Soil carbon stocks not linked to aboveground litter input and chemistry of old-growth forest and adjacent prairie

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Abstract

The long-standing assumption that aboveground plant litter inputs have a substantial influence on soil organic carbon storage (SOC) and dynamics has been challenged by a new paradigm for SOC formation and persistence. We tested the importance of plant litter chemistry on SOC storage, distribution, composition, and age by comparing two highly contrasting ecosystems: an old-growth coast redwood (*Sequoia sempervirens*) forest, with highly aromatic litter, and an adjacent coastal prairie, with more easily decomposed litter. We hypothesized that if plant litter chemistry was the primary driver, redwood would store more and older SOC that was less microbially processed than prairie. Total soil carbon stocks to 110 cm depth were higher in prairie (35 kg C m^{-2}) than redwood (28 kg C m^{-2}). Radiocarbon values indicated shorter SOC residence times in redwood than prairie throughout the profile. Higher amounts of pyrogenic carbon and a higher degree of microbial processing of SOC appear to be instrumental for soil carbon storage and persistence in prairie, while differences in fine-root carbon inputs likely contribute to younger SOC in redwood. We conclude that at these sites fire residues, root inputs, and soil properties influence soil carbon dynamics to a greater degree than the properties of aboveground litter.

Keywords: soil organic matter; soil carbon; density fractionation; radiocarbon; grassland; 13C-NMR spectroscopy

INTRODUCTION

Old-growth coast redwood (*Sequoia sempervirens*) are among the world's largest trees, capable of living over 2000 years because of their shade tolerance, resistance to fungi, and resilience to fire and flood (Sawyer et al. 2000). Old-growth redwoods are highly productive, with increasing wood production with age (Sillett et al. 2010), large amounts of aboveground litterfall (Pillers and Stuart 1993), and large accumulations of detrital material (Busing and Fujimori 2005) because their highly aromatic tissues are resistant to decomposition (Anderson et al. 1968). Redwood tissues are particularly rich in complex lipid compounds such as terpenes (Hall and Langenheim 1986) and the polyphenolic compounds lignin and tannin (Hergert 1992). Despite the importance of these forests for C storage in aboveground biomass, little is known about belowground C storage and cycling in these ecosystems. Furthermore, throughout much of the redwood range, redwood forest is interspersed with coastal prairie, providing a striking contrast to old-growth redwood forest in terms of plant stature, productivity, and tissue chemistry. This creates a unique opportunity to investigate the effects of litter input chemistry on soil carbon storage and persistence.

The effect of litter chemistry, particularly lignin and nitrogen content, on litter decomposition is well documented (Cusack et al. 2009; Prescott 2010; Zhang et al. 2008) and incorporated into ecosystem and land surface models (Bonan et al. 2013; Ricciuto et al. 2021). High amounts of aromatics, particularly polyphenols, decrease initial decomposition rates and form secondary metabolite complexes that further inhibit decomposition (Hättenschwiler and Vitousek 2000; Horner et al. 1988). In contrast, litter from grasses and other prairie plants are comparatively depleted in aromatics but rich in polysaccharides and N compared to forest litters, characteristics that result in high decomposition rates (Osono et al. 2013; Zhang et al. 2013). Different plant tissues within a plant also decompose at different rates, with aboveground tissues generally decomposing faster than roots (Bird and Torn 2006; Ziter and MacDougall 2012), likely because aboveground tissues tend to have more water-soluble carbohydrates and cellulose (Cusack et al. 2009). Additionally, fine roots decompose more quickly than coarse roots (Wang et al. 2014) and decomposition of root litter slows with depth (Hicks Pries et al. 2018). Furthermore, higher soil organic carbon (SOC) storage in sites with higher litter polyphenols (Northup et al. 1998) and hydrophobic lipid (Ostertag et al. 2008) contents has been observed.

However, the importance of chemical recalcitrance of plant litter inputs to soil carbon storage and persistence is challenged by a growing body of research emphasizing the importance of root inputs over aboveground inputs, pyrogenic (fire-derived) carbon (PyC), microbial processing of organic matter, physical disconnection, and organo-mineral associations (Lehmann and Kleber 2015; Schmidt et al. 2011). For example, studies have demonstrated that compounds identified as resistent to decay decompose, in some cases more rapidly than bulk organic matter or other labile compounds such as sugars (Amelung et al. 2008). In turn, isotopic labeling experiments showed that pure glucose persisted longer than wheat straw (Vorony et al. 1989) and proteins had lower turnover than bulk soil C (Miltner et al. 2009).

Many factors influence soil C storage and cycling, including climate (Post et al. 1982) and soil physical and chemical properties such as texture (Jobbágy and Jackson 2000) and mineralogy (Torn et al. 1997). Physical protection by soil aggregates can also increase soil organic matter (SOM) storage, particularly in grasslands and prairies (Ewing et al. 2006; Pérès et al. 2013) where aggregation is attributed to dense root systems (Young et al. 1998). Grasslands and prairies also tend to have considerable amounts of PyC, because of high fire frequencies (Glaser and Amelung 2003; Schmidt and Noack 2000). Pyrogenic C consists primarily of aromatic compounds (Schmidt and Noack 2000) with slower initial decay rates than most direct plant inputs. Thus, differences in litter chemistry, C allocation above- and belowground, root morphology, PyC inputs, and soil properties may contribute to differences in SOC storage and dynamics between forests and prairies.

We assessed the importance of the type of C inputs on soil C storage and cycling in an old-growth coast redwood stand and an adjacent coastal prairie. Since climate and parent material are also major controls of SOC storage and dynamics, we conducted our comparison at one location where these ecosystem properties were shared between the two vegetation types. We hypothesized that if the chemistry of plant litter inputs was the primary control on SOC storage and dynamics, the redwood forest would store more SOC that was less microbially processed than the prairie, that SOC would be older on average under old-growth redwood, and that differences in SOC would be more pronounced near the surface where plant litter inputs are concentrated. We also tested the relationship between light density fraction molecular composition and ^{14}C values to see if there were differences in these relationships between redwood forest and prairie. Specifically, we hypothesized that older fractions would show evidence of being more microbially processed than younger fractions in prairie and that this relationship would be stronger in prairie than in redwood.

METHODS

Study Site

This study was conducted at Prairie Creek Redwoods State Park in northwestern California (Table S1). The region has a Mediterranean climate. Local mean annual precipitation is 1709 mm and mean annual temperature is 11°C (Western Regional Climate Center 2010). The redwood forest and prairie sampling locations were 550 m apart on soils derived from alluvial deposits. The redwood grove is dominated by old-growth coast redwood, while perennial grasses dominate the prairie. The prairie results from waterlogged conditions in winter followed by rapid drying in spring and summer, which favors dry season dormant grasses and herbs (Veirs 1987). The prairie was extensively grazed from approximately 1885 until the park was established in 1923 and grazing by wild elk continues. Both sites were subject to fires historically, and the prairie was subject to light-severity prescribed fires between 1983 and 2005 (Stassia Samuels, personal communication, 2011).

Field Sampling and Sample Processing

Belowground samples were collected in July 2009 from 5 equally spaced plots along a 50 m sampling transect in prairie and 7 randomly selected plots within a 0.2 ha area in redwood. More plots were used in redwood because we expected greater spatial variability there. O horizon samples and standing biomass from prairie plots were collected in a 0.0625 m^2 quadrat. Aboveground litterfall was collected in redwood using eight 0.135 m^2 litter traps placed near our soil sampling plots. O horizon and litter samples were dried, weighed, and ground for chemical analysis.

We were not able to attain permits to dig soil pits in this ecologically and culturally significant park, so mineral soils and roots were sampled using a hammer-driven 7.5 cm diameter corer. One core was sampled from each plot in 10 cm increments to 30 cm and in 20 cm increments from 30 to 110 cm depth. At both sites, a gravelly layer was encountered at 110 cm. Bulk density and soil C and N concentrations were determined for all depths and plots. Three cores from each site were selected for further analysis.

Fine roots (< 2 mm diameter) were hand-picked using a combination of dry and wet sieving and sorted into < 0.25 mm, 0.25–0.50 mm, and 0.50–2.0 mm diameter size classes. Coarse roots were sorted for chemical analyses into 2–5 mm and > 5 mm (redwood only). Redwood roots < 0.25 mm in diameter were virtually non-existent. Roots were thoroughly cleaned with tap water, dried, and weighed. A subset of roots was ground for chemical analysis.

Sieved soil samples from 0–10 cm and 50–70 cm depths were fractionated into free light (fLF), occluded light (oLF), and dense (DF) density fractions sodium polytungstate (SPT-0, TC Tungsten Compounds) adjusted to a density of 1.65 g cm^{-3} using the procedure described in detail in McFarlane et al. 2013. The fLF is comprised of free particulate organic matter, oLF contains light-density organic matter occluded in aggregates, and DF includes mineral-associated organic matter. During soil density fractionation, some C and N are dissolved in SPT solution or during water rinses and is lost from the solid sample. The reported proportions of bulk soil C and N in different fractions are based on total C and N recovered following density fractionation (< 9 % of bulk soil C and < 4 % of bulk soil N were lost in this procedure).

