



## Interactive effects of climate change and fungal communities on wood-derived carbon in forest soils



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### ABSTRACT

Although wood makes up the majority of forest biomass, the importance of wood contributions to stable soil carbon (C) pools is uncertain. Complex interactions among climate, soil physical properties, intrinsic properties of woody residues, and biological processes all exert dynamic controls over the stabilization, destabilization and transport of wood-derived C in soils. Many studies have demonstrated the strong physical controls on decomposition rates in soils, but little work has been done to relate these to changes in decomposer community composition and how this influences the fate of wood-derived C in soils. Here, we examine the effects of initial fungal inoculation, temperature, soil texture, Free Air CO<sub>2</sub> Enrichment (FACE) wood type, and location of wood residue in the soil, with an experiment investigating the fate of wood-derived C from soils in the first two years following clear-cut harvest in aspen (*Populus tremuloides* Michx.) forests. We applied <sup>13</sup>C-depleted aspen wood chips in 168 experimental plots across six sites in northern Michigan, USA, and tracked the depleted <sup>13</sup>C signature through the mineral soil as DOC and from the soil surface as CO<sub>2</sub>.

Wood residue location had the largest impact on soil CO<sub>2</sub> efflux, with surface wood treatments having more than twice as much wood-derived soil CO<sub>2</sub> efflux as buried wood treatments (1.20 g CO<sub>2</sub> m<sup>-2</sup> h<sup>-1</sup> versus 0.49 g CO<sub>2</sub> m<sup>-2</sup> h<sup>-1</sup>, respectively;  $p < 0.001$ ). Initial fungal decomposers had a significant effect on DOC quantity and quality, with higher wood-derived DOC concentrations, levels of humification, and tannin content for white-rot treatments compared with brown-rot treatments. Buried chip treatments within open-top chambers had one-third higher wood-derived soil CO<sub>2</sub> efflux than buried chips in ambient temperature treatments ( $p < 0.002$ ). FACE wood type also influenced soil C fluxes from the decomposing wood chips. The average wood-derived soil CO<sub>2</sub> efflux and the average percentage of wood-derived soil CO<sub>2</sub> efflux were significantly greater from wood grown under elevated CO<sub>2</sub> than wood grown under elevated CO<sub>2</sub> + O<sub>3</sub> ( $p = 0.002$  and  $p = 0.004$ , respectively). Furthermore, wood grown under elevated CO<sub>2</sub> had increased DOC aromaticity relative to wood grown in ambient conditions. Taken together, these results show that wood-derived C sources and the decomposers that process them are significant determinants of C fluxes from and transformations within the soil following harvest in aspen forests.

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## 1. Introduction

Soils are the largest terrestrial sink for carbon (C), containing about two-thirds of the total terrestrial C pool (Schlesinger, 1977; Scharlemann et al., 2014). About 17% of the total soil C pool (1500–2000 Pg) is in forests (Amthor et al., 1998). In forested ecosystems, the potential C inputs to the soil are derived from aboveground litter, woody debris, and roots. Although wood makes up the majority of forest biomass, and woody debris is ~73 Pg (or

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### Abbreviations

C	Carbon
DOC	Dissolved Organic Carbon
FACE	Free Air CO <sub>2</sub> Enrichment
OTC	Open-top chamber
SOC	Soil organic carbon

8%) of the global forest C stock (Pan et al., 2011), the importance of wood contributions to stable soil C pools is uncertain (Magnússon et al., 2016).

Previous research has demonstrated that decomposition products from wood residue tend to accumulate in the soil during early stages of organic matter formation (Williams and Gray, 1974; Kalbitz et al., 2006), but conclusions from studies examining the long-term effects of woody residues on soil organic C (SOC) stocks are mixed. For example, some studies claim that repeated wood harvests reduce soil C inputs and therefore decrease SOC over time (Johnson et al., 2010). This is especially true when whole tree harvest removal systems are used (Johnson and Curtis, 2001). Other studies show that soil C can recover after a harvest and may even increase in certain wood removal systems (Johnson and Curtis, 2001; Fahey et al., 2005). Inconsistent effects of the stabilization of wood-derived C are likely due to variation in stabilization mechanisms with mineralogy (Oades, 1984) and soil horizon genesis (e.g., Torn et al., 2009). For example, stabilization of the water-soluble products of wood decomposition could be expected to occur in illuvial soil horizons (Lajtha et al., 2005), but there is much uncertainty surrounding the mechanisms. There are complex interactions among climate, soil physical properties, and biological processing of woody residues that exert dynamic controls over the stabilization or destabilization of illuvial organic matter (Prescott, 2010).

The effects of climatic change on the relative balance of C inputs and outputs in forest soils are complex and poorly resolved. The IPCC (Intergovernmental Panel on Climate Change) predicts that climate change will include widespread increases in air temperature, changes in the timing and amounts of precipitation, and increases in atmospheric CO<sub>2</sub> and O<sub>3</sub> levels (IPCC, 2014). Generally, warmer soil temperatures should increase organic matter decomposition rates (Kirschbaum, 1995; Chen et al., 2013), but interactions with other limitations of microbial activity make predictions difficult (e.g., Swift et al., 1979). Adding to this complexity, changes in atmospheric CO<sub>2</sub> and O<sub>3</sub> are likely to affect rates of primary production and the intrinsic properties of woody biomass (Runion et al., 1999; Cotrufo and Ineson, 2000; Blaschke et al., 2002; Atwell et al., 2003; Kaakinen et al., 2004; Niklaus and Falloon, 2006), but these factors are not yet considered as drivers of change in large scale models of SOC storage (Niklaus and Falloon, 2006).

