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Research Article

Warming and elevated CO_2 alter the suberin chemistry in roots of photosynthetically divergent grass species

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Abstract. A majority of soil carbon (C) is either directly or indirectly derived from fine roots, yet roots remain the least understood component of the terrestrial carbon cycle. The decomposability of fine roots and their potential to contribute to soil C is partly regulated by their tissue chemical composition. Roots rely heavily on heteropolymers such as suberins, lignins and tannins to adapt to various environmental pressures and to maximize their resource uptake functions. Since the chemical construction of roots is partly shaped by their immediate biotic/abiotic soil environments, global changes that perturb soil resource availability and plant growth could potentially alter root chemistry, and hence the decomposability of roots. However, the effect of global change on the quantity and composition of root heteropolymers are seldom investigated. We examined the effects of elevated CO_2 and warming on the quantity and composition of suberin in roots of *Bouteloua gracilis* (C4) and *Hesperostipa comata* (C3) grass species at the Prairie Heating and CO_2 Enrichment (PHACE) experiment at Wyoming, USA. Roots of *B. gracilis* exposed to elevated CO_2 and warming had higher abundances of suberin and lignin than those exposed to ambient climate treatments. In addition to changes in their abundance, roots exposed to warming and elevated CO_2 had higher ω-hydroxy acids compared to plants grown under ambient conditions. The suberin content and composition in roots of *H. comata* was less responsive to climate treatments. In *H. comata*, α,ω-dioic acids increased with the main effect of elevated CO_2, whereas the total quantity of suberin exhibited an increasing trend with the main effect of warming and elevated CO_2. The increase in suberin content and altered composition could lower root decomposition rates with implications for root-derived soil carbon under global change. Our study also suggests that the climate change induced alterations in species composition will further mediate potential suberin contributions to soil carbon pools.

Keywords: Climate change; elevated CO_2; fine roots; grassland; lignin; suberin; warming.

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Introduction

Fine roots (with diameter < 2 mm) not only help plants in the uptake of resources, but also contribute to 20–67 % of the terrestrial net primary productivity (Jackson *et al.* 1997; McCormack *et al.* 2015). Along with the root biomass, fine roots also input a considerable amount of carbon through root exudates that support high microbial biomass and diversity (Jones *et al.* 2009), thus enabling greater rhizosphere activity. Unlike leaves, roots are heavily protected against biotic and abiotic stressors by the organized deposition of heteropolymeric compounds such as lignins, tannins and suberins along the tissue matrix. These polymeric compounds are relatively slow to decompose and are associated with higher residence time of C in roots than in leaves (Feng and Simpson 2007; Filley *et al.* 2008). Also, due to their close proximity to soil, the decomposition products from roots are better incorporated to soil matrix. Thus, fine roots contribute significantly to the sequestration of atmospheric CO_2 in soils (Mendez-Millan *et al.* 2010). Roots achieve varied functions and resistance to a myriad of biotic and abiotic stresses by altering the quantity and composition of heteropolymers; hence the composition of heteropolymers in roots is primarily governed by the soil environment to which the roots are exposed, and by the resource demand of the plant. Thus, global changes that impose constraints on soil resources and plant growth demands could potentially alter fine root tissue chemistry, which in turn could alter soil carbon sequestration. Although, it is widely recognized that a majority of carbon sequestered in soil is root derived (Russell *et al.* 2004; Rasse *et al.* 2005), remarkably little is known about the effect of global change on the quality of root biopolymers.

Global change factors such as warming and elevated CO_2 can affect fine root dynamics directly by influencing soil resource availabilities and indirectly through feedbacks from changes in above-ground productivity. For example, a warming-induced increase in N mineralization can reduce the allocation of resources to roots (Melillo *et al.* 2011) leading to lower root biomass. However, an increase in photosynthesis due to elevated CO_2 could increase root biomass (de Graaff *et al.* 2006; Dieleman *et al.* 2010; Nie *et al.* 2013), thus enabling plants to capture the nutrients necessary to sustain above-ground growth (Luo *et al.* 2004; Norby *et al.* 2010). Under these environmental conditions, fine roots adopt several morphological and physiological strategies that effectively facilitate their nutrient and water uptake functions while concurrently protecting their tissues from biotic and abiotic stresses. For example, fine roots alter their diameter, length and tissue density to improve their resource uptake functions under global change factors (Nie *et al.* 2013; Carrillo *et al.* 2014; Nelson *et al.* 2017).

