



# Distribution of lignin phenols in comparison with plant-derived lipids in the alpine versus temperate grassland soils

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## Abstract

**Aims** As a major plant-derived soil organic carbon (SOC) component, lignin-derived phenolic compounds show varying biogeochemical characteristics compared to plant-derived lipid moieties. Comparing their distribution patterns can provide information on mechanisms governing SOC preservation and dynamics. However,

the large-scale distribution pattern and stability of lignin versus plant-derived lipids are still poorly constrained. Here we investigated the distribution of lignin phenols versus plant-derived lipids in the surface soils across the alpine versus temperate grasslands of China and Mongolia. **Methods** Lignin phenols were isolated by cupric oxide oxidation method and compared with the previously

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analyzed plant-derived lipids (cutin and suberin). A comprehensive list of environmental variables was compiled to disentangle the climatic, edaphic and vegetation influences on lignin phenols' distribution in the soil.

**Results** Lignin phenols showed similar SOC-normalized concentrations in the alpine and temperate grassland soils despite a higher plant input to the latter, suggesting better lignin preservation in the cold region. However, compared with plant-derived lipids (cutin and suberin), lignin seems to be less stabilized. The variation of lipid versus lignin components is mainly related to climate (particularly aridity) in the alpine grassland soils, while the relative abundance of plant lipids and lignin phenols is more related to reactive mineral contents in the temperate grassland soils.

**Conclusions** Lignin contributes differentially to SOC accumulation in the alpine and temperate soils: while lignin seems to be better preserved in the cold region, lignin phenols decrease relative to other carbon components with SOC accrual in the temperate region. Overall, lignin distribution and fate may be more sensitive to carbon source variations than temperature shifts in the grasslands.

**Keywords** Biomarker · Stability · Soil organic carbon · Qinghai-Tibetan plateau · Mongolian plateau

## Introduction

The Qinghai-Tibetan Plateau (QTP) and Mongolian Plateau represent two major areas in the world with vast coverage by alpine and temperate grasslands, respectively. Soil organic carbon (SOC) stored in these two regions accounts for over 90% of organic carbon (OC) stocks in the respective ecosystems (Fang et al. 2010) and is considered to be sensitive to global changes (Liu et al. 2012). Hence, the distribution and dynamics of SOC in both grasslands have attracted considerable attention recently (Liu et al. 2017; Wang et al. 2016). Previous studies have mainly focused on the distribution of bulk SOC across these two regions, which is mainly regulated by soil moisture and root mass in the QTP alpine grasslands (Fang et al. 2010; Liu et al. 2012) and by mean annual temperature (MAT) in the Mongolian temperate grasslands (Liu et al. 2017; Shi et al. 2012). However, little is known about the composition and stability variations of SOC molecular components. It is not clear whether different SOC distribution in the

alpine versus temperate grasslands is tied to its compositional differences and whether specific SOC components (such as plant versus microbial derived carbon) dominate SOC variability across the region. Such information is important for understanding mechanisms governing grassland SOC dynamics and its response to global changes.

To fill the knowledge gap, we have recently employed source-specific biomarkers to investigate the large-scale distribution of molecular components in the surface soils across the QTP and Mongolian Plateau. We have demonstrated a higher abundance as well as better preservation of plant-derived lipid components in the alpine relative to temperate grassland soils, including plant waxes (Dai et al. 2018), cutin- and suberin-derived hydrolysable lipids (Ma et al. 2019). In addition, while plant biomass exerts the strongest control on the soil concentration of plant wax lipids in the alpine grasslands, climate-mediated decomposition processes strongly influence their distribution in the temperate grassland soils (Dai et al. 2018). These observations suggest that plant lipid components are better preserved in the alpine grasslands with a potentially stronger contribution to bulk SOC in the region. In contrast, microbial residues (indicated by amino sugars predominantly derived from microbial necromass; Joergensen 2018) increase with increasing SOC content across the Mongolian temperate grasslands while plant-derived lignin phenols decrease (Ma et al. 2018). This implies that plant-derived carbon, while being in a higher degradation stage in the warmer region, may be less important in SOC accumulation in the temperate grasslands. Hence, it is necessary to compare and contrast the distribution and preservation of plant-derived SOC components in the alpine versus temperate regions to better understand mechanisms influencing SOC accumulation and stability.

Compared to the previously mentioned lipid components, lignin is much more abundant in terrestrial vascular plants and is traditionally regarded as a key contributor to the stable SOC pool (Thevenot et al. 2010; and references therein). However, emerging evidence has revealed that lignin is not as stable as conventionally thought and that the fate of lignin in soils can be distinct from other SOC components (Feng et al. 2008; Schmidt et al. 2011; Thevenot et al. 2010). Therefore, it is vital to investigate the distribution pattern of lignin in soils to reveal the environmental variables regulating its preservation and its relationship with SOC accumulation.

Lignin phenols released by cupric oxide (CuO) oxidation are widely used biomarkers for lignin, providing unparalleled insight into the plant source and degradation stage of lignin in the soil (Feng and Simpson 2007; Guggenberger et al. 1995; Otto and Simpson 2006). The abundance of lignin phenols can be influenced by climate (Amelung et al. 1999; Otto and Simpson 2006) and edaphic properties such as pH (Kirk and Farrell 1987), soil grain size (Grünwald et al. 2006), soil aggregation (Angst et al. 2017; Mueller et al. 2014; Yu et al. 2015) and mineral contents (Miltner and Zech 1998). In contrast to aliphatic lipids which tend to form strong association with mineral surfaces (Feng et al. 2005) and are better preserved in fine particles, lignin phenols are more abundant in decomposing litter and coarse soil fractions (Kögel-Knabner et al. 2008; Sollins et al. 2009). However, most of the previous studies focused on site-level variations or specific environmental variables. A comprehensive assessment of climatic, edaphic and vegetation influences on lignin phenol distribution is still lacking.

Here we extend our previous study of lignin distribution on the Mongolian Plateau (10 sites; Ma et al. 2018) to include 8 additional sites along the arid and semiarid grasslands of China and 30 alpine sites on the QTP in order to compare the large-scale distribution of lignin phenols as well as its influencing factors in the surface soils of alpine versus temperate grasslands. In addition, we compare lignin phenols with the previously analyzed plant lipid components (i.e., cutin and suberin; Ma et al. 2019) in the same set of samples to assess their relative preservation and contribution to SOC. We hypothesize that: (1) similar to plant-derived lipids, lignin phenols have a better preservation in the alpine than temperate grassland soils; (2) however, lignin is less preserved compared to cutin and suberin that are strongly associated with clay minerals.