Chemical and Isotopic Analysis

Plant material, litter, soil, and soil fraction C and N concentrations were measured on dry, ground samples using a Carlo Erba Elantech elemental analyzer at UC Berkeley. Soil texture was measured using the micropipette method (Burt et al. 1993; Miller and Miller 1987). Bulk soil pH was measured in water and 0.01M $CaCl_2$ (Thomas 1996). Soil and soil fractions were analyzed for ^{14}C on the Van de Graaff FN accelerator mass spectrometer (AMS) at the Center for AMS at Lawrence Livermore National Laboratory. Samples were prepared for ^{14}C measurement as described in Vogel et al. 1984. Aliquots of CO_2 were analyzed for ^{13}C at the Department of Geological Sciences Stable Isotope Laboratory, University of California Davis (GVI Optima Stable Isotope Ratio Mass Spectrometer). Measured ^{13}C values were used to correct for mass-dependent fractionation of ^{14}C, and $\delta\ ^{13}C$ is reported relative to V-PDB. Radiocarbon values are reported in $\Delta\ ^{14}C$ notation, had an average AMS precision of 3 ‰, and were corrected for ^{14}C decay since 1950 and the year of measurement, 2011 (Stuiver and Polach 1977).

The amount of PyC in a subset of samples was determined by analyzing benzene polycarboxylic acids (BPCA) molecular markers by high-performance liquid chromatography at the University of Zurich (Wiedemeier et al. 2013). We present PyC results “as measured” without the use of a conversion factor and should therefore be considered low-end estimates of total PyC contents. They provide a conservative and very robust basis to compare PyC contents in our study soils as was shown for diverse environmental materials (Hammes et al. 2008; Wiedemeier et al. 2013).

Molecular Characterization of Plant Tissues, Litter, and Light Density Fractions

We assessed differences in the molecular composition of above and belowground biomass and light-density fractions in redwood and prairie using solid-state ^{13}C nuclear magnetic resonance spectroscopy (NMR), which can be applied to a wide range of organic materials without relying on extensive chemical extraction procedures and used to assess differences between the chemistry of different organic materials or transformations in the chemical composition of organic matter during decomposition (Nelson and Baldock 2005). The chemical structure of aboveground litter and biomass, roots, and light density fractions was characterized by variable amplitude cross-polarization magic-angle spinning (VACPMAS) ^{13}C NMR spectroscopy at the Pacific Northwest National Laboratory (Agilent/Varian VNMRS solid-state 300 MHz spectrometer and 5 mm HXY Chemagnetics MAS probe). We selected all light fractions from 0–10 cm (n = 3 cores) and 2 samples each of redwood needles, wood, and bark; prairie grass and mixed aboveground biomass; each of the fine root classes at each site; and light fractions from 50–70 cm from each site. Useful ^{13}C-NMR spectra for dense fractions could not be attained because low C concentrations and interference from the iron present in the soil minerals resulted in low C signal strength, a common challenge for organic matter characterization of mineral-rich soil samples (Kögel-Knabner 2000; Yeasmin et al. 2020). 80–100 mg of sample was packed into 5 mm zirconia rotors using Kel-F spacers and a vespel drive tip. Samples were spun at 10 kHz to reduce interference due to spinning side bands. The VACP pulse program was optimized using hexamethylbenzene and glycine to achieve maximum intensity for all peaks. The contact time for samples was 1 ms, the proton 90 was 3 μs, the decoupling power was 62.5 kHz for 25 ms, and the recycle delay was 1–2 seconds. The number of scans for litters was 3000 and for light fractions was about 12000. Examples of representative spectra are provided in Figure S1. Spectra were digitally processed using MNova NMR software (Mestrelab Research SL, Spain) to integrate peak areas in the following chemical shift regions: 0–45 ppm (alkyl), 45–110 ppm (O-alkyl), 110–165 ppm (aromatic), 165–210 ppm (carbonyl). Integrated spectral areas were normalized to the total signal intensity for each spectrum.

Sample "aliphaticity" (A/O-a), defined as the ratio alkyl to O-alkyl (C peak area in the region 0–45 ppm / C peak area in the region 45–110 ppm), was used to infer the degree of microbial processing in soils where a higher ratio indicates higher processing (Baldock et al. 1997). This approach assumes that as decomposition progresses: 1) carbohydrates are degraded resulting in a decrease in the concentration of O-alkyl C, and 2) the metabolic products of decomposers (including lipids and long-chain aliphatic compounds) accumulate resulting in an increase in the concentration of alkyl C (Baldock et al. 1990; Baldock and Preston 1995; Baldock et al. 1997; Webster et al. 2000). Sample aromaticity (AR) was defined as the ratio of aromatic to alkyl plus O-alkyl and aromatic (C peak area in the region 110–165 ppm / C peak areas in the region 0–165 ppm) where a higher ratio indicates higher aromaticity (Kögel-Knabner 1997). A "combined" index (CI) was defined as the ratio of alkyl and aromatic to O-alkyl (C peak area in the region 0–45 plus 110–165 ppm / C peak areas in the region 45–110 ppm) (Baldock and Preston

1995; Baldock et al. 1997). Alkyl and aromatic C are considered less preferred C substrates, thus we interpreted litter A/O-a, CI and AR as indices of substrate quality for microbes as well as the extent of microbial processing of SOM fractions (Baldock and Preston 1995).

We measured a significant amount of PyC at our sites, especially at depth (Figure 2d in Results section). The presence of char in the 110–165 ppm region affects the interpretation of CI and AR as indexes of the extent of decomposition. Therefore, we controlled for the influence of char in CI and AR by subtracting the percentage of signal intensity from char according to Baldock et al. (2004) (64.9% from 110-145 ppm and 17.5% from 145-165 ppm). These char-corrected indexes are presented as CI* and AR*.

^{13}C NMR spectroscopy does not provide a quantitative measure of the molecular composition of organic materials, so we applied a molecular mixing model (MMM) for terrestrial soils (Baldock et al. 2004) to infer the molecular structure of our samples based on spectral intensities. We used a 5-component model (carbohydrate, lignin, lipid, protein, and carbonyl) for the litter samples and a 6-component model (5-component model plus char) for soil fractions. This model iteratively determines the linear combination of components that best fit the integrated regions of the NMR spectra constrained with the molar N:C ratio for each sample.

Data Analysis

Results are reported as means followed by standard errors. Statistical tests were performed in R 3.6.1 and effects were considered significant at $\alpha = 0.05$. Depth, ecosystem, root size, and density fraction effects were tested by analysis of variance

(ANOVA) with repeated measures for depth accounted for using mixed-effect models with the nlme package (Pinheiro et al. 2019; R Core Team 2019) and interaction effects were investigated using Phia (De Rosario-Martinez 2015). ^{13}C NMR results were compared by site and litter/fraction type using Type III ANOVA to account for imbalanced design with regards to the numbers of tissue types analyzed. Modest heterogeneities in variances for aromatic signal intensity, combined index, carbohydrate content, and char content were improved with a log transformation. Post hoc comparisons were performed using a Tukey adjustment with the Multcomp package (Hothorn et al. 2008). Relationships between soil density fraction molecular composition and ^{14}C values were investigated using correlation analysis and linear regression.

RESULTS AND DISCUSSION

Aboveground Biomass, Litter, and Fine Root Biomass

Aboveground biomass in our coast redwood forest was previously reported as 428 kg m^{-2} (Sillett and Van Pelt 2007). Annual litterfall in redwood consisted mainly of needles (81 ± 2 % by mass) and was similar in dry mass to standing aboveground biomass in prairie at the time of sampling, which was mostly grasses (97 ± 3 % by mass) and dead or senesced material (91± 1 % by mass). Total C and N mass, as indicators of inputs to the soil, were similar between plant types, but redwood aboveground litter had higher C concentration, lower N concentration, and higher C:N ratio than prairie (Table 1). Total fine root biomass to 110 cm, an indicator of belowground C inputs, was more than double in redwood than in prairie and fine-root C and N stocks were similarly higher in redwood than prairie. Redwood roots tended to be larger diameter and declined less strongly with depth than prairie roots (Figure S2). Like aboveground plant tissues, redwood roots had lower N concentration and higher C:N ratio than prairie roots (Table 1).

Table 1. Aboveground litter and fine root mass and general chemistry characteristics in Coast Redwood Forest and Coastal Prairie. Letters indicate differences between plant litter or biomass types within a column. N = 6 for redwood and n=3 for prairie.

Site	Litter Type	Dry Mass (g m^{-2})	C (%)	N (%)	C:N ratio	C Mass (g m^{-2})	N Mass (g m^{-2})
Aboveground							
Redwood	Annual Aboveground litterfall	780 (57)^{a}	48.8 (0.2)^{a}	0.58 (0.02)^{a}	90 (2)^{a}	380 (28)^{a}	8.1 (0.4)^{a}
Prairie	Standing Aboveground Biomass	1041 (81)^{a}	44.1 (0.1)^{b}	0.79 (0.03)^{b}	56 (2)^{b}	459 (35)^{a}	4.5 (0.3)^{a}
Belowground							
Redwood	Fine root biomass to 110 cm	1794 (274)^{b}	35.9 (1.2)^{c}	1.1 (0.0)^{c}	63 (4)^{c}	776 (115)^{b}	18 (3)^{b}
Prairie	Fine root biomass to 110 cm	775 (251)^{a}	36.8 (0.3)^{c}	2.3 (0.1)^{b}	44 (0.2)^{b}	334 (94)^{a}	5 (1)^{a}

Aromaticity and combined (aliphatic plus aromatic) indices were higher in litter and tissues from redwood than prairie (Figure 1), reflecting a higher abundance of aromatics and lipids and lower abundance of carbohydrates (Figure S3). The molecular mixing model (MMM) indicated that carbohydrates and lignin were the most prominent litter compounds (Figure S3). Also estimated by the MMM, carbohydrates were 73 ± 1 % of observed C in prairie litters, but only 58 ± 2 % in redwood litters, while lignin was nearly double in redwood (34 ± 1 %) than prairie (18 ± 1 %). Protein and lipid content did not differ between sites and no carbonyl C was detectable in litters by the MMM.