Along with the morphological changes, roots may undergo changes in tissue chemistry as they rely on heteropolymers such as suberins and lignins to protect their tissues from pests and pathogens. Although, there is mounting evidence that fine roots alter their specific root area, specific root length and tissue density in response to warming, elevated CO_2 and resource availabilities (Nie *et al.* 2013; Pilon *et al.* 2013; Nelson *et al.* 2017), there is sparse knowledge about the accompanying changes in the quantity and composition of heteropolymers of roots (Brunner *et al.* 2015). The chemistry of roots is a key factor regulating microbial carbon use efficiency and tissue decomposition, so a molecular-level understanding of root chemistry in response to global change factors is a prerequisite for accurately predicting root-derived soil carbon under future climates.

Our knowledge of the chemistry of roots exposed to warming and elevated CO_2 is mostly limited to carbon, nitrogen and lignin. However, less is known about suberin, a dynamic biopolymer of roots that functions as a barrier to reduce uncontrolled transport of water, dissolved ions and gasses, and also defend against pathogens and toxic compounds in the rhizosphere (Baxter *et al.* 2009; Graca 2015). Suberin is an extracellular biopolymer comprised of polyaliphatic and polyphenolic domains. The predominant aliphatic components are ω-hydroxy acids, α,ω-dioic acids, fatty acids and primary alcohols, whereas the polyphenolic domain is mainly composed of hydroxycinnamic acid, especially ferulic acid (Schreiber *et al.*, 1999; Bernards 2002). Along with their quantity, the function and biological reactivity of heteropolymers such as suberin are also governed by the identity of their monomeric units and the linkages connecting these units.

In this study, we investigated the effect of global change factors such as elevated CO_2 and warming on root chemistry particularly focusing on suberin. We hypothesized that warming and elevated CO_2 would alter the quantity and chemical composition of suberin in grass roots. As elevated CO_2 increases below-ground resource allocation, we predicted that elevated CO_2 alone or in combination with warming would have a greater effect on the content and composition of suberin than warming alone. To test this hypothesis, we used common grass species belonging to C3 (*Hesperostipa comata*) and C4 (*Bouteloua gracilis*) functional types exposed to factorial combinations of warming and elevated CO_2 at the Prairie Heating and CO_2 Enrichment (PHACE) experiment in Wyoming, USA (Morgan *et al.* 2011).

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Methods

Study site

The PHACE experiment (Cheyenne, WY, USA; latitude 41°11′N, longitude 104°54′W) subjects a semi-arid mixed-grass prairie to a factorial combination of two levels of CO_2 treatment (400 p.p.m.v. and elevated to 600 p.p.m.v.; abbreviated as c and C, respectively) and two levels of warming treatment (ambient and +1.5/3.0 °C warmer in the day/night; abbreviated as t and T, respectively). The study site has a mean temperature of 17.5 °C in July and a mean of −2.5 °C in January. The site has a mean annual precipitation of 384 mm. Free-air CO_2 enrichment (FACE) technology was used to apply the CO_2 treatments in 3.3-m diameter FACE rings and infrared heaters were used to raise the canopy temperature in the warming treatments (Dijkstra *et al.* 2010; Morgan *et al.* 2011). There were five replicates for each treatment. The most dominant species at the study site included *B. gracilis* (C4 grass), *H. comata*, *Pascopyrum smithii* and *Koeleria macrantha* (C3 grasses) and *Artemisia frigida* (shrub species; Zelikova *et al.* 2014).

Litter chemistry analyses (sequential extraction)

Live, pigmented fine roots of *B. gracilis* and *H. comata* were collected from *ct* (ambient CO_2, ambient temperature), *Ct* (elevated CO_2, ambient temperature), *cT* (ambient CO_2, warming) and *CT* (elevated CO_2, warming) treatments at the PHACE site in July 2013, following 7 full years of treatments. From each treatment plot, blocks of soil from an area of 25 cm^2 that contained live plants and roots of the study species were excavated to a depth of 10 cm. In each block, the study species were separated by identifying the grasses based on the above-ground leaf tissues followed by tracing the roots of the corresponding species in the soil block (Nelson *et al.* 2017). The roots were washed in deionized (DI) water, oven-dried at 60 °C, finely powdered using a genogrinder (Spex Sample Prep, Metuchen, NJ, USA). About 200 mg of the powdered samples were added to 10 mL glass tubes and were subjected to a sequential extraction procedure. The samples were first subjected to solvent extraction using an equal mixture of methylene chloride and methanol followed by sonicating the samples for 5 min. The samples were shaken overnight and then centrifuged at 2500 r.p.m. for 5 min and the supernatant was removed. The roots remaining after the solvent extraction were subjected to base hydrolysis with 1 M methanolic KOH (pre-sparged with Ar for 20 min) at 20 °C and were shaken overnight. The samples were then centrifuged at 2500 r.p.m. for 5 min and the supernatant was removed. The roots subjected to base hydrolysis were further incubated at 100 °C for 3 h in the presence of 1 M methanolic KOH (pre-sparged with argon for 20 min; Tamura and Tharayil 2014; Wang

Table 1. Suberin monomers identified from the roots of C3 and C4 grass species exposed to different climatic treatments.