## Materials and methods

### Study area

This study includes two grassland-dominated transects across the alpine and temperate regions of China and Mongolia (see map of sampling sites in Ma et al. 2019). Detailed descriptions of the sampling sites can be found in our previous studies (Ma et al. 2019). Briefly, the alpine grassland transect spans >3200 km with MATs of

−4.0 to 7.8 °C and mean annual precipitation (MAP) of 147 to 446 mm. The aridity index (AI; <http://www.cgiar-csi.org/>), calculated as the ratio of precipitation to potential evapotranspiration, ranges from 0.19 to 0.56. The main vegetation types include alpine meadow (dominated by *Kobresia pygmaea*, *Kobresia humilis* and *Kobresia tibetica*), alpine steppe (dominated by *Stipa purpurea*, *Carex lanceolata* and *Stip subsessili*) and alpine desert steppe (dominated by *Oxytropis ochrocephala*, etc.). Soils along the transects included Leptosols, Cambisols and Phaeozems according to the IUSS Working Group World Reference Base (WRB) for Soil Resources (IUSS Working Group WRB 2015). The temperate grassland transect spans >4700 km in the arid and semi-arid regions of northern China and Mongolia (AI of 0.17–0.57). MAT and MAP range from −2.6 to 5.5 °C and from 151 to 436 mm, respectively. The dominant vegetation type shifts with increasing aridity from east to west in the order of temperate meadow steppe (dominated by *Leymus chinensis*, *Stipa baicalensis* and *Carex pediformis*), temperate typical steppe (dominated by *Stipa grandis*, *Leymus chinensis* and *Achnatherum sibiricum*), temperate desert steppe (dominated by *Stipa klemenzii*, *Agropyron michnoi* and *Cleistogenes squarrosa*). Soils include Arenosols, Kastanozems and Chernozems according to the IUSS Working Group WRB (2015).

### Field sampling

Soil sampling was conducted in the summer of 2011 and 2012 (July–August) in a collaborative effort to quantify soil carbon distribution across China. For the lignin analysis, we selected 18 sites (including 10 sites with lignin phenols reported in our previous study; Ma et al. 2018) in the arid and semiarid grasslands across China and Mongolia, and 30 sites in the alpine transect on the Qinghai-Tibetan Plateau. These sites have minimal human disturbances and overlap completely with those in Ma et al. (2019) and partially with those in Dai et al. (2018). The alpine transect had a larger grassland area and higher spatial heterogeneity in vegetation coverage (Fang et al. 2010; Yang et al. 2010) and hence more sites compared to the temperate transect. Details related to field sampling and bulk soil measurements can be found in our previous studies (Dai et al. 2018; Ma et al. 2019). Briefly, at each site (10 m × 10 m), five random plots (1 m × 1 m) were established. Three soil cores were randomly collected within the five plots and mixed in situ as one composite

sample for the depth of 0–10 cm. A total of 72 new samples were analyzed for lignin phenols in this study (Table S1), including three field replicate samples for 16 alpine grassland sites and two for another 2 alpine grassland sites. For the remaining 20 sites (including 12 and 8 sites in the alpine and temperate grasslands, respectively), soils from 3 plots were mixed in equal proportions to constitute a single representative sample due to limited sample availability. Previously published lignin data on the temperate grasslands (10 sites; Ma et al. 2018) are also included to compare with the alpine grasslands. Soils were air-dried and sieved to <2 mm. Soil bulk properties including bulk density, pH, texture, aboveground biomass (AGB), belowground biomass (BGB, for temperate grasslands), total root mass (for alpine grasslands), SOC, nitrogen (N), reactive iron (Fe) and aluminum (Al) extracted by dithionite were measured and described previously (Dai et al. 2018; Ma et al. 2019). In addition, total root mass for temperate grasslands were calculated based on the average living-to-total root mass ratios of different vegetation types (0.74 for temperate meadow steppe, 0.50 for temperate typical steppe and 0.45 for temperate desert steppe; Ma et al. 2019). It should be mentioned that we re-analyzed the SOC content for 30 Mongolian soil samples using the same method as for the alpine soils (i.e., as total carbon subtracted by inorganic carbon; Dai et al. 2018), which varied from the Walkley-Black method used in Ma et al. (2018). Specifically, SOC content was calculated by subtracting inorganic carbon from total carbon, with the former analyzed volumetrically by reaction with hydrochloric acid and the latter determined by dry combustion at 500 °C in this study. Nonetheless, our method gave quite consistent results as in Ma et al. (Ma et al. 2018; Table S2).

In addition, whole plants (> 10 individual plants for each species) of five dominant grass species in the alpine grasslands (*Kobresia pygmaea*, *Kobresia humilis*, *Kobresia tibetica*, *Carex moorcroftii* and *Oxytropis ochrocephala*) and five dominant species in the temperate grasslands (*Leymus chinensis*, *Stipa grandis*, *Agropyron michnoi*, *Cleistogenes squarrosa* and *Achnatherum sibiricum*) were collected during the summer of 2016 to compare lignin phenol compositions in the dominant vegetation. The average coverage of the five dominant species is 10–60% in the alpine grasslands (Liu et al. 2016) and 5–50% for the temperate grassland (Li et al. 1988). The aboveground and belowground (root) tissues of fresh plants were separated, cleaned, oven-dried at 65 °C and crushed with a ball mill prior to chemical analysis.

## Extraction and quantification of lignin phenols

Lignin phenols in 30 replicate samples for 10 sites on the Mongolian Plateau have been previously reported in Ma et al. (Ma et al. 2018; see Table S1). All other soils (72 samples) were analysed using the exact same protocol here. Briefly, air-dried soil (as well as above- and belowground plant tissue) samples were first subjected to solvent extraction and alkaline hydrolysis to remove free and hydrolysable lipids, respectively (Otto and Simpson 2006). Lignin phenols were subsequently isolated from the dried soil or plant residues using the CuO oxidation method (Feng et al. 2016; Hedges and Mann 1979). About 1–2 g soil residues or 50 mg plant tissue residues were mixed with 1 g CuO, 100 mg ammonium iron (II) sulfate hexahydrate  $[\text{Fe}(\text{NH}_4)_2(\text{SO}_4)_2 \cdot 6\text{H}_2\text{O}]$  and 20 mL of 2 M  $\text{N}_2$ -purged sodium hydroxide solution in Teflon-lined bombs. All bombs were flushed with  $\text{N}_2$  in the headspace for 10 min and heated at 150 °C for 2.5 h. The oxidation products were spiked with a recovery standard (ethyl vanillin), acidified to pH 1 with 6 M hydrochloric acid (HCl), and kept in the dark for 1 h. After centrifugation, oxidation products were liquid-liquid extracted from the clear supernatant with ethyl acetate three times and concentrated under  $\text{N}_2$  for further analysis.