These results confirm that litters and plant tissues in redwood are more aromatic and depleted in N compared to those in prairie. A global synthesis found the rate of litter decomposition decreased with increasing C:N ratio and was higher in grasslands than in coniferous forests (Zhang et al. 2008). As redwood litter contains particularly high amounts of lignin and tannin that slow decomposition, plant inputs likely decompose more slowly in the redwood forest than prairie, which should facilitate the accumulation of soil organic matter that has undergone relatively little decomposition and microbial processing.

Bulk Soils

Contrary to expectations, total soil C stock to 110 cm was lower in redwood (28 ± 1 kg C m^{-2}) than prairie (35 ± 1 kg C m^{-2}), as was N stock (Figure 2a-b, $p < 0.01$). Redwood mineral soils had lower C and N concentrations in the top 30 cm (Figure S5a-b, $p < 0.01$) and layer-specific stocks were higher in prairie only in the top 50 cm – soil C and N concentrations and stocks converged at depth. Redwood mineral soils had higher C:N ratios than prairie throughout the profile, consistent with higher C:N ratios in redwood litters (Figure 2c, $p < 0.01$).

At both sites, $\delta\ ^{13}C$ values increased and $\Delta\ ^{14}C$ values decreased with depth (Figure 2e-f), indicating a presence of older and more decomposed carbon at depth. Bulk soil $\delta\ ^{13}C$ and $\Delta\ ^{14}C$ values were higher in redwood than prairie throughout the profile (Figure 2e-f, $p < 0.05$). Only redwood forest floor and 0–10 cm mineral soils had $\Delta\ ^{14}C$ values higher than 0 ‰, indicating the presence of ^{14}C associated with atmospheric weapons testing. Therefore, the difference in $\Delta\ ^{14}C$ values between sites indicates the presence of younger C throughout the soil profile in redwood than prairie. This is consistent with recent comparisons of forests and grasslands, which have found $\Delta^{14}C$ of soil organic carbon to be less depleted in forests than grasslands (Heckman et al. 2020; Moreland et al. 2021).

Pyrogenic C constituted a larger amount (Figure S5d) and percentage of total C in prairie than redwood ($p < 0.01$). This percentage increased slightly with depth at both sites (Figure 2d). At least 20 % of the difference in total mineral-soil C stocks can be attributed to higher PyC stocks in prairie. With the commonly used multiplier of 2.27 to convert the conservative BPCA measurements to more realistic PyC content (Schneider et al. 2011), PyC explains at least 40 % of this difference. Larger amounts of PyC in prairie may also contribute to older soil C in prairie than redwood as fire-derived C has been found to be among the oldest and most chemically refractory components of soil organic matter, though its fate in soils depends on conditions during formation as well as physical and chemical interactions with organic matter and minerals (Cusack et al. 2012; Czimczik and Masiello 2007; Eckmeier et al. 2010; Preston and Schmidt 2006; Schmidt et al. 2011).

Despite their proximity, there were some differences in soil characteristics between the redwood and prairie that could influence soil C storage and age. Specifically, pH of shallow soil (0–30 cm) was slightly higher in redwood than prairie (Figure S6a-b), redwood soils had higher sand contents throughout the profile, and deep prairie soils (below 50 cm) had higher clay content than redwood (Figure S6c-d). While these differences in pH are likely too small to impact soil carbon storage, chemistry, and persistence, a tendency for soil C to be higher in finer textured soils is well documented in the literature (Homann et al. 2007; McFarlane et al. 2010; Slessarev et al. 2020) and this difference in soil texture may partly explain our observation of more and older soil C in prairie than redwood. For our sites, however, differences in soil texture between sites were most pronounced below 50 cm where soil C stocks were similar.

Alternatively, the presence of younger soil C in redwood may result from a higher rate of recently fixed C inputs in redwood, particularly to deep soils. There is growing evidence that belowground C inputs through root turnover and rhizosphere deposition are more important C sources to soils than aboveground litter (Schmidt et al. 2011). We did not quantify belowground input rates, but redwood fine-root biomass to 110 cm depth was more than double that in prairie, and below 50 cm depth redwood had nearly 10 times the fine-root density of prairie. The few direct comparisons of belowground litter production between paired prairie and forest suggest that forests have higher root turnover (Pärtel and Wilson 2002), higher root productivity and belowground C inputs (Zhang et al. 2013), and that belowground inputs occur deeper in the soil profile in forest than in prairie (Steinaker and Wilson 2005).

Soil Fractions

Larger bulk soil C and N stocks in prairie than redwood were attributed to larger DF stocks in prairie ($p < 0.01$), as DF contained most of the soil C (71–91 %) and N (84–95 %, Figure S7). Light fraction C and N stocks were similar between vegetation types, but there was a shift in the proportion of C distributed across light fractions in the surface; fLF contained a larger portion of soil C and N in prairie (17 ± 1 % of C and 12 ± 1 % of N) while oLF contained a larger proportion of soil C and N in redwood (21 ± 6 % of C and 10 ± 3 % of N).

Nitrogen concentrations were higher in prairie than redwood for all fractions, though this difference was more pronounced in the surface and in oLF (Table 2). Light fraction C:N ratios were lower in prairie than redwood ($p < 0.01$), but DF had similar C:N ratios between sites. Like bulk soil, fraction δ^{13}C values became more enriched with depth and were more enriched in redwood than prairie at the surface (Table 2). Regardless of depth, δ^{13}C values were more enriched in DF than in light fractions, possibly reflecting a greater degree of microbial processing in this mineral-associated fraction. These results suggest that light fractions in redwood may be less microbially processed than those in prairie.

Table 2. Soil fraction C and N concentration and isotopes for 0–10 and 50–70 cm depths (means followed by standard error, n = 3). Letters indicate differences among fractions, sites, and depths within a column.

Site	Depth (cm)	Fraction	C (%)	N (%)	C:N Ratio	δ^{13}C (‰)	Δ^{14}C (‰)
Redwood	0–10	fLF	34.0 (1.9)^{ab}	1.0 (0.1)^{ab}	35.6 (1.7)^{a}	-27.0 (0.3)^{ab}	44 (15)^{a}
		oLF	35.9 (1.2)^{ac}	1.1 (0.0)^{bc}	32.6 (1.7)^{a}	-27.5 (0.2)^{a}	25 (13)^{a}
		DF	5.1 (0.4)^{de}	0.4 (0.0)^{e}	13.5 (0.4)^{b}	-25.3 (0.3)^{c}	82 (7)^{a}
	50–70	fLF	32.7 (1.2)^{ab}	0.7 (0.0)^{f}	49.0 (3.8)^{c}	-27.4 (0.1)^{a}	-67 (43)^{ab}
		oLF	38.4 (2.8)^{bc}	0.8 (0.0)^{af}	46.9 (2.5)^{c}	-26.3 (0.0)^{b}	-302 (94)^{cd}
		DF	1.5 (0.1)^{de}	0.1 (0.0)^{d}	13.2 (0.4)^{b}	-24.9 (0.1)^{c}	-218 (40)^{bd}
Prairie	0–10	fLF	23.1 (0.6)^{f}	1.3 (0.0)^{c}	18.4 (0.6)^{b}	-28.7 (0.2)^{d}	63 (9)^{a}
		oLF	36.8 (0.3)^{ac}	2.3 (0.1)^{g}	16.3 (0.7)^{b}	-28.7 (0.1)^{d}	31 (6)^{a}
		DF	8.9 (0.7)^{e}	0.7 (0.1)^{ad}	12.2 (0.1)^{b}	-27.6 (0.2)^{a}	-2 (11)^{ae}
	50–70	fLF	32.1 (1.6)^{ac}	0.9 (0.1)^{bf}	36.0 (4.3)^{a}	-27.3 (0.2)^{ae}	-197 (98)^{bed}
		oLF	40.8 (1.7)^{c}	1.1 (0.1)^{bc}	37.5 (0.9)^{a}	-26.5 (0.0)^{be}	-505 (31)^{c}
		DF	1.4 (0.2)^{d}	0.1 (0.0)^{de}	11.3 (0.6)^{b}	-25.3 (0.1)^{c}	-344 (20)^{cd}

Except for redwood fLF, fraction Δ^{14}C values declined with depth (Table 2). This lack of change in Δ^{14}C values with depth for fLF in redwood may results from higher rates of recently fixed root-C inputs to deep soils in redwood, as described above, while fresh plant inputs may be more limited to near-surface soils in prairie. Significant two-way interactions between depth and site and depth and fraction showed that: 1) in the top 10 cm, Δ^{14}C values were similar amongst fractions and sites and 2) at 50–70 cm depth, Δ^{14}C values were highest for fLF and lowest for oLF and were higher in redwood than prairie at depth ($p < 0.01$).