No	Suberin monomers	m/z	Monomer class	Abbreviation
1	Docosane-1,22-dioic acid	514, 499, 147, 129, 117, 73	α,ω-Dioic acid	DOCO
2	Hexacosane-1,26-dioic acid	570, 555, 439, 217, 204, 147, 129	α,ω-Dioic acid	HAD
3	Octadec-9-ene-1,18-dioic acid	456, 441, 217, 204, 170	α,ω-Dioic acid	OCTA
4	Tetracosane-1,24-dioic acid	542, 527, 411, 217, 204, 147, 129	α,ω-Dioic acid	TETRA
5	4-Hydroxy-3-methaxybenzoic acid	254, 239, 224, 193, 179	Aromatics	4H3MBA
6	4-Hydroxy-3-methoxybenzaldehyde	224, 209, 194, 179, 165, 163	Aromatics	4H3MBAL
7	Coumaric acid	308, 293, 249, 219, 179	Aromatics	CA
8	Ferulic acid	338, 323, 308, 293, 279, 249	Aromatics	FA
9	16-Hydroxyhexadecanoic acid	401, 385, 311, 217, 204	ω-Hydroxy acid	16HHDA
10	18-Hydroxyoctadec-9-enoic acid	442, 427, 411, 383, 337	ω-Hydroxy acid	18HOD9EA
11	18-Hydroxyoctadecanic acid	444, 429, 413, 439, 217	ω-Hydroxy acid	18HODA
12	22-Hydroxydocosanoic acid	485, 217, 204, 147, 129	ω-Hydroxy acid	22HDSA
13	24-Hydroxytetracosanoic acid	513, 497, 423, 217, 204	ω-Hydroxy acid	24HHCSA
14	26-Hydroxyhexacosanoic acid	541, 525, 451, 147, 117	ω-Hydroxy acid	26HHCSA
15	Hexadecanoic acid	328, 313, 201, 145, 132, 117, 73	ω-Hydroxy acid	HEXA
16	Octadecanoic acid	341, 201, 145, 132, 117, 73	ω-Hydroxy acid	ODA

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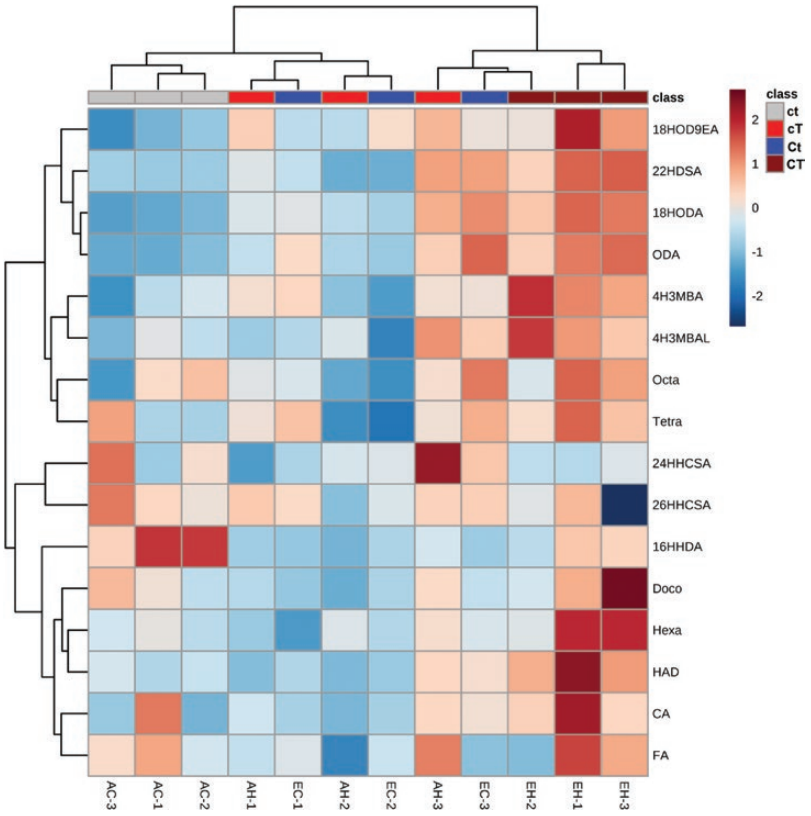


Figure 1. Heatmap and two-way hierarchical clustering of suberin monomers of roots of *B. gracilis* exposed to different climate treatments. Each column represents a sample from a climate treatment, and each row represents a positively identified compound, based on GC-MS (Table 1). Key: *ct* (ambient CO_2, ambient temperature), *Ct* (elevated CO_2, ambient temperature), *cT* (ambient CO_2, warming) and *CT* (elevated CO_2, warming).