An aliquot (30–50%) of the oxidation products was derivatized with N,O-bis-(trimethylsilyl)trifluoroacetamide (BSTFA) and pyridine (70 °C, 1 h) to yield trimethylsilyl (TMS) derivatives. The derivatives were examined on a Trace 1310 gas chromatograph (GC) coupled to an ISQ mass spectrometer (MS; Thermo Fisher Scientific, USA) using a DB-5MS column (30 m × 0.25 mm i.d., film thickness, 0.25 μm) for separation (Dai et al. 2018). The oven temperature was held at 65 °C for 2 min, increased from 65 to 300 °C at a rate of 6 °C min<sup>-1</sup> with final isothermal hold at 300 °C for 20 min. Helium was used as carrier gas (0.8 mL min<sup>-1</sup>). The mass spectrometer was operated in the electron impact mode (EI) at 70 eV and scanned from 50 to 650 Da. Phenols were identified by comparing the mass spectra with the authentic standards of all typical lignin phenols and MS libraries. Quantification was achieved by comparison with the recovery standard (ethyl vanillin) using selected ions typical of target compounds after accounting for response variations using external phenol standards. Coefficient of variation associated with phenol concentrations are typically <10% based on replicate analysis of the same sample (Table S3).

## Lignin phenol indicators

CuO oxidation releases eight characteristic lignin phenols (Feng et al. 2016; Hedges and Mann 1979), including vanillyl (V; vanillin, acetovanillone, vanillic acid), syringyl (S; syringaldehyde, acetosyringone, syringic acid), and cinnamyl (C; *p*-coumaric acid, ferulic acid) units. The OC-normalized concentration of these monomers was used to indicate lignin concentrations in soils. In addition, CuO oxidation releases *p*-hydroxyl (P) phenols (including *p*-hydroxybenzaldehyde, *p*-hydroxyacetophenone, and *p*-hydroxybenzoic acid) and 3,5-dihydroxybenzoic acid (3,5 Bd) that may derive from proteins and “tannin-like” compounds or from demethylation of lignin (Feng et al. 2016; Prahl et al. 1994). Ratios of syringyl-to-vanillyl phenols (S/V) and cinnamyl-to-vanillyl phenols (C/V) are often used to indicate the relative input of angiosperm and non-woody tissues versus gymnosperm wood, respectively, because S and C phenols are considered to mainly derive from angiosperm and non-woody tissues, respectively (Jex et al. 2014; Moingt et al. 2016). Both ratios have also been observed to decrease with the preferential degradation of S and C relative to V phenols (Thevenot et al. 2010; and references therein). Demethylation of lignin (e.g., by brown-rot decay) leads to a selective loss of methoxylated phenols (such as V and S) with nonmethoxylated phenols (such as P phenols) unaffected (Feng et al. 2016; Goñi and Hedges 1995). The ratio of P/(V + S) may therefore reflect the diagenetic state of lignin when the other sources of P phenols (such as protein and tannin) are relatively constant (Dittmar and Lara 2001). By comparison, the ratio of 3,5 Bd/V is usually considered to reflect organic matter (OM) transformation in soils (Houel et al. 2006) as plants allegedly contain very low levels of 3,5 Bd. The acid-to-aldehyde (Ad/Al) ratios of V and S phenols are used to indicate lignin degradation and increases with increasing lignin oxidation (Feng and Simpson 2007; Otto and Simpson 2006). In addition, ratios of cutin/lignin and suberin/lignin are calculated using cutin and suberin data emanating from the same batch of soil samples (Ma et al. 2019) to investigate the stability of lignin relative to plant lipid components in the soil. Cutin-specific markers include C<sub>14</sub> and C<sub>16</sub> hydroxyalkanoic acids in the alpine grasslands and C<sub>14</sub> hydroxyalkanoic acid and C<sub>15</sub> dihydroxyalkanoic acid in the temperate grasslands, while suberin-specific markers include C<sub>20</sub>–C<sub>32</sub> ω-hydroxyalkanoic acids, C<sub>20</sub>–C<sub>32</sub> α, ω-dioic

acids and C<sub>18</sub> 9,10-epoxy-dioic acid in both regions (Ma et al. 2019). Lignin-specific biomarkers (V + S + C) are used to calculate the cutin/lignin and suberin/lignin ratios.

Similar to Dai et al. (2018), the following equation is used to estimate annual plant inputs of lignin phenols to surface soils (0–10 cm):

$$\text{Input} = [C_L \times 1/(1 + R) + C_R \times R/(1 + R)] \times \text{NPP}/1000 \quad (1)$$

where Input is the plant input of lignin phenols in the units of g m<sup>-2</sup> yr.<sup>-1</sup>; C<sub>L</sub> and C<sub>R</sub> is the OC-normalized lignin phenol concentrations (mg g<sup>-1</sup> OC) in the aboveground and belowground tissues of the dominant species, respectively; R is the ratio of BGB to AGB at each sampling site; NPP is the net primary productivity (g C m<sup>-2</sup> yr.<sup>-1</sup>). Similar to our previous studies (Dai et al. 2018; Ma et al. 2019), to calculate R, BGB in the alpine sites was estimated using the living to dead root mass of 1.167 that was used in a similar alpine grassland transect previously (Jing et al. 2015).

## Statistical analyses

Mean values (± standard error) were used for sites with spatial replicates in this paper. Differences in lignin phenol concentrations and parameters were examined using independent sample *t*-tests between the alpine and temperate grassland soils and using one-way ANOVA followed by post-hoc analysis (Dunnnett’s test) for varied vegetation types. Differences in cutin/lignin and suberin/lignin ratios of grass tissues and soils between the alpine and temperate grasslands were examined using Kruskal-Wallis test with mean rank’s post-hoc comparison due to non-normal distribution of variance and different sample sizes (IBM SPSS Statistics 20.0, Chicago, IL). To delineate influencing factors on the distribution of lignin phenols, Pearson’s correlation was performed for lignin phenol concentrations, cutin/lignin and suberin/lignin ratios with environmental factors. Environmental variables showing significant correlations with the cutin/lignin and suberin/lignin ratios were selected for multiple stepwise regression to further elucidate the most correlated factors. For the multiple stepwise regression analysis, 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). Variables were natural

logarithm transformed before analysis to achieve normal distribution. To ensure that the statistical results obtained using log-transformed data are relevant for the original, non-transformed data, we also performed all statistical analyses on the original, non-transformed data. Differences and correlations were considered to be significant at a level of  $p < 0.05$ .