Carbohydrates and aromatics were the most prominent compounds in light fractions (Figure S3). In general, aliphaticity, aromaticity, and combined indices were highest in oLF and lowest in biomass and litters (Figure 1). Char-corrected decomposition indices (AR* and CI*) followed similar patterns to the indices that included char (Figure 1 and Figure S8). Light fractions tended to be enriched in alkyl, aromatic, and carbonyl C and depleted in O-alkyl C compared to biomass and litters (Figure S3). The molecular mixing model indicated that light fractions tended to be depleted in carbohydrates and enriched in lipids and proteins compared to biomass and litters (Figure S4). These shifts in the molecular composition from litter to fLF and oLF are consistent with expected changes as organic matter decomposes.

Deep light fractions tended to have higher aromaticity and combined indices than surface fractions because of lower Alkyl C and higher aromatic C than surface light fractions (Figure S3). Char content, derived from the molecular mixing model, increased with depth for oLF at both sites from an average of 17 ± 2 % to 60 ± 12 % and for fLF in prairie (Figure S8). At 50–70 cm depth, char accounted for most aromatic C in the oLF, but a considerable amount of char (37 ± 14 % of total C) was also present in prairie fLF. Char-corrected aromaticity also increased with depth (Figure S8).

Molecular composition of light fractions did not differ greatly between redwood and prairie, with a few key exceptions. The molecular mixing model suggested that lignin content was about double in redwood than prairie and that protein content was higher in surface fractions from prairie than redwood (Figure S4). Deep olf from prairie appeared to be more decomposed than that from redwood as it had higher decomposition indices (aliphaticity, char-corrected aromaticity, combined, and char-corrected combined indices), lower lignin content, and higher carbohydrate content.

Relationships between SOM Chemistry and ^{14}C Values

We found that light fractions with higher ^{14}C values (indicating a younger C average age) had higher carbohydrate content (Figure 3). Fractions with higher ^{14}C values also had higher lignin content regardless of depth in prairie but only at 50–70 cm depth in redwood. The presence of more carbohydrate and more lignin in younger soil fractions suggests the presence of relatively recent plant C inputs and that these compounds, including lignin, are not retained as organic matter decomposes even *in situ*. In contrast, fractions with lower ^{14}C values had higher char content (Figure 3) and combination indices, though the relationship between older C and higher aromaticity was only significant for deep soils (Figure 4). This further demonstrates that PyC helps to explain the presence of older C in prairie than redwood.

We hypothesized that relationships between SOC molecular composition and age would be weaker in redwood than prairie. We found that some relationships between molecular composition and age were consistent across sites, but that overall, there were more significant relationships in prairie than redwood. The most striking difference was that fractions with lower ^{14}C values (older C) also had higher lipid content and higher aliphaticity in prairie only (Figure 4), suggesting an accumulation of lipids in these fractions over time. These results support an indirect role of litter chemical structure in soil carbon formation, wherein plant inputs are processed by microbes and microbial processing promotes SOC persistence (Cotrufo et al. 2013; Gleixner 2013; Mambelli et al. 2011; Olagoke et al. 2022). Fast degrading litter may be transformed more efficiently into SOC by soil microbes (Bradford et al. 2013; Kallenbach et al. 2015; Manzoni et al. 2012), resulting in greater accumulation of microbial products compared to less labile litter (Cotrufo et al. 2013). This transformation of plant-derived substrates to microbial products appears to be key for the persistence of soil C especially in grasslands (Angst et al. 2021; Kallenbach et al. 2016). However, we did not identify the source (plant or microbial) of lipids in our study and research addressing this hypothesis in complex natural systems is sparse.

Conclusion

We compared the storage, age, and molecular characterization of SOC in old-growth coast redwood forest and adjacent prairie. These systems have highly contrasting amounts, types, and chemistry of plant litter inputs, allowing us to assess the role of plant litter in driving soil carbon storage and persistence in sites selected to minimize differences in climate and soil characteristics. As expected, redwood forest plant litters included more aromatic compounds, less nitrogen, and less carbohydrates than prairie litters. Despite having more easily degradable plant litter, prairie stored more and older soil C than redwood. Our observation of larger soil carbon stocks and higher $\Delta^{14}C$ values in bulk soils and density fractions in redwood forest than prairie, implies the presence of more recently fixed, faster cycling C in redwood soils and/or longer residence time of soil carbon in prairie soils. Greater amounts of fire residues account for up to 40% of the larger soil carbon stocks, and likely contribute to longer soil C residence times, in prairie than redwood. Greater physicochemical protection of SOC may contribute to larger stocks and older soil carbon in prairie than redwood as most soil C was found in mineral-associated fractions and we found evidence for an increase in lipid content in older prairie light density fractions. Litter chemistry may indirectly influence soil carbon dynamics in redwood forest and prairie, but litter recalcitrance does not drive soil carbon storage and persistence in these ecosystems. Instead, differences in root inputs with depth, the amount of fire-residue, and microbial processing likely contribute to differences in soil carbon storage and age between old-growth redwood forest and coastal prairie.

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Figures for

Soil carbon stocks not linked to aboveground litter input and chemistry of old-growth forest and adjacent prairie

Karis J. McFarlane[1], Stefania Mambelli[2], Rachel C. Porras[3], Daniel B. Wiedemeier[4,5], Michael W. I. Schmidt[4], Todd E. Dawson[2], and Margaret S. Torn[3]

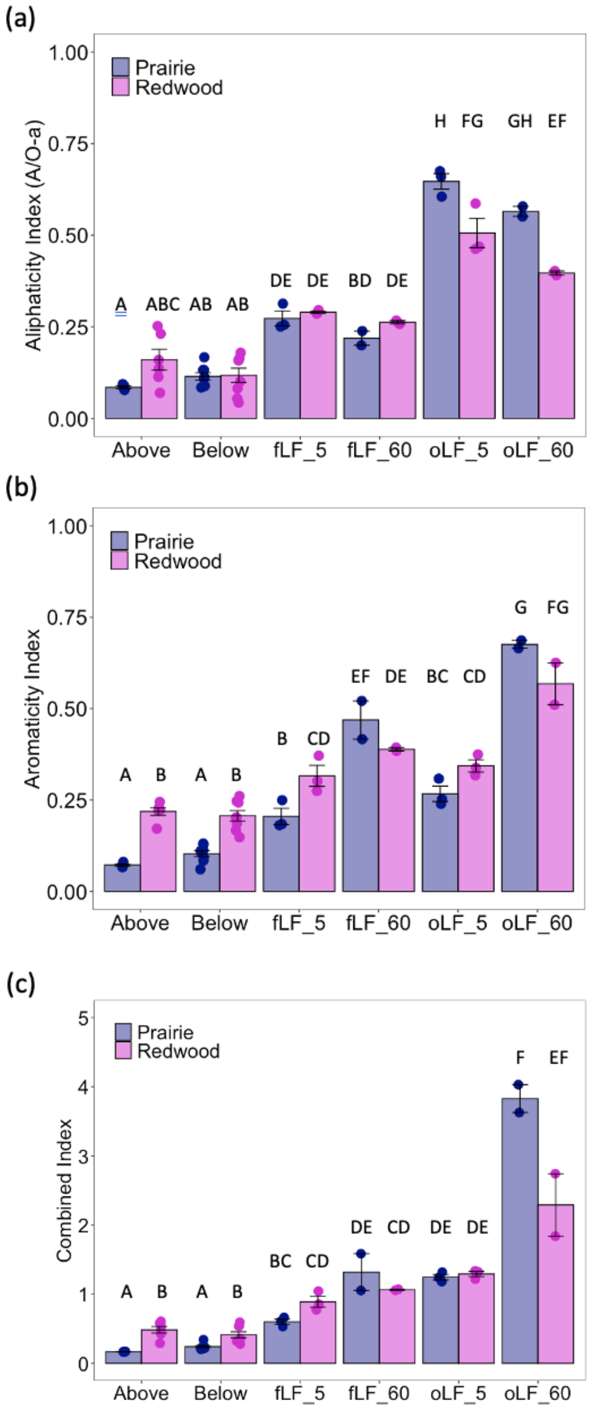


Figure 1. (a) Aliphaticity, (b) Aromaticity, and (c) Combined Indices calculated from ^{13}C-NMR spectroscopy. Letters indicate statistically significant differences at α = 0.05 among organic matter fraction (aboveground litter and biomass, belowground biomass, free light fractions, and occluded light fractions) and vegetation cover (prairie and redwood) as there was a significant interaction between organic matter fraction and vegetation cover. Values are means ± standard error and n ranges from 2 to 8 as samples were pooled into the categories shown.