et al. 2015). The hydrolysates were cooled in ice, centrifuged at 2500 r.p.m. for 5 min and the supernatant was removed. To the supernatants 15 mL of DI water and 600 µL of 50 % HCl were added. The tubes were gently shaken and then added a mixture of methylene chloride and methanol (1:1) and put in ice bath for 15 min. The tubes were gently shaken for 10 min and put at 4 °C overnight for phase separation. The methylene chloride fraction settled at the bottom of the tube; ~200 µL of this fraction from each sample were added to a low volume GC vial to which 5 µL of C19 (methyl nonadecanoate; 1000 p.p.m. in hexane) were added as internal standard. The vials were then completely dried under nitrogen, followed by derivatization using 100 µL of *N*-methyl-*N*-methyl-*N*-(trimethylsilyl)-trifluoroacetamide with 1 % trimethylchlorosilane (MSTFA + 1 % TMCS) and incubation at 60 °C for 30 min. The derivatized samples were analysed within 8 h using gas-chromatography mass spectrometry (GC-MS) as per Tamura and Tharayil (2014) and compounds were positively identified based on comparison of mass fragmentation patterns (Table 1) with Wiley 9th + NIST08 MS Libraries (Agilent Technologies) and in comparison with external standards and the literature (Jarvinen *et al.* 2009).

Diffuse reflectance infrared Fourier transform spectroscopy

The solvent-extracted tissues of *B. gracilis* were further analysed using diffuse reflectance infrared Fourier transform (DRIFT) spectroscopy (Suseela *et al.* 2013, 2014*a*). DRIFT is a complementary technique to wet chemistry analysis, because it provides the overall chemical composition based on the molecular vibration of the tissue matrix. The DRIFT spectra were acquired in transmission mode using a Perkin-Elmer Spectrum One DRIFT spectrometer with a deuterated triglycine sulfate detector. For acquiring the DRIFT spectra, the solvent-extracted root tissues were mixed with spectral grade KBr in a ratio of 1:50 using an agate mortar and pestle and packed in a macrocup accessory. The spectra were collected from 4000 to 650 cm^{-1} at 4 cm^{-1} resolution. For each sample, we acquired 40 interferograms and the spectra were then transformed to Kubelka–Munk units, and the baseline was corrected (ACD Spec Manager; Advanced

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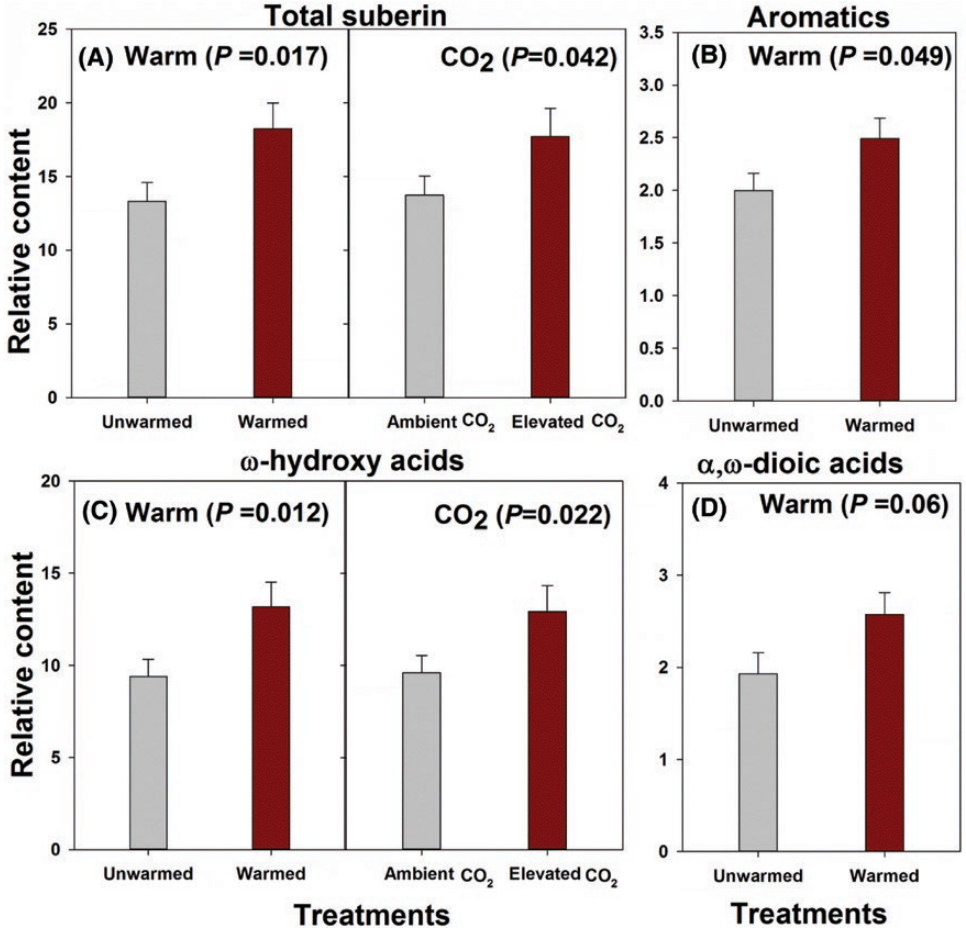