## Results

### Lignin phenols in plants and inputs to soils

Despite large variabilities among species, lignin phenols showed higher OC-normalized concentrations in the temperate than alpine grass roots ( $p < 0.05$ ) but similar concentrations in the aboveground tissues from both regions ( $p > 0.05$ ; Figs. 1a–b). Notably, C phenols showed a higher abundance relative to V phenols in the belowground rather than aboveground tissues of all alpine and three temperate species (*Stipa grandis*, *Leymus chinensis*, *Achnatherum sibiricum*;  $p < 0.05$ ). Consequently, a higher C/V ratio was found for the belowground ( $0.53 \pm 0.12$ ) than aboveground tissues of alpine grasses ( $0.20 \pm 0.05$ ;  $p < 0.05$ ). A higher S/V ratio was also found for the alpine grass roots ( $1.81 \pm 0.21$ ) than the temperate counterparts ( $0.90 \pm 0.20$ ;  $p < 0.05$ ). In addition, a considerable amount of P phenols and 3,5 Bd were detected in the alpine grass roots, leading to higher ratios of P/(V + S) and 3,5 Bd/V in the alpine than temperate grass roots ( $p < 0.05$ ; Figs. 1c–d) while aboveground tissues contained a low amount of P phenols and 3,5 Bd in both regions. No significant difference in the (Ad/Al)<sub>V</sub> or (Ad/Al)<sub>S</sub> ratios was found for the aboveground or belowground tissues between alpine and temperate grasses ( $p > 0.05$ ; Figs. 1e–f). Consistent with Ma et al. (2018), the Ad/Al ratios were rather invariant in the temperate grass tissues with slight variations in the aboveground tissues for (Ad/Al)<sub>S</sub>. However, the belowground tissues of *Kobresia humilis* and the aboveground tissues of *Oxytropis ochrocephala* had much higher (Ad/Al)<sub>V</sub> ratios than all other species.

Using Eq. (1), we calculated the upper and lower ranges for the plant inputs of lignin phenols, P phenols and 3,5 Bd at every sampling site, assuming a 100% coverage by the dominating plant species typical for each vegetation type (in the absence of detailed species coverage data). The results showed that plant inputs of lignin phenols were significantly higher in the temperate

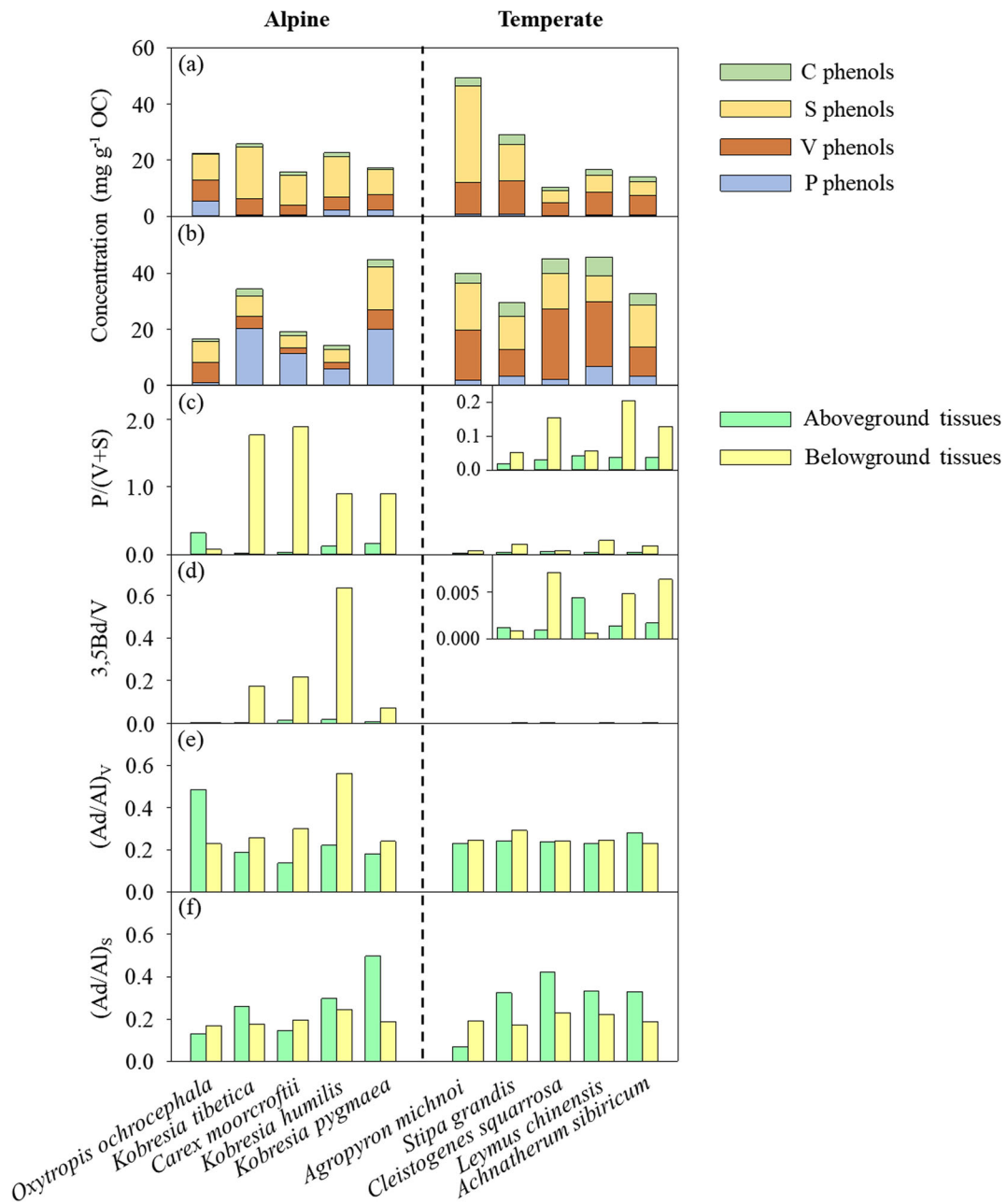
than alpine grasslands ( $p < 0.05$ ; Fig. S1) mainly due to the higher NPP in temperate grasslands. Plant inputs of P phenols did not differ between the two regions ( $p > 0.05$ ) whereas 3,5 Bd had a higher input in the alpine than temperate grasslands ( $p < 0.05$ ) due to its high abundance in the alpine plant roots.

