plant roots on SOC dynamics in the future.

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