Figure 2. Effect of warming and CO_2 treatments on the relative contents of (A) total suberin, (B) aromatics, (C) ω-hydroxy acids and (D) α,ω-dioic acids in roots of *B. gracilis*. Values represent means ± SE (n = 4).

Chemistry Development, Toronto, ON, Canada). The peaks between 3000 and 2800 cm^{-1} correspond to C–H stretching contributed by aliphatic compounds such as suberin. The other important peaks identified were 1510 cm^{-1} (C=C aromatic skeletal vibration attributed to lignin); 1375–1370 cm^{-1} (C–H symmetrical bending attributed to phenols and aliphatic compounds) and 1200–800 cm^{-1} (C–O stretching and O–H deformation, representing carbohydrates).

Statistical analysis

The data on the chemical composition of suberin in roots were analysed using a two-way analysis of variance with warming and CO_2 as the main factors (SAS v.9.2; SAS Institute Inc., Cary, NC, USA). Differences between climate change treatments were analysed using Tukey's HSD test with significance inferred for $P < 0.05$. We did a hierarchical cluster analysis on both treatments and the suberin monomers and generated a heat map (Metaboanalyst; Xia *et al.* 2012) to visualize the difference in the relative abundance of suberin monomers in roots exposed to different treatments. The DRIFT data were subjected to principal component analysis (PCA) to compare samples across different treatments. For the PCA analysis, we used the relative peak heights of 11 DRIFT peaks that corresponded to important carbon functional groups. In each sample, the relative peak heights were computed as the ratio of the intensity of each of the individual peaks to the sum of intensities of the 11 peaks (Suseela *et al.* 2014*a*).

Results

We identified 16 monomers of suberin in both the C3 and C4 species, of which eight belonged to ω-hydroxy acids and four each to α,ω-dioic acids and aromatic monomers (Table 1). The *B. gracilis* roots exposed to ambient conditions (*ct*; control) clustered separately from all other treatments and those exposed to combination of warming and elevated CO_2 had higher abundance of monomers

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belonging to ω-hydroxy acids and aromatic monomers (Fig. 1). Moreover, the total quantity of suberin increased with warming (36 %) and elevated CO_2 (28 %; Fig. 2A; $P < 0.05$; Table 2). Among the different compound classes, ω-hydroxy acids followed the same trend as total suberin, and increased with warming (40 %) and elevated CO_2 (35 %; Fig. 2C; $P < 0.05$). However, other compound classes such as the aromatic compounds increased only with warming (24 %; Fig. 2B; $P < 0.05$). Warming had a marginal influence on α,ω-dioic acids (34 %; Fig. 2D; $P = 0.06$).

The climate treatments affected the chemical composition of roots of *B. gracilis* subjected to factorial combinations of warming and elevated CO_2, as indicated by the relative intensities of the DRIFT peaks (Fig. 3). The PC axis 1 explained 89 % of the variation in the data and separated the treatments based on the abundance of different carbon functional groups. The DRIFT analysis of *B. gracilis* roots also revealed that plants exposed to combination of warming and elevated CO_2 (*CT*) had higher abundance of alkyl compounds (2944, 2890 cm^{-1}; potentially arising from the methylene groups of aliphatic suberin monomers; Zeier and Schreiber 1999; Lopes *et al.* 2000) and lignin (1511 cm^{-1}) compared to those exposed to the control (*ct*) treatment which had higher abundance of polysaccharides (1039, 800 cm^{-1}; Fig. 3; $P < 0.05$). However, *B. gracilis* exposed to either warming (*cT*) or elevated CO_2 (*Ct*) alone did not differ from plants exposed to the control treatment (*ct*) and combined warming and elevated CO_2 (*CT*) treatment.

Roots of *H. comata* were less responsive to global change treatments compared to *B. gracilis*. In *H. comata*, the total quantity of suberin showed an increasing trend with the main effect of warming and elevated CO_2 ($P = 0.07$; Table 3). Unlike *B. gracilis*, in *H. comata*, α,ω-dioic acids increased with the main effect of elevated CO_2. The ω-hydroxy acids marginally varied by an interaction of warming and elevated CO_2 (Fig. 4).