### Lignin phenols in the soil

Lignin phenols had concentrations of 1.66–37.12 mg g<sup>-1</sup> OC in the investigated soils (Fig. 2a), comparable to other grassland soils (0.20–64 mg g<sup>-1</sup> OC; Thevenot et al. 2010; and references therein). The average abundance of lignin phenols was similar in the alpine and temperate grasslands ( $p > 0.05$ ). Both P/(V + S) and 3,5 Bd/V ratios were highest in the alpine meadows with slightly varying values in the other vegetation types (Figs. 2b–c). Both ratios were on average higher in the alpine than temperate grasslands ( $p < 0.05$ ). The (Ad/Al)<sub>V</sub> ratio was higher in the alpine desert steppes and comparable in all other soils, while (Ad/Al)<sub>S</sub> ratios were not significantly different in all soils ( $p > 0.05$ , Figs. 2d–e).

### Distribution of lignin phenols relative to cutin and suberin

To compare the preservation of lignin phenols relative to plant lipid components, we calculated the relative proportion of cutin, suberin and lignin phenols isolated from the same plant and soil samples (Ma et al. 2019; Fig. 3a). Overall, lignin phenols accounted for  $65 \pm 3\%$  and  $45 \pm 4\%$  of these components in the aboveground and belowground tissues of alpine grasses, respectively and for  $71 \pm 7\%$  and  $75 \pm 5\%$  in the temperate counterparts. In the surface soils, the relative proportion of lignin phenols were comparable among all vegetation types ( $p > 0.05$ ) except alpine meadows, which showed a slightly lower proportion of lignin phenols and higher ratios of cutin/lignin and suberin/lignin ( $p < 0.05$ ). Nonetheless, the average proportion of lignin phenols decreased to  $42 \pm 3\%$  and  $53 \pm 2\%$  in the alpine and temperate grassland soils, respectively. Conversely, the proportion of cutin and suberin derived lipids were higher in soils, with suberin-specific compounds accounting for  $42 \pm 2\%$  and  $39 \pm 2\%$  of the analyzed components in the alpine and temperate soils, respectively. This trend resulted



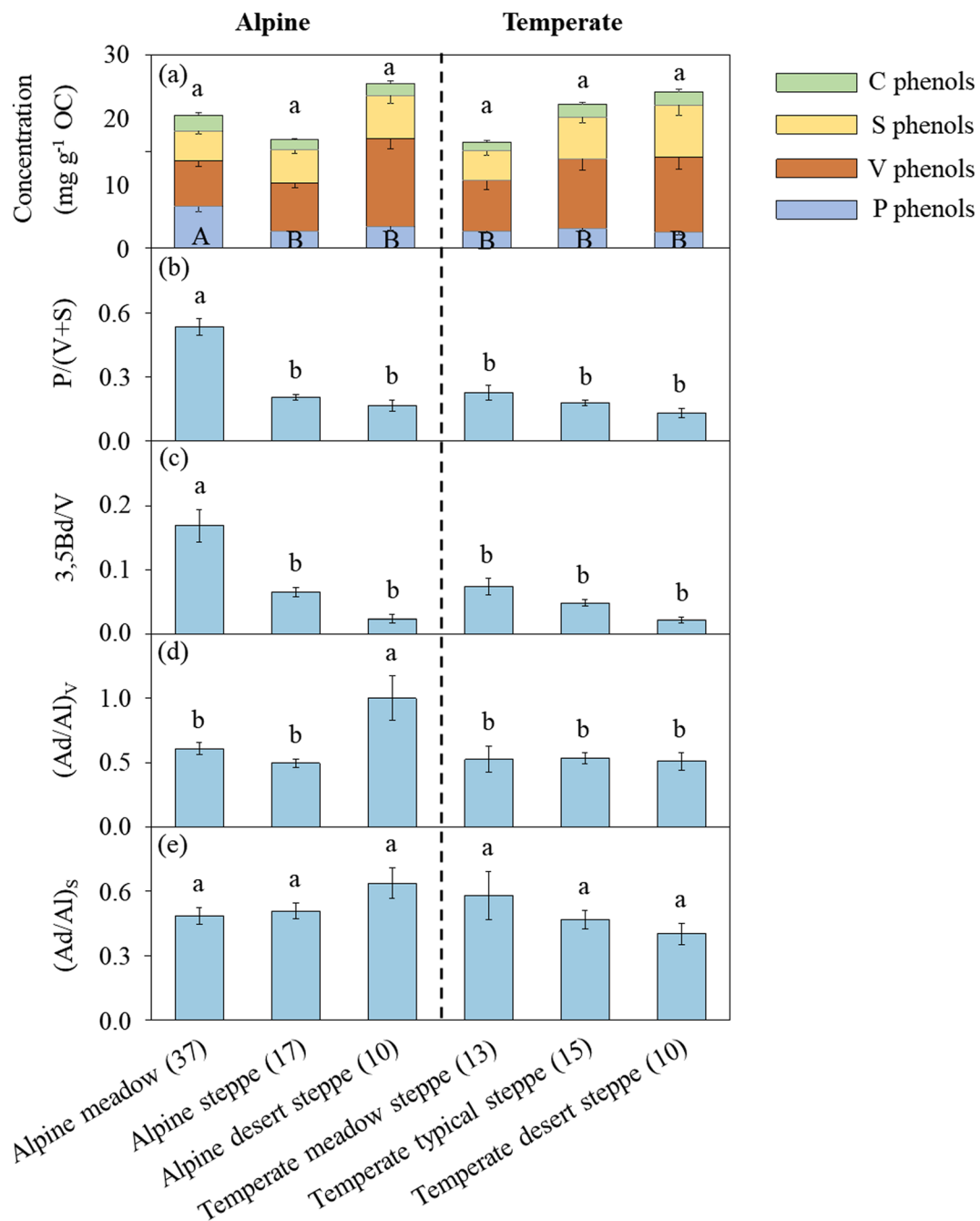
**Fig. 1** Organic carbon (OC)-normalized concentration of cinnamyl (C), syringyl (S), vanillyl (V) and *p*-hydroxyl (P) phenols in the leaves (a) and roots (b) as well as the ratios of P/(V + S) (c), 3,5-dihydroxybenzoic acid/V phenols (3,5 Bd/V; d), acid-to-

aldehyde (Ad/Al) ratios of V ((Ad/Al)<sub>v</sub>; e) and S phenols ((Ad/Al)<sub>s</sub>; f) in the leaves and roots of dominant grasses in the alpine and temperate grasslands

in significant increases in the ratios of cutin/lignin and suberin/lignin from grass tissues to soils (Figs. 3b-c). Moreover, ratios of cutin/lignin and suberin/lignin were significantly higher in the alpine grass tissues and soils than those in the temperate counterparts ( $p < 0.05$ ).