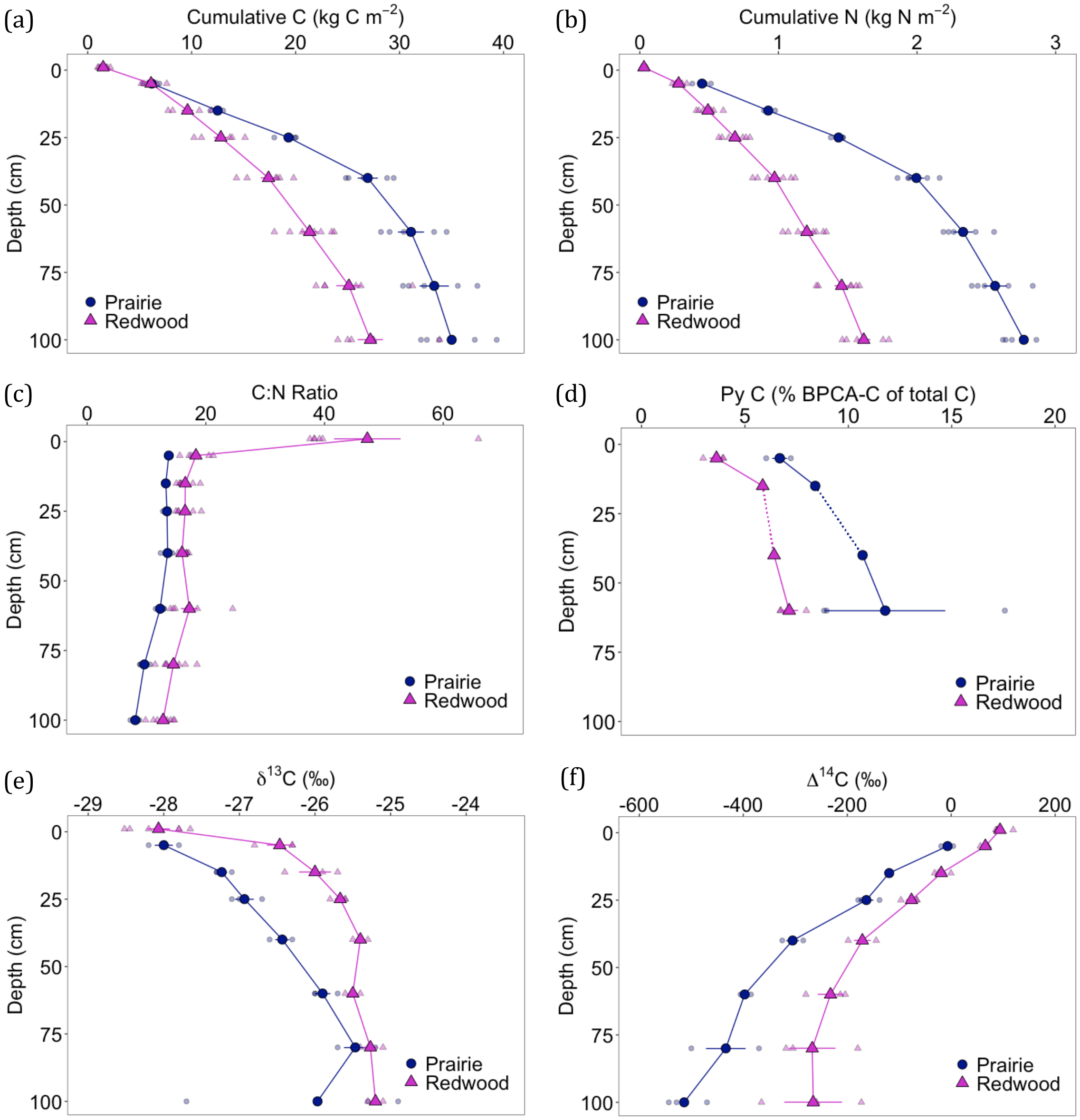


Figure 2. Bulk soil characteristics by middle increment depth for Coastal Redwood Forest and Coastal Prairie. Data are means ± 1 SE. n = 7 for Redwood and 5 for Prairie and 7 for (a) Cumulative C stock, (b) Cumulative N stock, and (c) C:N Ratio. For (d) Py C measured as BPCA, n = 3 for 0–10 cm and 50–70 cm and n = 1 for 10–20 cm and 30–50 cm for each site. Cumulative C stock. N = 3 for both sites for (e) δ^{13}C and (f) Δ^{14}C. Depths > 0 cm are for the forest floor (O-horizon), which was only present in redwood forest.

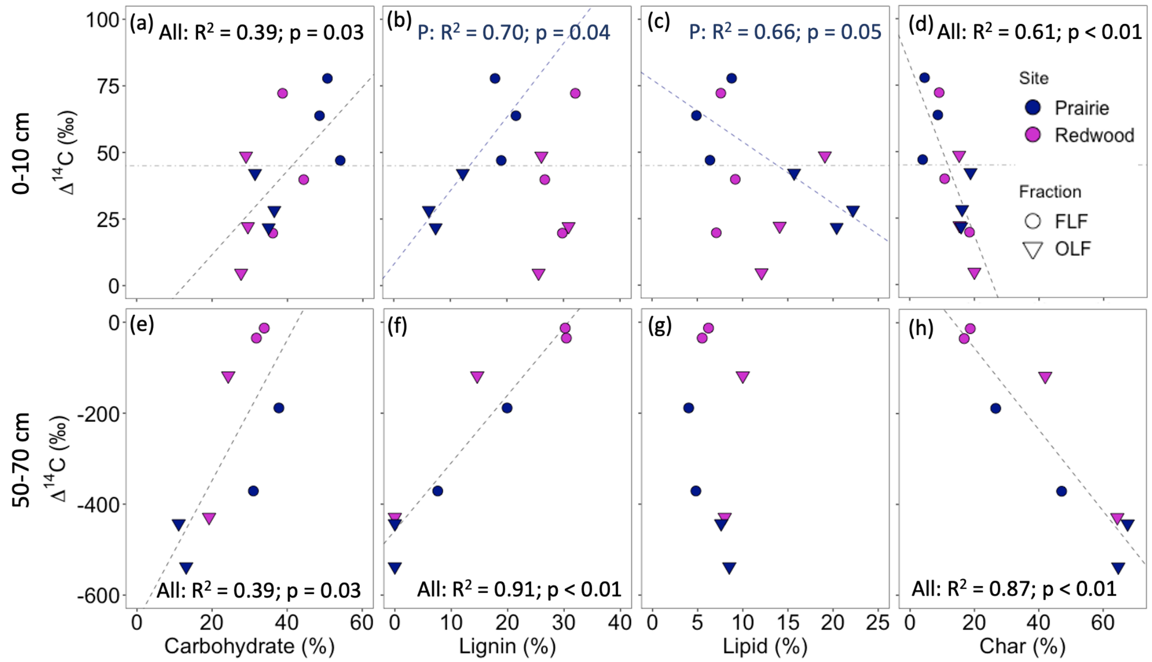


Figure 3. Light density fraction ^{14}C and molecular composition for 0–10 cm (top) and 50–70 cm (bottom) depths. A reference line is provided for the approximate atmospheric ^{14}C value in 2009, the year of sampling (gray horizontal dash-dotted line). Regression lines, R^2, and p values are provided for regressions with $p < 0.05$. Dashed lines show statistically significant linear regressions for all points (black, denoted “All”) or prairie only (blue, denoted “P”).

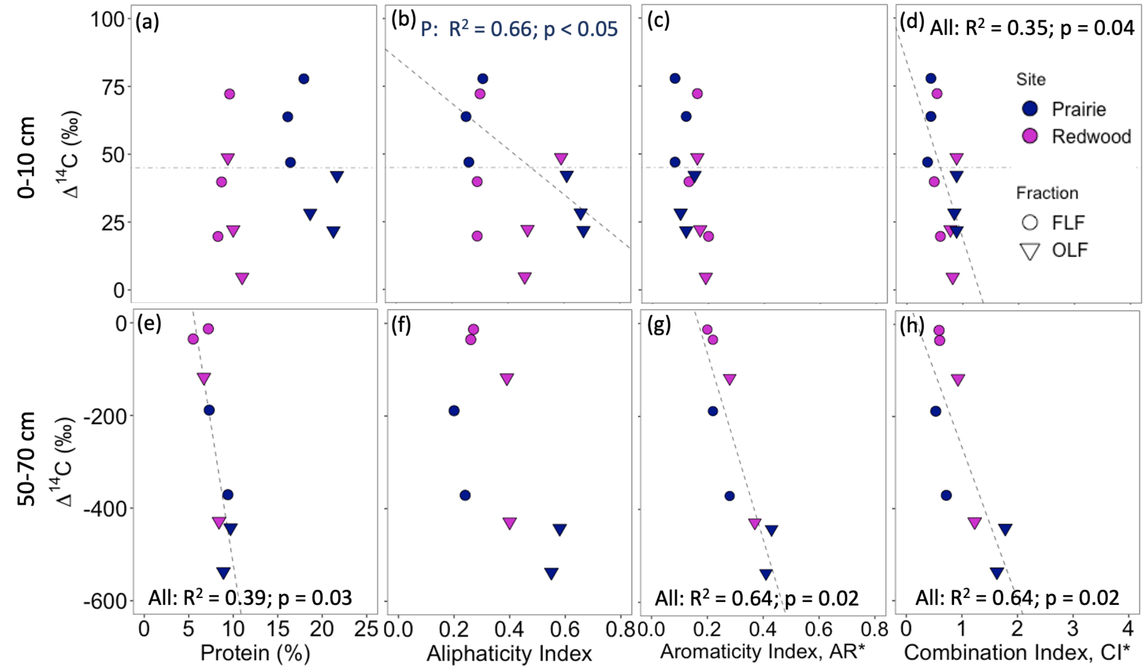


Figure 4. Light density fraction ^{14}C and molecular composition or indices for 0–10 cm (top) and 50–70 cm (bottom) depths. A reference line is provided for the approximate atmospheric ^{14}C value in 2009, the year of sampling (gray horizontal dash-dotted line). Regression lines, R^2, and p values are provided for regressions with $p < 0.05$. Dashed lines show statistically significant linear regressions for all points (black, denoted "All") or prairie only (blue, denoted "P").

