

9-2017

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Recommended Citation

Suseela V, Tharayil N, Pendall E, Rao AM. 2017. Warming and elevated CO₂ alter the suberin chemistry in roots of photosynthetically divergent grass species. *AoB PLANTS*9: plx041; doi: 10.1093/aobpla/plx041

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Research Article

Warming and elevated CO₂ alter the suberin chemistry in roots of photosynthetically divergent grass species

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Received: 14 April 2017 Editorial decision: 25 April 2017 Accepted: 29 August 2017 Published: 1 September 2017

Associate Editor: Astrid Volder

Citation: Suseela V, Tharayil N, Pendall E, Rao AM. 2017. Warming and elevated CO₂ alter the suberin chemistry in roots of photosynthetically divergent grass species. *AoB PLANTS* 9: plx041; doi: 10.1093/aobpla/plx041

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 CO₂ 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 CO₂ Enrichment (PHACE) experiment at Wyoming, USA. Roots of *B. gracilis* exposed to elevated CO₂ 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 CO₂ 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 CO₂, whereas the total quantity of suberin exhibited an increasing trend with the main effect of warming and elevated CO₂. 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 CO₂; 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 CO₂ 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 CO₂ 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 CO₂ 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 CO₂ 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 CO₂ 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 CO₂ and warming on root chemistry particularly focusing on suberin. We hypothesized that warming and elevated CO₂ would alter the quantity and chemical composition of suberin in grass roots. As elevated CO₂ increases below-ground resource allocation, we predicted that elevated CO₂ 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 CO₂ at the Prairie Heating and CO₂ Enrichment (PHACE) experiment in Wyoming, USA (Morgan *et al.* 2011).

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 CO₂ 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 CO₂ enrichment (FACE) technology was used to apply the CO₂ 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 CO₂, ambient temperature), Ct (elevated CO₂, ambient temperature), cT

(ambient CO₂, warming) and CT (elevated CO₂, 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 cm² 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-methoxybenzoic 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-Hydroxyoctadecanoic 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 |