#### Environmental influences

In the alpine grassland soils, lignin phenols are only negatively correlated with AI ( $p < 0.05$ ; Fig. 4). In the temperate grassland soils, lignin phenols are negatively correlated with AI, SOC, N and AI contents and



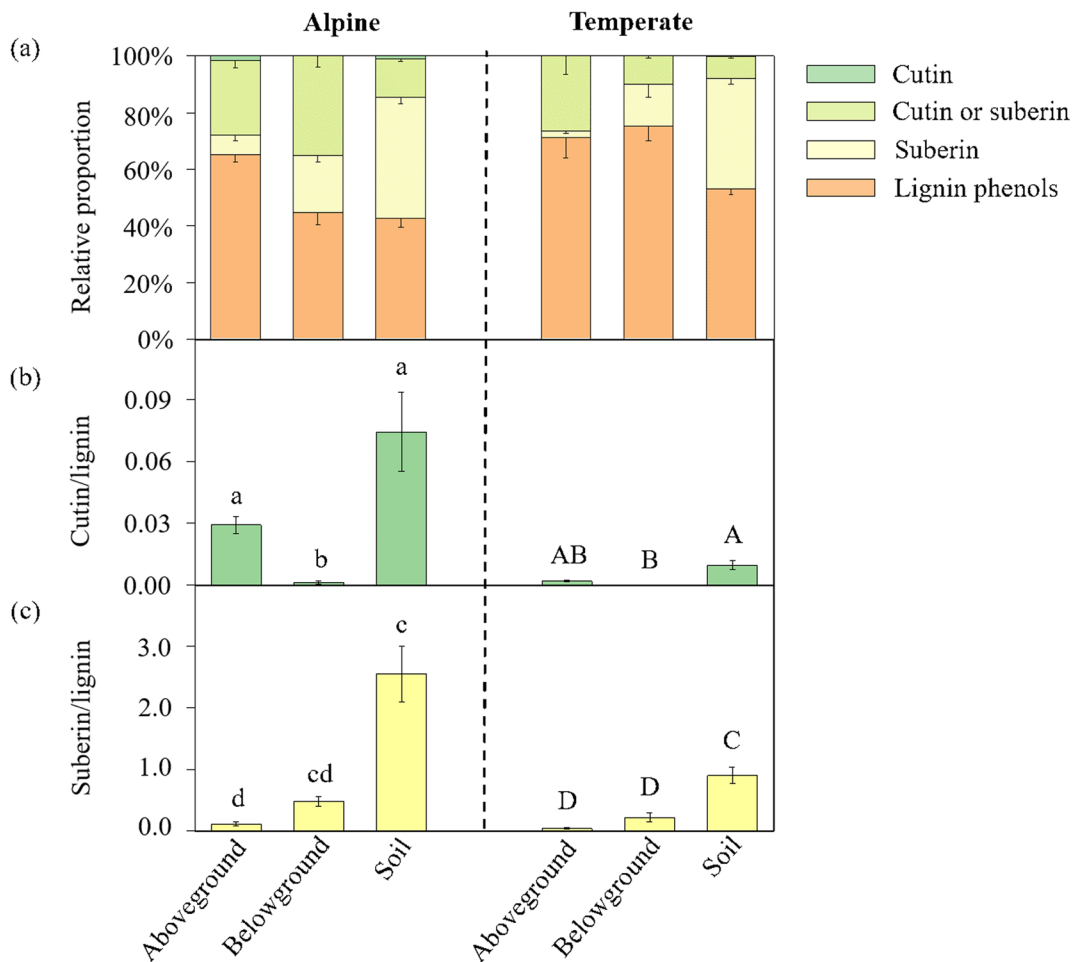
**Fig. 2** Organic carbon (OC)-normalized concentration of cinnamyl (C), syringyl (S), vanillyl (V) and *p*-hydroxyl (P) phenols (a), ratios of P/(V+S) (b), 3,5 Bd/V (c), (Ad/Al)<sub>V</sub> (d) and (Ad/Al)<sub>S</sub> (e) in the surface soils (0–10 cm) under different vegetation types. Numbers in parentheses represent for the number of

samples analyzed. Abbreviations for the parameters are listed in Fig. 1. Letters indicate different levels among vegetation types, a-b for lignin phenols (V+S+C) and A-B for P phenols in (a) ( $p < 0.05$ )

positively correlated with soil pH and sand contents ( $p < 0.05$ ). The suberin/lignin ratio is only positively related to SOC in the alpine grassland soils, while the cutin/lignin ratio is positively correlated with AI,

SOC, N and AI contents ( $p < 0.05$ ; Fig. 4). By comparison, the suberin/lignin ratio is positively correlated with SOC, N, Fe and AI contents and negatively correlated with sand and soil pH in the temperate





**Fig. 3** Relative proportion of lignin phenols, cutin and suberin markers (a) as well as ratios of cutin to lignin phenols (cutin/lignin; b) and suberin to lignin phenols (suberin/lignin; c) in the grass leaves, roots and soils of alpine and temperate grasslands. Letters

indicate different levels for the leaf, root and soil: a-b for cutin/lignin and c-d for suberin/lignin in the alpine grasslands; A-B for the cutin/lignin and C-D for suberin/lignin in the temperate grasslands ( $p < 0.05$ )

grassland soils ( $p < 0.05$ ). Multiple stepwise regression analysis shows that AI and SOC are most tightly correlated with the cutin/lignin ratios, while SOC is most tightly correlated with the suberin/lignin ratio in the alpine grassland soils (Table 1). In the temperate grassland soils, sand contents are primarily correlated with the suberin/lignin ratio followed by Fe contents.