Supplementary materials for

Soil carbon stocks not linked to aboveground litter input and chemistry of old-growth forest and adjacent prairie

Karis J. McFarlane[1], Stefania Mambelli[2], Rachel C. Porras[3], Daniel B. Wiedemeier[4,5],
Michael W. I. Schmidt[4], Todd E. Dawson[2], and Margaret S. Torn[3]

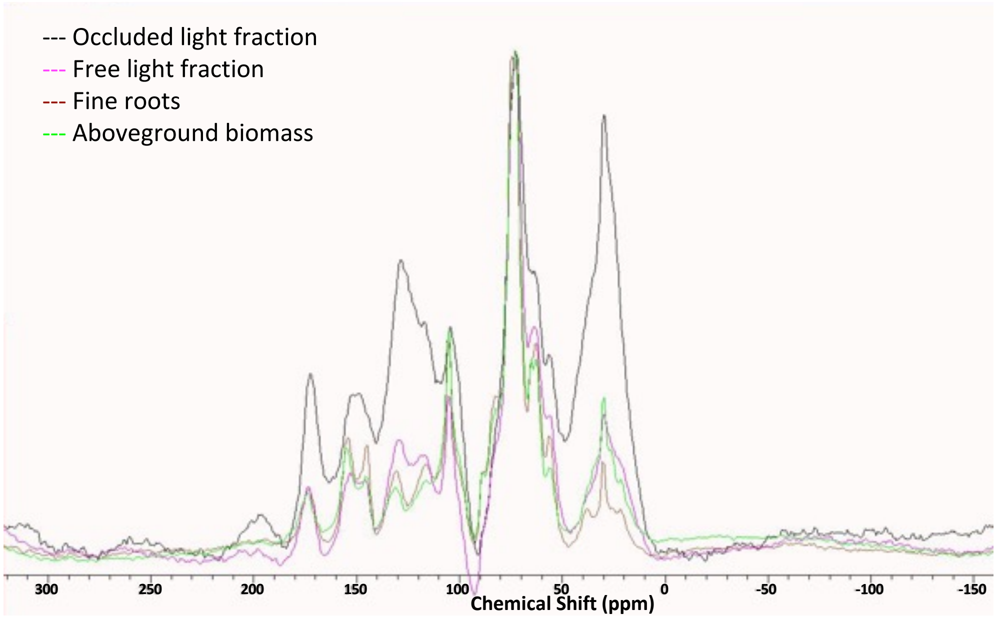
Table S1. Study Site Characteristics

	Atlas Grove Redwood	Boyes Prairie
Latitude/Longitude (°)	41.3625/-124.0203	41.3667/-124.0167
Elevation (m)	55	35
USDA Soil Series	Mystery	Ferndale
USDA Soil Taxonomic Class	Coarse-loamy, mixed, superactive, isomesic, Oxyaquic Eutrudepts	Fine-silty, mixed, superactive, nonacid mesic Typic Udifluvents
Dominant vegetation species[1]	*Sequoia sempervirens, Acer macrophyllum, Rhamnus purshiana, Pseudotsuga menziesii, Tsuga heterophylla, Umbellularia californica*	*Danthonia californica*, *Poa pratensis*, *Holcus lanatus, Carex* sp., *Hypochoeris radicata*, *Plantago lanceolata*, *Pteridium aquilinium*, *Dactilis glomerata*, *Rubus vitifolius*, *Rosaceae*
Understory vegetation species[1]	*Polystichum munitum, Gaultheria shallon, Vaccinium ovatum*, *Oxalis oregana*	NA

[1] Redwood vegetation from Sillet and Van Pelt, 2007. Prairie vegetation from Veirs, 1987 and Stassia Samuels, personal communication, 2011.

Figure S1. Example ^{13}C-NMR spectra for (a) Coast Redwood Forest and (b) Coastal Prairie occluded light fraction (black), free light fraction (magenta), fine roots (brown), and aboveground biomass (green).

(a) Redwood



(b) Prairie

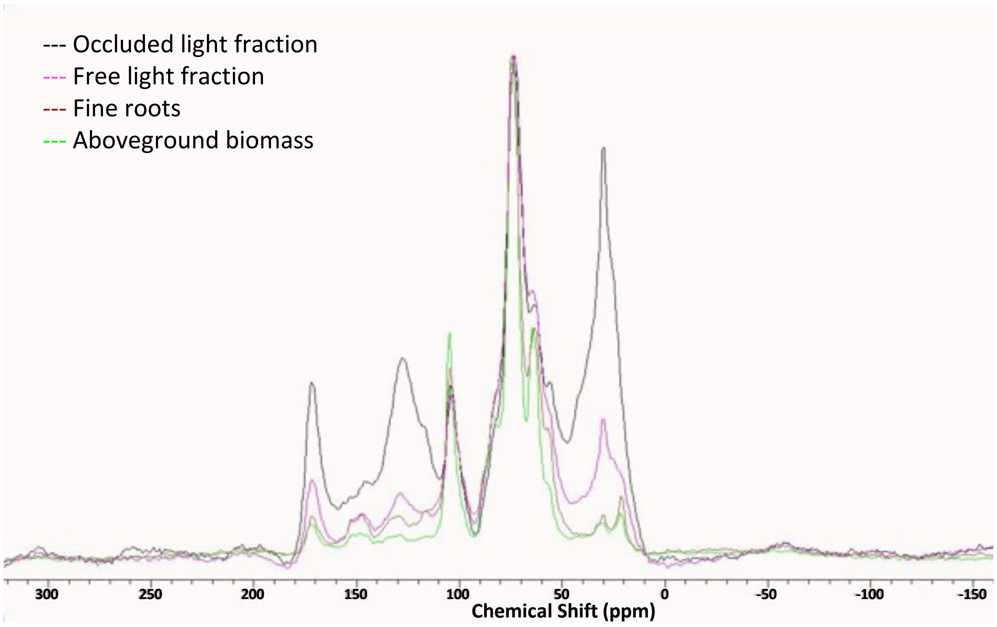
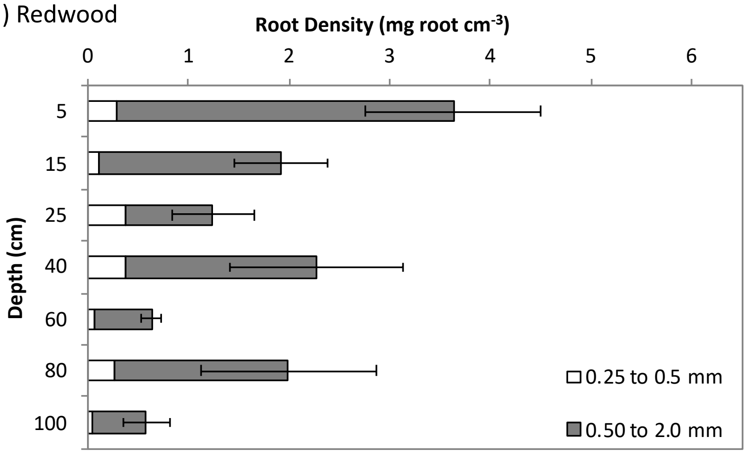
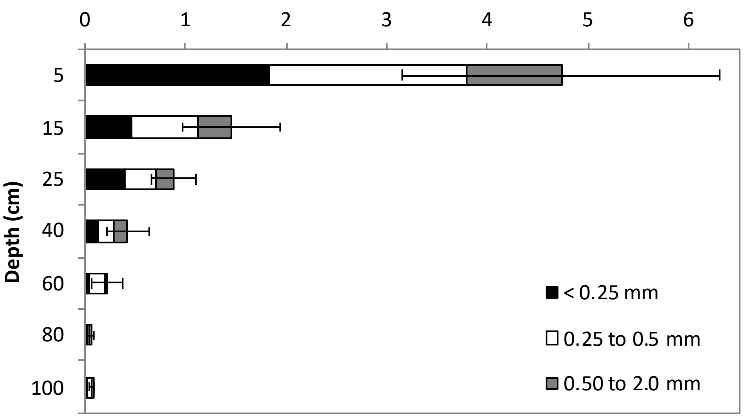


Figure S2. Fine root biomass in (a) Coast Redwood Forest and (b) Coastal Prairie. Data are means ± SE of the total mean. n = 3 cores for Prairie and 6 for Redwood. The distribution of roots among diameter classes did not change significantly with depth.

(a) Redwood



(b) Prairie



A subset of redwood roots was sorted into live and dead roots based on tensile strength and root morphology. By mass, 42 % was live root biomass while 58 % was necromass. The proportion of live vs. dead roots did not differ among diameter classes or soil depths and root C and N concentrations did not differ between live and dead roots. A subset of Redwood roots was taken to holocellulose following the Soxhlet solvent extraction described in Gaudinski et al 2005. No consistent patterns with depth or root characteristics and ^{14}C were observed. Data are available in the supplementary data file.