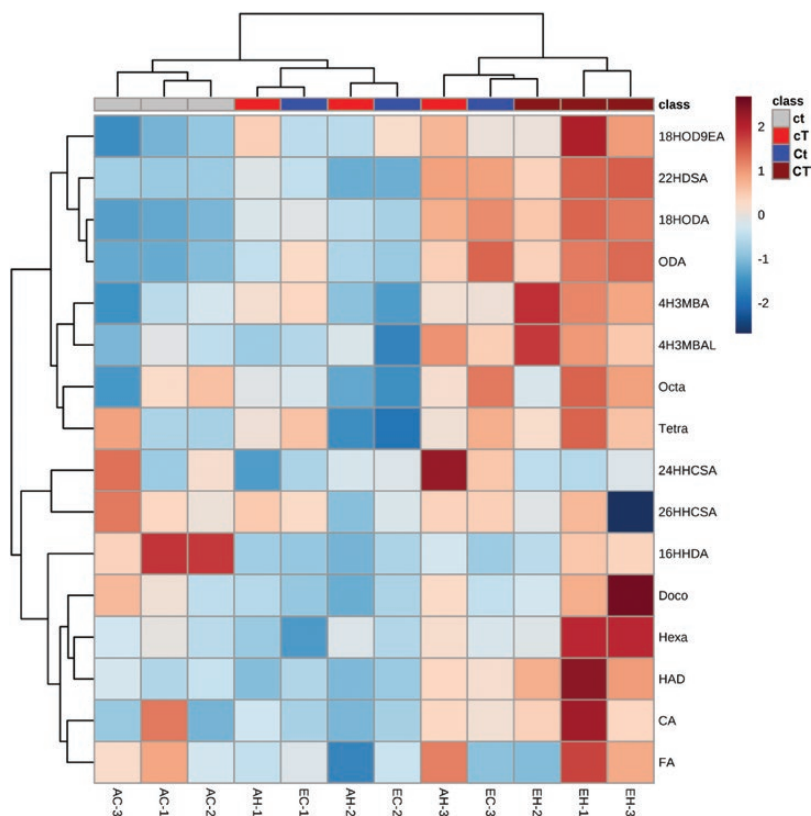


Figure 1. Heatmap and two-way hierarchical clustering of suberin monomers of roots of *B. gracilis* exposed to different climate treatments. Each column represents a sample from a climate treatment, and each row represents a positively identified compound, based on GC-MS (Table 1). Key: ct (ambient CO₂, ambient temperature), Ct (elevated CO₂, ambient temperature), cT (ambient CO₂, warming) and CT (elevated CO₂, 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 μ L 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 μ L of this fraction from each sample were added to a low volume GC vial to which 5 μ L 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 μ L 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, 2014a). 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

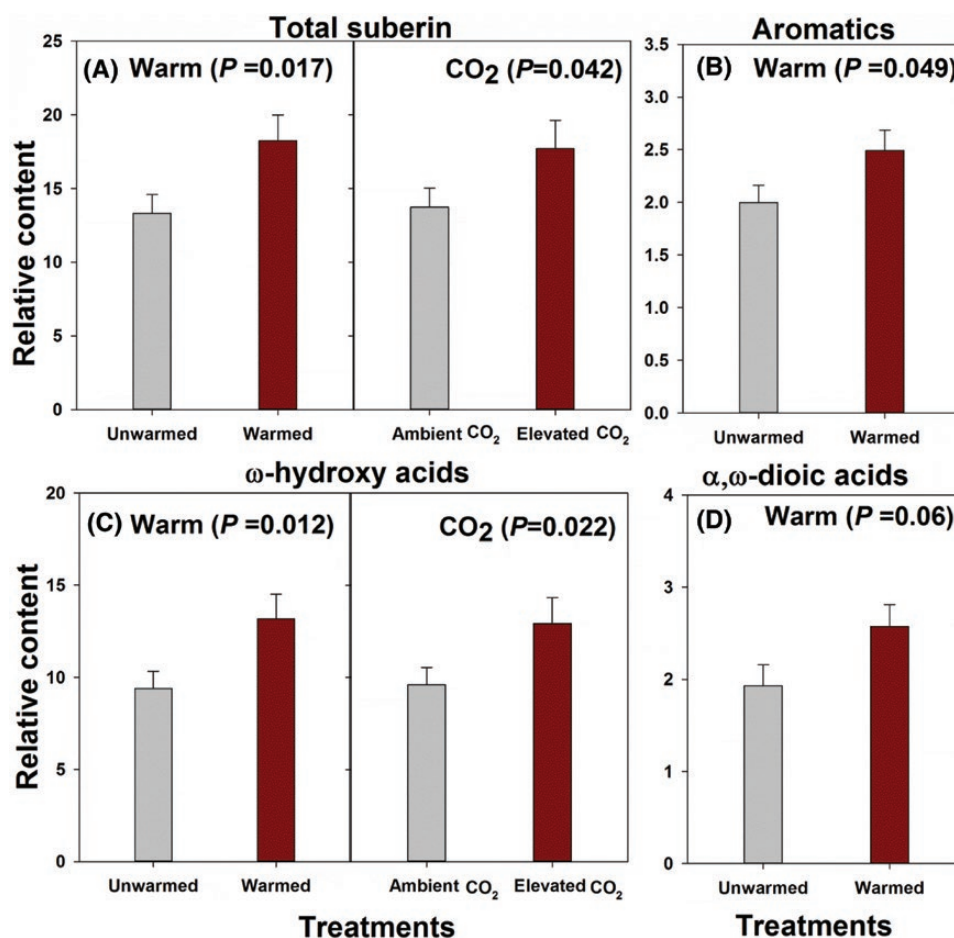


Figure 2. Effect of warming and CO₂ treatments on the relative contents of (A) total suberin, (B) aromatics, (C) ω-hydroxy acids and (D) α,ω-dioic acids in roots of *B. gracilis*. Values represent means ± SE (n = 4).