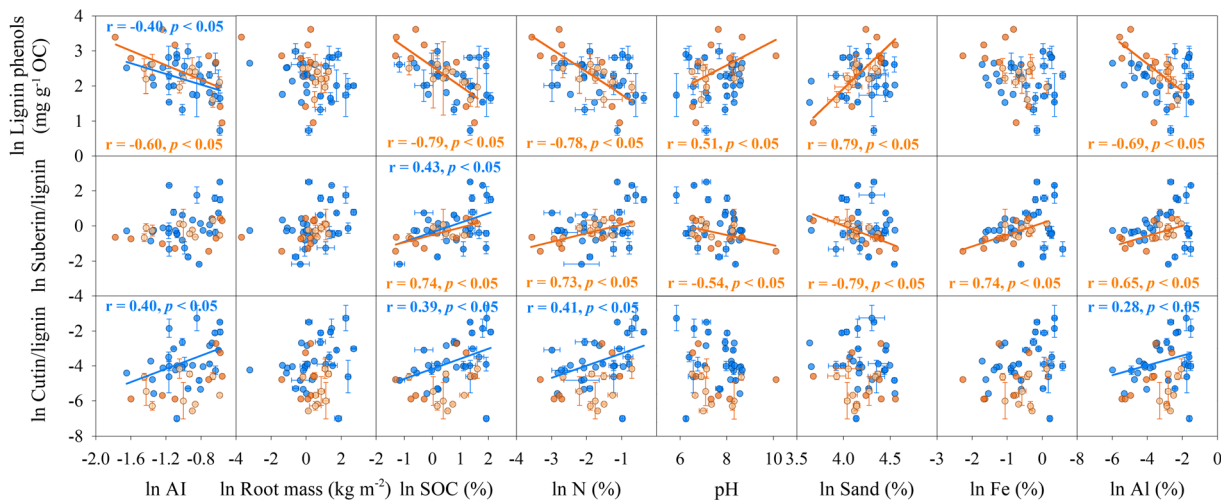
## Discussion

**Caveats in lignin phenol applications: C and P phenols in grass tissues**

Conventionally, lignin C phenols are thought to originate mainly from plant leaves (non-woody parts) in grassland

ecosystems (Filley et al. 2008; Hedges and Mann 1979) and the ratio of C/V is commonly used to indicate the relative contribution of non-woody species in paleovegetation reconstructions (Jex et al. 2014; and references therein). However, our study shows that compared to plant aboveground tissues, roots have higher (for alpine species) or similar (for temperate species) C/V ratios (Figs. 1a-b). This result is consistent with a recent study reporting high amounts of C phenols in the woody parts or roots of angiosperms (Moingt et al. 2016), urging caution in the use of C/V ratio as a proxy for non-woody sources.

Another interesting observation is that 3,5 Bd and P phenols occurred in a sizable amount in the alpine grass roots (Figs. 1c-d). These phenols may derive from proteins and tannin upon CuO oxidation (Houel et al. 2006; Prah et al. 1994). However, up till now, they have been



**Fig. 4** Pearson's correlations of lignin phenol concentrations, suberin/lignin and cutin/lignin ratios with environmental variables in the grassland soils. Blue dots and lines represent alpine sites ( $n = 30$ ), and orange dots and lines represent temperate sites ( $n = 18$ ). Light orange dots are data from Ma et al. (Ma et al. 2018) and

dark orange dots are newly analyzed data. Data presented are all natural logarithm transformed except pH. AI, aridity index; SOC, soil organic carbon; N, nitrogen; Fe: dithionite-extractable iron; Al: dithionite-extractable aluminum. Error bars represent standard error of mean for sites with spatial replicates

mainly treated as a byproduct of soil OM degradation (Dittmar and Lara 2001; Feng et al. 2016), although their occurrence in plant tissues has been reported previously (Goñi and Hedges 1995; Moingt et al. 2016; Zaccane et al. 2008). Their significant occurrence in our studied grass roots adds to the mounting evidence that these compounds are not specific markers for OM transformation and should be used with caution.

Tannins and other phenolic compounds are involved in plants' ultraviolet (UV) light absorption, tolerance to water shortage, thermal protection and defense against herbivores or pathogens (Cisneros et al. 2013). Hence, we postulate that the observed high abundance of 3,5 Bd and P phenols in the alpine grass roots may be related to

plants' adaptation to the strong UV radiation and/or low temperatures in the alpine regions (Zhao et al. 2006). This postulation agrees with the high content of antioxidant tannin in the alpine plants on the QTP, which tends to increase with elevation due to plants' adaptation to higher oxidative stress (Cui et al. 2016). Unlike other four alpine grasses (*Kobresia pygmaea*, *Kobresia humilis*, *Kobresia tibetica* and *Carex moorcroftii*), *Oxytropis ochrocephala*, mainly present in the alpine desert and desert steppe, contained high amounts of alkaloids rather than phenolics as defensive compounds (Tan et al. 2017). This may explain the low level of 3,5 Bd and P phenols in its root compared with other alpine grasses (Figs. 1c-d). The high ratios of 3,5 Bd/V and P/(V + S) in the alpine grassland

**Table 1** Standardized partial regression coefficient of multiple stepwise regression analysis for the cutin/lignin and suberin/lignin ratios with environmental variables in the alpine ( $n = 30$ ) and temperate grassland soils ( $n = 18$ ). Bold values denote the highest regression coefficient

Region	Ratio	Standardized partial regression coefficient						model $R^2$	$p$ value
		AI	Root mass	SOC	pH	Sand	Fe		
Alpine	Cutin/lignin	<b>0.37</b>	ns	0.35	ns	ns	ns	0.29	< 0.05
	Suberin/lignin	ns	ns	<b>0.43</b>	ns	ns	ns	0.19	< 0.05
Temperate	Suberin/lignin	ns	ns	ns	ns	<b>-0.54</b>	0.44	0.75	< 0.01

In the temperate grassland soils, multiple stepwise regression analysis was not conducted for the cutin/lignin ratios, since they were not correlated to any environmental variables (Fig. 4)

AI: aridity index; SOC: soil organic carbon; Fe: dithionite-extractable iron; ns: not significant

soils hence may reflect phenol compositions in the source plants rather than enhanced SOC decomposition compared to the temperate grassland soils.

#### Patterns of lignin distribution in the alpine versus temperate grassland soils

Lignin phenol distribution in the temperate grasslands is highly consistent with that described in Ma et al. (2018) with the inclusion of eight additional sites (Fig. 4). As such, lignin phenols decrease per unit of SOC with increasing AI and SOC (and N) accumulation likely due to lignin phenol decrease relative to other SOC components, such as microbial carbon (Kallenbach et al. 2016; Liang et al. 2017), as microbial derived amino sugars increase with AI and SOC along the Mongolian transect (Ma et al. 2018). Moreover, the positive correlations of suberin/lignin and cutin/lignin ratios with SOC in both regions (Fig. 4) indicates the decrease of lignin phenols relative to other more resistant plant components (such as hydrolysable lipids; Feng and Simpson 2007; Lorenz et al. 2007) with SOC accrual. As SOC associated with minerals (hence presumably fine particles) is reportedly depleted in lignin and enriched in lipids (Guggenberger et al. 1994; Kögel-Knabner et al. 2008), the decrease of lignin phenols relative to lipid components also explains lignin phenols' increase with sand contents in the temperate grasslands (Fig. 4). In addition, the decrease of lignin phenols with increasing AI can be attributed to enhanced lignin oxidation with increasing moisture conditions (Ma et al. 2018). Soil pH also has a positive effect on lignin phenols likely due to reduced fungal activities, since fungal richness and activities decrease with increasing soil pH in alkaline soils (Maestre et al. 2015; Tedersoo et al. 2014).