Table 2. Results of the two-way analysis of variance of the responses of a) total suberin, b) aromatics, c) ω-hydroxy acids and d) α,ω-dioic acids in roots of *B. gracilis* to warming and CO_2 treatments. *P* values < 0.05 are given in bold. *P* values < 0.1 but >0.05 are given in italics. DF = degrees of freedom; MS = mean squares; SS = sum of squares.

a) Total suberin

Source of variation	DF	SS	MS	*F*	*P*
CO_2	1	71.364	71.364	5.201	**0.042**
Warming	1	105.788	105.788	7.71	**0.017**
CO_2 × warming	1	14.123	14.123	1.029	0.33
Residual	12	164.643	13.72		
Total	15	338.441	22.563		

b) Aromatics

Source of variation	DF	SS	MS	*F*	*P*
CO_2	1	0.318	0.318	1.36	0.266
Warming	1	1.118	1.118	4.779	**0.049**
CO_2 × warming	1	0.414	0.414	1.771	0.208
Residual	12	2.807	0.234		
Total	15	4.407	0.294		

c) ω-hydroxy acids

Source of variation	DF	SS	MS	*F*	*P*
CO_2	1	49.037	49.037	6.978	**0.022**
Warming	1	62.028	62.028	8.826	**0.012**
CO_2 × warming	1	6.67	6.67	0.949	0.349
Residual	12	84.332	7.028		
Total	15	192.49	12.833		

d) α,ω-dioic acids

Source of variation	DF	SS	MS	*F*	*P*
CO_2	1	0.776	0.776	1.785	0.206
Warming	1	1.829	1.829	4.204	*0.063*
CO_2 × warming	1	0.283	0.283	0.65	0.436
Residual	12	5.219	0.435		
Total	15	7.816	0.521		

Discussion

Environmental cues exert a major influence on the deposition of suberin along root tissues (Schreiber *et al.* 1999; Kolattukudy *et al.* 2001; Baxter *et al.* 2009; Ranathunge *et al.* 2011). For example, abiotic stresses such as drought and salinity increased the total quantity of suberin in roots (Franke and Schreiber 2007). Our results revealed that warming and elevated CO_2 can alter the total quantity of suberin in plant roots (Fig. 2). The above- and below-ground changes in resource availabilities induced by warming and elevated CO_2 alter the morphological and physiological traits of roots (Nie *et al.* 2013; Carrillo *et al.* 2014; Nelson *et al.* 2017). Previous study from the PHACE site reported that elevated CO_2 with warming increased the length and decreased the diameter of fine roots of grasses resulting in greater specific root length and specific root area (Carrillo *et al.* 2014). As the surface area of roots increases, the content of suberin per unit mass could also increase. As roots become thinner,

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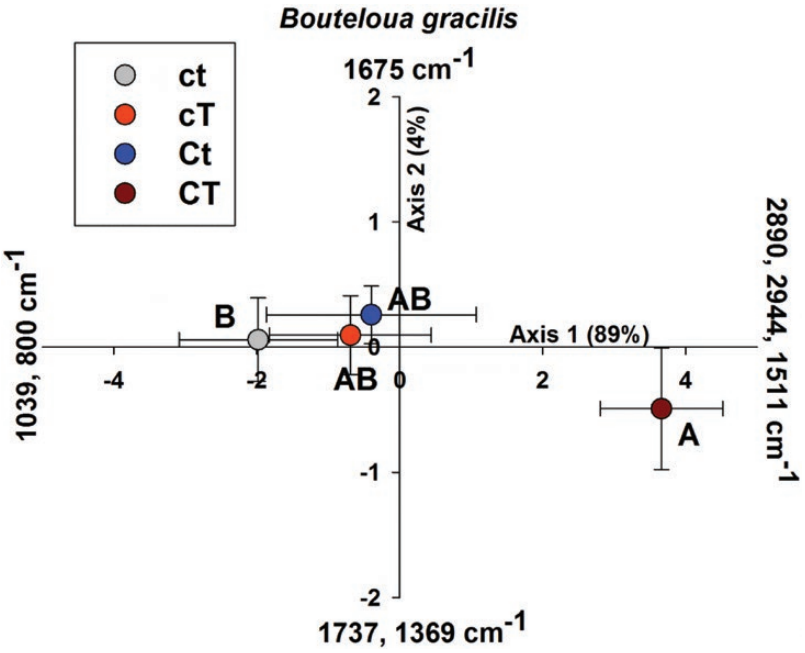


Figure 3. Principal component analysis of the relative intensities of the dominant DRIFT peaks of roots of *B. gracilis* from different climatic treatments. The wave numbers (carbon functional groups in the litter corresponding to different compounds) with the highest eigenvector loadings are listed on each principal component axis. Each point represents a mean of five replicates. Letters 'A', 'B' and 'C' indicate Tukey's difference between treatments separated by PC1 axis.