Gaudinski, J. B., Dawson, T. E., Quideau, S., Schuur, E. A. G., Roden, J. S., Trumbore, S. E., Sandquist, D. R., Oh, S.-W., and Wasylishen, R. E.: Comparative Analysis of Cellulose Preparation Techniques for Use with 13C, 14C, and 18O Isotopic Measurements, Analytical Chemistry, 77, 7212-7224, 10.1021/ac050548u, 2005.

Figure S3. Signal intensities in the (a) Alkyl, (b) O-alkyl, (c) Aromatic, and (d) Carbonyl chemical shift ranges from ^{13}C-NMR spectroscopy. Lowercase letters indicate differences among organic matter fraction (aboveground litter and biomass, belowground biomass, free light fractions, and occluded light fractions). Uppercase letters indicate differences among organic matter fraction and vegetation cover as there was a significant interaction between organic matter fraction and vegetation cover. Values are means ± standard error and n ranges from 2 to 8 as samples were pooled into the categories shown.

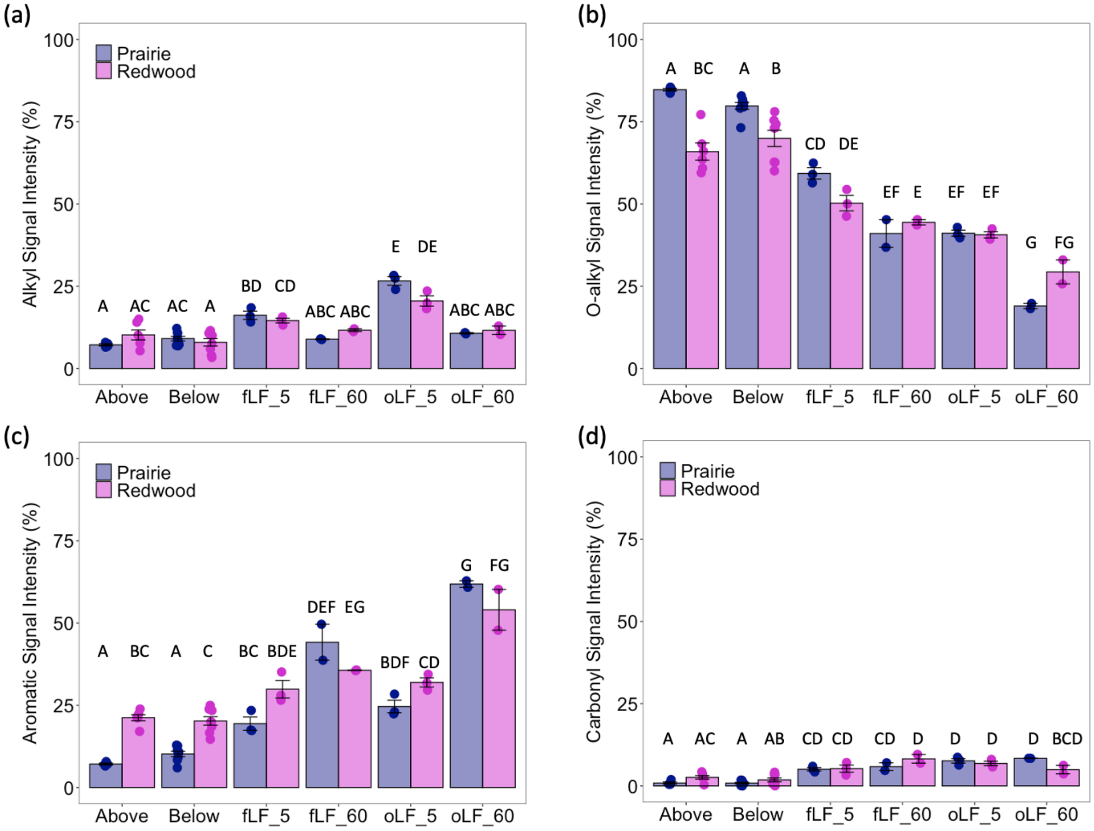


Figure S4. (a) Carbohydrate, (b) Lipid, (c) Lignin, and (d) Protein as a portion of total observed C from the molecular mixing model derived from ^{13}C-NMR spectroscopy. Letters indicate statistically significant differences at $\alpha = 0.05$. Lowercase letters indicate differences among organic matter fraction (aboveground litter and biomass, belowground biomass, free light fractions, and occluded light fractions). Uppercase letters indicate differences among organic matter fraction and vegetation cover as there was a significant interaction between organic matter fraction and vegetation cover. Values are means ± standard error and n ranges from 2 to 8 as samples were pooled into the categories shown.

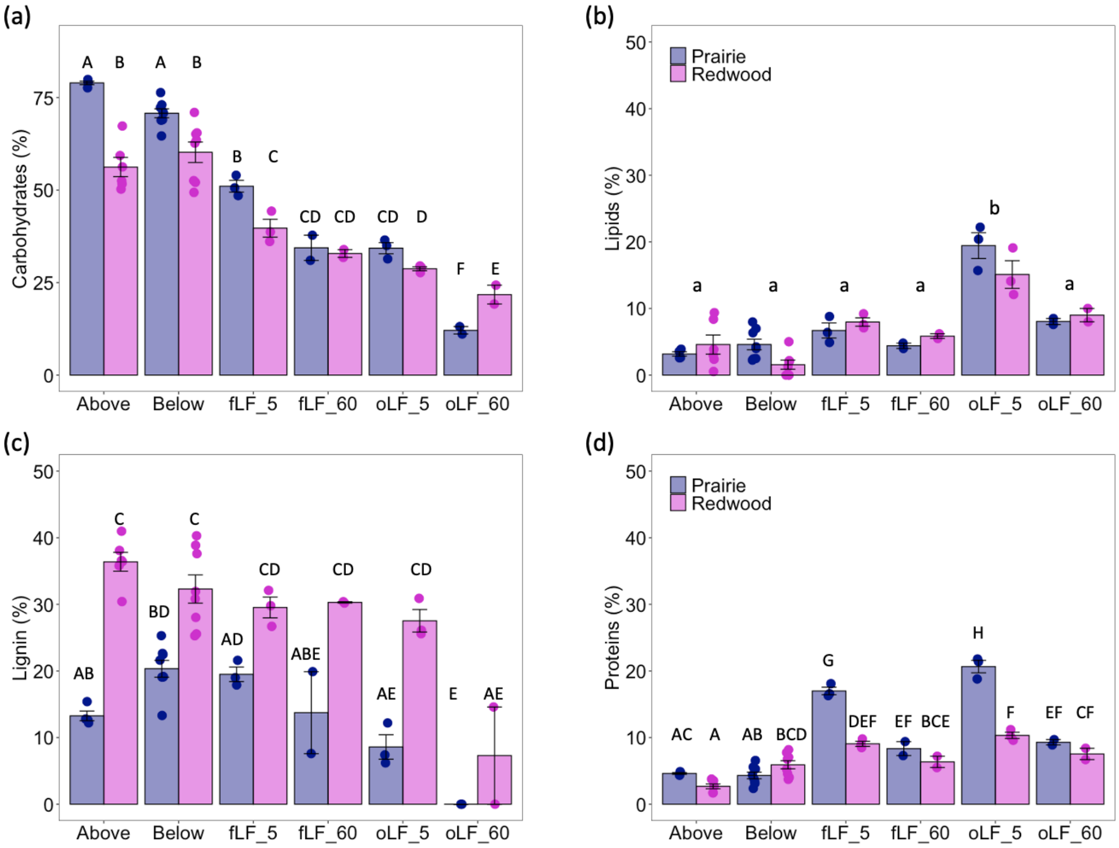


Figure S5. Bulk soil characteristics by middle increment depth for Coast Redwood Forest and Coastal Prairie. Data are means ± 1 SE. n = 7 for Redwood and 5 for Prairie for (a) C concentration, (b) N concentration, and (c) bulk density. For (d) Py C measured as BPCA, n = 3 for 0–10 cm and 50–70 cm and n = 1 for 10–20 cm and 30–50 cm. Depths > 0 cm are for the forest floor (O-horizon), which was only present in redwood forest. Data are means ± 1 SE.