By comparison, lignin phenols are not correlated to any soil properties in the alpine grasslands despite similar ranges of edaphic variables (Fig. 4). This discrepancy may be attributed to the varied stage of OM transformation as SOC exhibits a lower degradation stage in the temperature-constrained alpine grasslands (Dai et al. 2018). Hence, lignin phenol variations may be more strongly influenced by plant inputs rather than decomposition processes that are closely related to edaphic properties. Nonetheless, similar to the temperate counterparts, lignin phenols are negatively correlated with AI in the alpine grassland soils, i.e., lignin phenols are more concentrated at drier sites. This phenomenon may be explained by two possible causes. First, with increasing

AI (i.e., humidity), plant biomass (especially AGB) and productivity (NPP) increase in the alpine grasslands ( $p < 0.05$ ), thus increasing root exudates and/or labile substrates such as carbohydrates (Amelung et al. 1999). The co-metabolic decomposition of lignin may thus accelerate (Kiem and Kögel-Knabner 2003). Increasing moisture also facilitates fungal degradation of lignin due to a higher dominance of fungi in moist soils (Maestre et al. 2015) as the primary decomposer of lignin (Jex et al. 2014; and references therein). Second, the positive relationships between AI and cutin/lignin in the alpine grasslands (Fig. 4) indicate a selective accumulation of hydrolysable lipids relative to lignin with increasing humidity, potentially resulting in the relative decrease of lignin phenols compared to cutin. Potential accumulation of microbial carbon in moist soils (Ma et al. 2018) may also contribute to the relative decrease of lignin phenols.

Notably, different from the temperate counterparts, the  $(Ad/AI)_V$  ratio was higher in the soils of alpine desert steppes than alpine steppes and alpine meadows in the alpine grasslands with higher AI values. Alpine desert steppes have a lower foliar coverage than other vegetation types (Liu et al. 2016), resulting in higher exposure of surface soils to UV radiations. Photo-oxidation is known to elevate  $Ad/AI$  ratios in soils (Feng et al. 2011), potentially contributing to higher  $(Ad/AI)_V$  and, to a lesser extent,  $(Ad/AI)_S$  ratios in the alpine desert steppes. The photo-oxidation effect on lignin decay may be particularly strong on the Qinghai-Tibetan Plateau compared to the temperate regions due to strong UV radiations at high elevations and temperature-constrained microbial decomposition. Alternatively, in contrast to the invariant  $(Ad/AI)_V$  ratios in temperate grasses, *Oxytropis ochrocephala* that dominates alpine desert steppes has a much higher  $(Ad/AI)_V$  ratio in its aboveground tissues than all other alpine species (Fig. 1e). The elevated  $(Ad/AI)_V$  ratio in the soils of alpine desert steppes may hence be attributed to source plant variations as well. Environmental influences on the  $Ad/AI$  ratios are not analyzed because different plant tissues showed varying ratios (Figs. 1e-f) and the exact proportion of plant species at each site was not collected during sampling.

#### Lignin preservation in comparison with plant lipids in grassland soils

One main objective of this study is to test whether, similar to plant-derived lipids, lignin phenols have a

better preservation in the alpine than temperate grassland soils. Despite higher plant inputs in the temperate grasslands (Fig. S1), the OC-normalized concentrations of lignin phenol were similar in the surface soils of the two areas (Fig. 2a). This result supports our first hypothesis that lignin is better preserved in the alpine soils. However, it also implies that, compared to plant lipids that show a higher OC-normalized concentration in the alpine soils (Dai et al. 2018; Ma et al. 2019), lignin phenols are less well preserved. The increasing proportion of cutin- and suberin-derived compounds from plant tissues to surface soils (Fig. 3) also suggests a selective preservation of plant lipids relative to lignin phenols.

The relative enrichment of plant lipids is governed by varied environmental variables in the alpine versus temperate grassland soils. In the alpine grasslands, regional humidity (AI) and SOC are positively correlated with the cutin/lignin ratio, while SOC content is most tightly correlated with the suberin/lignin ratio (Table 1). Additionally, root mass and regional humidity (AI) also have non-negligible effects on the suberin/lignin ratio (Table S4). As mentioned previously, these relationships may be associated with an enhanced co-metabolic degradation of lignin (Amelung et al. 1999; Kiem and Kögel-Knabner 2003) under wetter climate as well as the relative decrease of lignin phenols compared to root-derived carbon since root is a more important contributor to SOC accrual in our grasslands (Ma et al. 2019). By comparison, in the temperate grasslands, sand and Fe contents are tightly correlated with the suberin/lignin ratio (Table 1) likely due to sorptive protection of hydrolysable lipids by reactive soil minerals (Guggenberger et al. 1994; Kögel-Knabner et al. 2008). These findings suggest that the variation of plant lipid (in particular, shoot-derived lipid) versus lignin components is mainly related to climate (in particular, aridity index) in the QTP alpine grassland soils via affecting their input and accumulation. By comparison, the relative abundance of plant-derived lipids and lignin phenols is more related to mineral protection in the temperate grassland soils in a higher degradation state.

## Conclusions

In summary, through measuring lignin phenols along two large-scale soil transects across the alpine and temperate grasslands of China and Mongolia, we show that lignin is better preserved in the alpine than temperate soils despite

a lower plant input. However, compared to plant-derived lipids, lignin is less stabilized, reflected by the enrichment of suberin relative to lignin with decreased sand and increased reactive Fe contents (in the temperate soils), and SOC (and N) accrual (in the alpine soils). Moreover, in contrast to previous studies that emphasize the temperature effect, our study shows that regional aridity rather than temperature has a strong impact on lignin distribution in the studied grasslands via affecting the input and accumulation of other SOC components. Hence, lignin decomposition may be more sensitive to variations in the source and input to SOC rather than temperature alone.

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## Compliance with ethical standards

**Conflict of interest** The authors have no conflict of interest to declare.

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