they are increasingly susceptible to pest and pathogen attack which could result in the increased deposition of defence compounds such as suberin and lignin (Emmett *et al.* 2014; Wang *et al.* 2015). In our study, in the roots of *B. gracilis* (C4 species), the relative abundance of suberin increased with the main effect of warming and elevated CO_2 where the magnitude of change was highest with warming. Similarly, the abundance of lignin also increased with a combination of warming and elevated CO_2 (Fig. 3). These changes in the content of suberin and lignin in the roots of *B. gracilis* may decrease their decomposability particularly if the suberin is associated with lignin (Angst *et al.* 2016). Although our study did not examine the association of different heteropolymers in roots, our previous research at the same PHACE study system revealed that the cell wall-bound phenolics that cross-link lignin to polysaccharides increased in the leaves of *B. gracilis* exposed to elevated CO_2 (Suseela *et al.* 2014b).

Along with changes in the content of suberin, our study also revealed changes in the composition of suberin in roots of plants exposed to different global change factors. Suberin composition is associated with important changes in root function (Schreiber *et al.* 2005; Ranathunge and Schreiber 2011), which may be a response to biotic/abiotic stress. For instance, higher amounts of aliphatic suberin in the exodermal cell walls in the roots of rice contributed to lower hydraulic conductivity (Schreiber *et al.* 2005). Similarly, the deposition of aliphatic suberin monomers preferentially increased resistance to fungal pathogens compared to those from the aromatic domain (Lulai and Corsini 1998). In our study, the preferential enhancement of ω-hydroxy acids (35–40 %) with warming and elevated CO_2 (Fig. 2) potentially indicates the relative importance of the aliphatic component in maintaining their protective functions (Schreiber *et al.* 1999; Thomas *et al.* 2007; Ranathunge *et al.* 2008). In general, the lipid composition of leaves and roots is indicative of their morphology and lifespan (Mueller *et al.* 2012) which largely depends on the biotic and abiotic environmental conditions. As the enzymatic susceptibility of heteropolymers depends on their chemical composition, the qualitative changes in suberin in response to elevated CO_2 and warming may also have implications on the decomposability of these tissues. Recent studies have suggested that the decomposability and potential of suberin to contribute to soil carbon depends on the chain length of the different monomers and the type and location of chemical functional groups in each monomer (Angst *et al.* 2016). For example, the decomposability of roots decreased with increase in the chain length of the monomers of suberin (Angst *et al.* 2016). Thus, the observed increase in ω-hydroxy acids with monomers of higher chain length (ω-C22, ω-C24, ω-C26) in *B. gracilis* may potentially decrease the decomposability of these tissues (Moucawi *et al.* 1981; Angst *et al.* 2016).

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Table 3. Results of the two-way analysis of variance of the responses of a) total suberin, b) aromatics, c) ω-hydroxy acids and d) α,ω-dioic acids in roots of *H. comata* to warming and CO_2 treatments. *P* values < 0.05 are given in bold. *P* values < 0.1 but >0.05 are given in italics. DF = degrees of freedom; MS = mean squares; SS = sum of squares.

a) Total suberin

Source of variation	DF	SS	MS	*F*	*P*
CO_2	1	9.709	9.709	4.198	*0.075*
Warming	1	9.514	9.514	4.113	*0.077*
CO_2 × warming	1	4.194	4.194	1.813	0.215
Residual	8	18.504	2.313		
Total	11	41.921	3.811		

b) Aromatics

Source of variation	DF	SS	MS	*F*	*P*
CO_2	1	0.0848	0.0848	0.385	0.552
Warming	1	0.0592	0.0592	0.269	0.618
CO_2 × warming	1	0.629	0.629	2.859	0.129
Residual	8	1.761	0.22		
Total	11	2.534	0.23		

c) ω-hydroxy acids

Source of variation	DF	SS	MS	*F*	*P*
CO_2	1	7.755	7.755	3.294	0.107
Warming	1	2.553	2.553	1.085	0.328
CO_2 × warming	1	9.794	9.794	4.16	*0.076*
Residual	8	18.834	2.354		
Total	11	38.937	3.54		

d) α,ω-dioic acids

Source of variation	DF	SS	MS	*F*	*P*
CO_2	1	0.656	0.656	7.741	**0.024**
Warming	1	0.224	0.224	2.645	0.142
CO_2 × warming	1	0.232	0.232	2.736	0.137
Residual	8	0.678	0.0847		
Total	11	1.789	0.163		