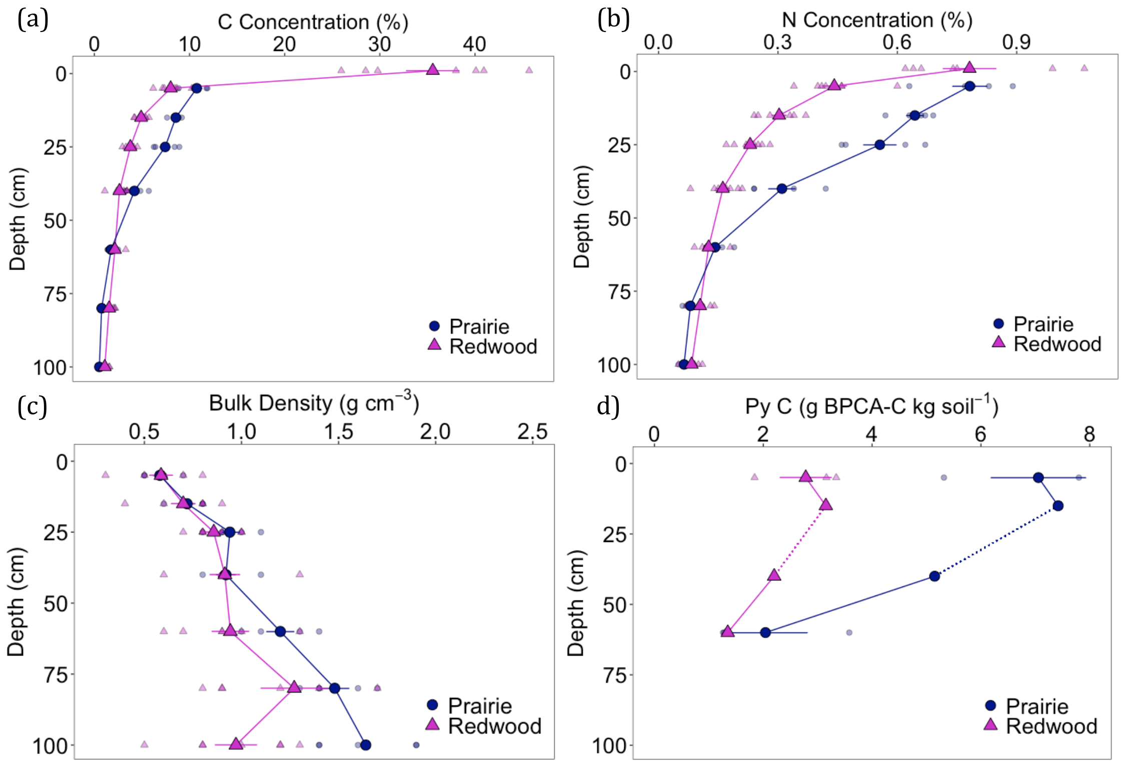


Figure S6. Bulk soil characteristics by middle increment depth for Coast Redwood Forest and Coastal Prairie. Data are means ± 1 SE and n = 3.

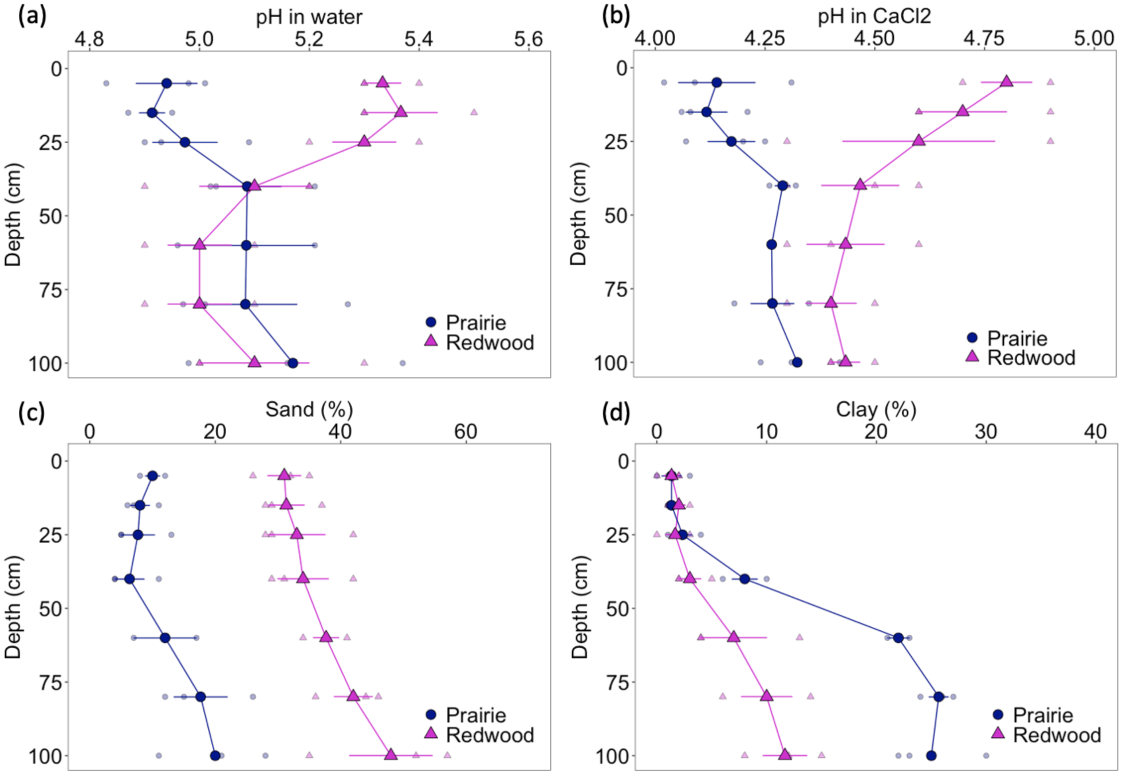


Figure S7. Distribution of (a) carbon and (b) nitrogen in density fractions from Coast Redwood Forest (R) and Coastal Prairie (P) for 0–10 cm and 50–70 cm depths. Data are means ± SE of the total mean. n = 3 for each site.

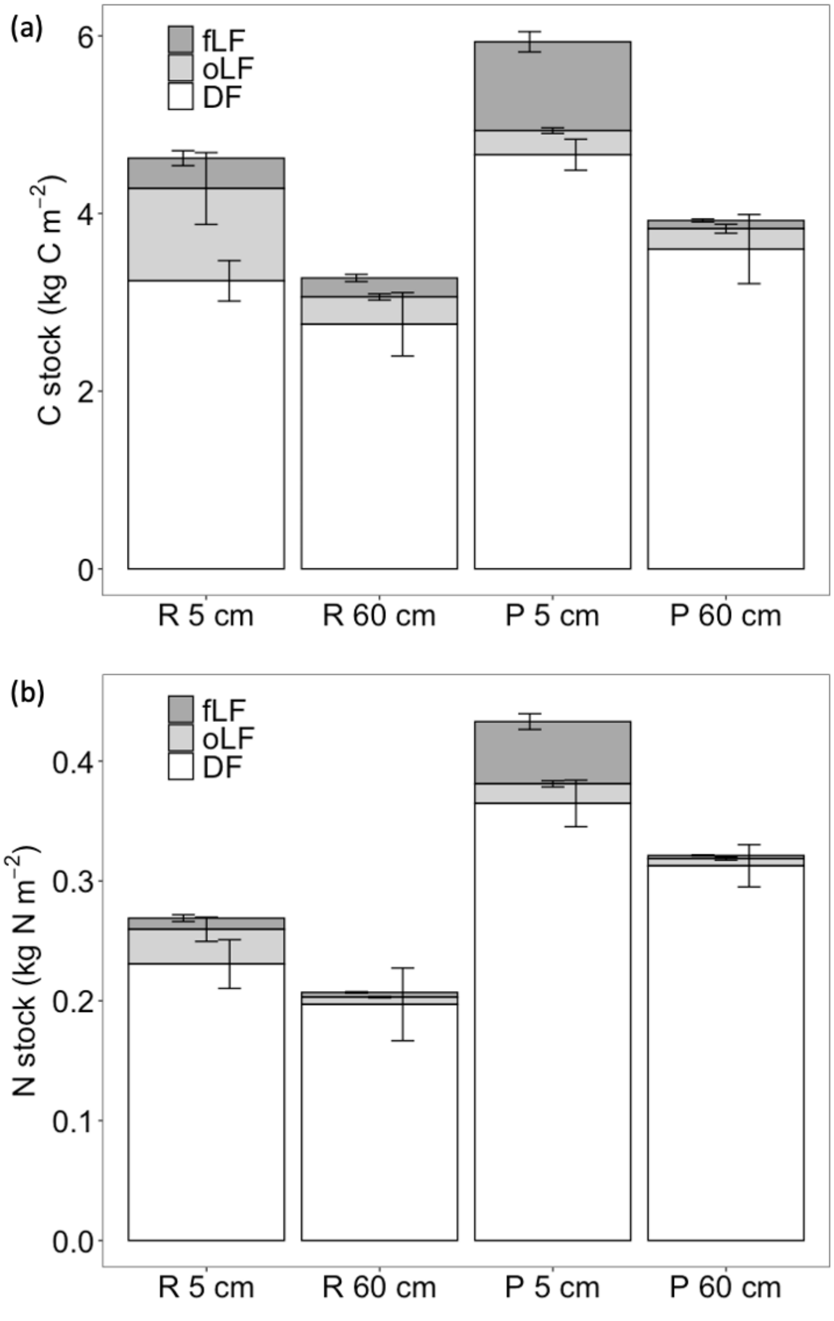


Figure S8. (a) Char and (b) Carbonyl C as a portion of total observed C from the molecular mixing model derived from ^{13}C-NMR spectroscopy. (c) Char-corrected Aromaticity (AR*) and (d) Char-corrected Combined Indices. Lowercase letters indicate differences among organic matter fraction and depth. Uppercase letters indicate differences among organic matter fraction, depth, and vegetation cover as there was a significant 3-way interaction. Values are means ± standard error and n is 3 for surface and 2 for deep light density fractions.