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 CO₂ 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. 2014a).

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 CO₂ had higher abundance of monomers

belonging to ω -hydroxy acids and aromatic monomers (Fig. 1). Moreover, the total quantity of suberin increased with warming (36 %) and elevated CO₂ (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 CO₂ (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 CO₂, 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 CO₂ (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 CO₂ (Ct) alone did not differ from plants exposed to the control treatment (ct) and combined warming and elevated CO₂ (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 CO₂ ($P = 0.07$; Table 3). Unlike *B. gracilis*, in *H. comata*, α,ω -dioic acids increased with the main effect of elevated CO₂. The ω -hydroxy acids marginally varied by an interaction of warming and elevated CO₂ (Fig. 4).

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 CO₂ 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 CO₂ 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 CO₂ with warming

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 CO₂ 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 | | |

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,

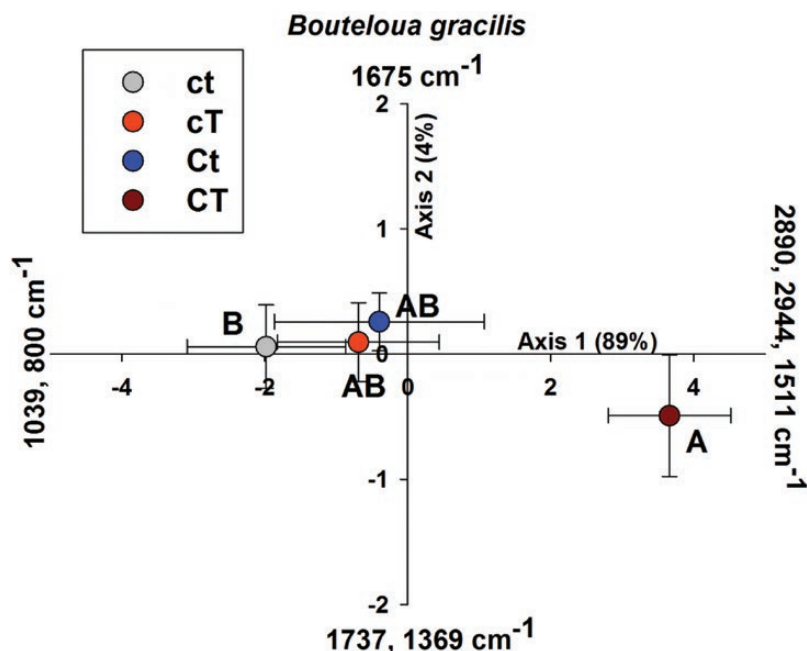


Figure 3. Principal component analysis of the relative intensities of the dominant DRIFT peaks of roots of *B. gracilis* from different climatic treatments. The wave numbers (carbon functional groups in the litter corresponding to different compounds) with the highest eigenvector loadings are listed 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 CO₂ where the magnitude of change was highest with warming. Similarly, the abundance of lignin also increased with a combination of warming and elevated CO₂ (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 CO₂ (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 CO₂ (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 CO₂ 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).

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 CO₂ 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 | <i>F</i> | <i>P</i> |
|---------------------------|----|--------|-------|----------|----------|
| 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 | <i>F</i> | <i>P</i> |
|---------------------------|----|--------|--------|----------|----------|
| 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 | <i>F</i> | <i>P</i> |
|---------------------------|----|--------|-------|----------|----------|
| 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 | <i>F</i> | <i>P</i> |
|---------------------------|----|-------|--------|----------|--------------|
| 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 | | |

In our study, the effect of warming and elevated CO₂ 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