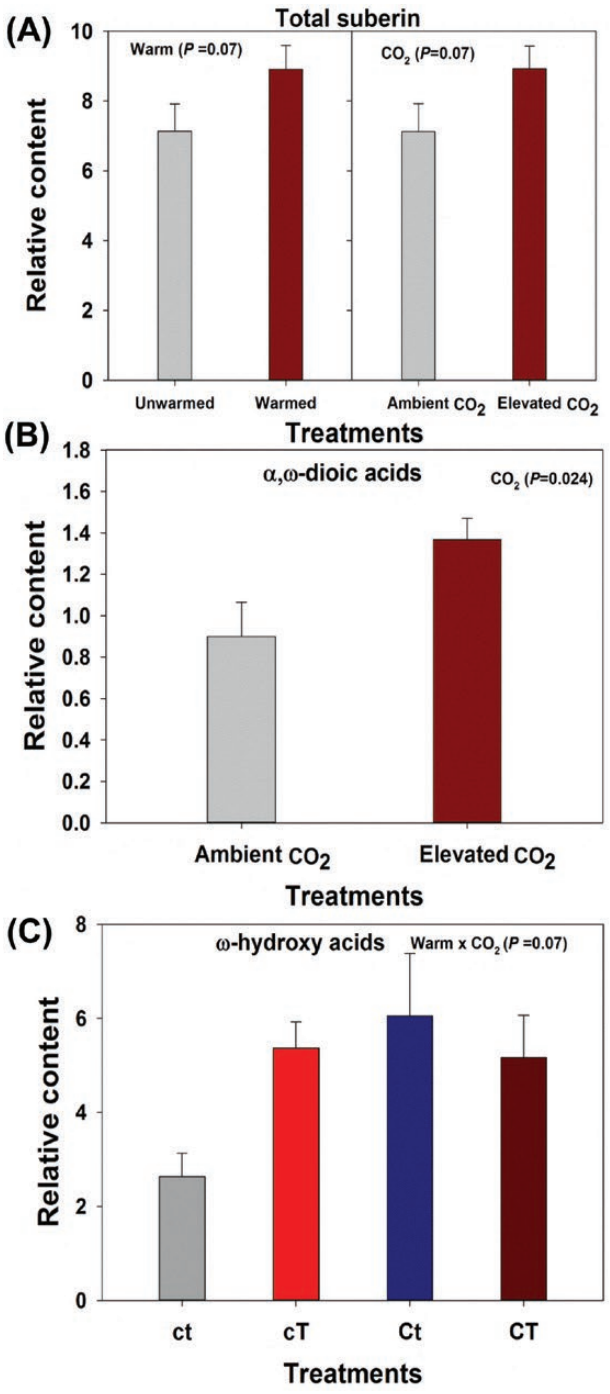


Figure 4. Effect of warming and CO_2 treatments on the relative contents of (A) total suberin, (B) α,ω-dioic acids and (C) ω-hydroxy acids in roots of *H. comata*. Values represent means ± SE (*n* = 3).

In our study, the effect of warming and elevated CO_2 on the content and composition of suberin varied with the C3 (*H. comata*) and C4 (*B. gracilis*) species. The content and composition of suberin in roots of *H. comata* was less responsive to warming and elevated CO_2 treatments. As our two study species varied in their photosynthetic carbon assimilation pathways, different climatic conditions could elicit differential metabolic responses in these species. For example, our previous research in the same study site evaluating the above-ground metabolic

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responses of a C3 (*P. smithii*) and C4 (*B. gracilis*) species revealed that the C4 species increased the content of lignin and cuticular matrix only under warming, whereas the C3 species exhibited similar response only under combination of warming and elevated CO_2. Similarly, the content of bound phenolics that cross-link lignin to polysaccharides within the tissue matrix increased with warming only in the C4 species (Suseela *et al.* 2014*b*). Thus, the differential response we observed to warming and elevated CO_2 in suberin in the roots of *H. comata* (C3) and *B. gracilis* (C4) could have arisen from different biochemical adaptations associated with their photosynthetic pathways.

Conclusion

Changes in the quantity and composition of suberin and an increase in the abundance of lignin could alter the contribution of roots to stable soil organic matter because root-derived aliphatic compounds have greater stability in soils (Nierop 1998; Crow *et al.* 2009; Xia *et al.* 2015). Our results suggest that the observed increase in the abundance of suberin, especially the aliphatic components, in roots could enhance below-ground soil carbon sequestration in grasslands under elevated CO_2 and warming. However, the results also revealed that suberin in roots of *B. gracilis* was more responsive to global change factors than *H. comata*. Thus, changing species composition with climate change (Morgan *et al.* 2011) would further mediate potential suberin contributions to soil carbon pools. A better understanding of how climatic factors modulate the biopolymer composition of fine roots would substantially improve our efforts to predict root-derived soil carbon storage under future climates.

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Contributions by the Authors

V.S. conceptualized the project, collected and analysed the data. N.T. and A.M.R. guided the analysis. E.P. provided the experimental frame work. V.S. drafted the article and all authors contributed to the writing of the article.

Conflicts of Interest

None declared.

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