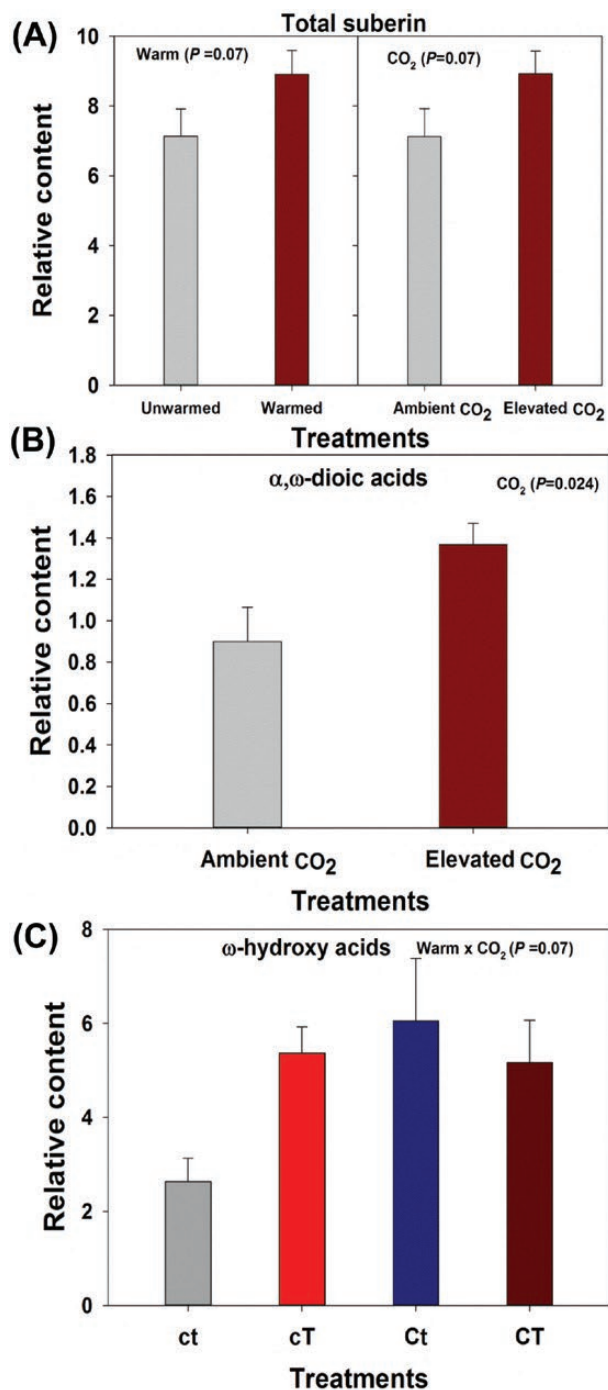


Figure 4. Effect of warming and CO₂ treatments on the relative contents of (A) total suberin, (B) α,ω -dioic acids and (C) ω -hydroxy acids in roots of *H. comata*. Values represent means \pm SE (*n* = 3).

less responsive to warming and elevated CO₂ 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

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 CO₂. 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. 2014b). Thus, the differential response we observed to warming and elevated CO₂ 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 CO₂ 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.

Sources of Funding

This study received financial support from the National Science Foundation (DBI-1306607) to V.S. The PHACE infrastructure and operation was supported by the United States Department of Agriculture (USDA)-Agricultural Research Service Climate Change, Soils and Emission Program USDA-CSREES Soil Processes Program (grant no. 2008-35107-18655), funding from US Department of Energy Office of Science (BER), through the Terrestrial Ecosystem Science program (DE-SC0006973), and by the National Science Foundation (DEB-1021559) to E.P.

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.

Acknowledgements

We thank L. Nelson, R. Dellinger and K. Koon for help with root collection and processing. We also thank J. Morgan, D. LeCain, D. Blumenthal, Y. Carrillo, and numerous students and technicians for their contributions to long-term research at the PHACE site. This is technical contribution No. 6575 of the Clemson University Experiment Station.

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