Growth of trees under elevated atmospheric CO<sub>2</sub> and O<sub>3</sub> can potentially alter the physical and chemical properties of the wood produced (i.e., wood quality), such as changes in nitrogen, carbohydrate, or lignin content. Quality tends to be important in the early stages of decomposition and can influence the rate at which wood is decomposed (von Lützow et al., 2006). Wood residues with higher C:N ratio, lignin:N ratio, lignin:cellulose ratio, and/or high lignin content have been found to decompose more slowly in some studies (Melillo et al., 1982; Taylor et al., 1989), while wood quality metrics have not been related to early decomposition rates in others (Cotrufo and Ineson, 2000; Niklaus and Falloon, 2006; Ebanyenle, 2012). Studies examining the extent to which wood

quality is altered by growth under elevated CO<sub>2</sub> and O<sub>3</sub> have also shown mixed results. Some studies demonstrated higher C:N and lignin:N ratios, while others found decreases in lignin and increases in non-structural carbohydrates in response to fumigation by CO<sub>2</sub> and/or O<sub>3</sub> (Cotrufo and Ineson, 2000; Blaschke et al., 2002; Kaakinen et al., 2004; Niklaus and Falloon, 2006). There are also reports of no chemical composition changes in wood in response to growth under elevated CO<sub>2</sub> and O<sub>3</sub> (Runion et al., 1999; Atwell et al., 2003). As such, directly ascribing a mechanistic linkage between wood quality metrics in response to altered growth environment and wood decomposition have been difficult to determine from the literature, particularly in natural soil environments. Nonetheless, a laboratory study suggests aspen wood grown under elevated CO<sub>2</sub> and O<sub>3</sub> may be more completely decomposed and less likely to become incorporated in soil organic matter (Loya et al., 2003), which certainly warrants further study.

Uncertainty pertaining to temperature and wood quality effects on decomposition rates is due in part to a poor understanding of how these factors affect the decomposer community composition, and how this in turn affects soil C balance. Fungi are important in the decomposition of wood in forested ecosystems. Two of the main groups of wood-decomposing fungi are brown-rot and white-rot fungi. Brown-rot fungi have the capacity to decompose cellulose, hemicellulose and other simple C compounds, but can only superficially alter lignin via partial oxidation (Boddy and Watkinson, 1995). In contrast, white-rot fungi have the capacity to decompose all components of wood, including lignin and other complex C compounds (Boddy and Watkinson, 1995). White-rot fungi have extracellular oxidative enzymes that are able to break down the lignocellulose complex (Baldrian and Valaskova, 2008), making cellulose accessible to hydrolytic enzymes (Osono, 2007). If it is true that the initial decomposing colonizers of woody substrate determine the trajectory of future colonization success (Hiscox et al., 2015), then this 'priority effect' may have ramifications for the rate of decomposition and the nature of partial breakdown products, altering their interaction with the soil minerals in complex ways (Hiscox et al., 2015; Magnússon et al., 2016). For example, white-rot fungi lead to more complete decomposition, and hence could increase the concentration of water-soluble products of decomposition, which may interact with secondary minerals in increasing stable SOM complexes. Thus, understanding the impact of decomposers of wood on dissolved organic C (DOC) quantity and quality is a critical first step in determining their impact on soil C stabilization.

In temperate forests, more root-derived C is found in soils than that of leaf, branch, or stem litter combined (Helgason et al., 2014). Residues found belowground, like lignified roots, are typically more recalcitrant and have a longer residence time in soils than aboveground leaf and fine litter biomass (Rasse et al., 2005; Trumbore, 2009). In the broad sense, wood decomposition rates are typically faster at the surface of the mineral soil than at depth owing in part to oxygen limitations, as is shown by fence post decay (Naidu, 2008). Belowground residues have more soil-to-residue contact than aboveground residues, increasing the chemical association formed between organic matter and mineral surfaces at greater depths (Rasse et al., 2001). Therefore, decomposing products from root residues are more likely to be stabilized in soil than those from aboveground litter inputs (Huang and Spohn, 2015; Xia et al., 2015). An additional consideration of wood residue location is that some forest management practices, such as whole-tree harvesting or harvesting of roots for bioenergy, could also affect soil C pools through the removal of these potentially important above and belowground C sources. However, there is still much uncertainty as to how changes in wood residues in aboveground and belowground pools affect soil C cycling after disturbances, such as timber

harvesting.

Taken together, many studies have demonstrated the importance of physical factors (temperature, moisture, and soil texture) on decomposition rates in soil, but little work has been done to relate these factors to changes in the decomposer communities, and how this influences the fate of wood-derived C in soils. Even less is known about how interactions among physical, chemical, and biological factors affect the stabilization of wood-derived C in soils, such as with changes in wood quality arising from increased atmospheric CO<sub>2</sub> and O<sub>3</sub> levels and resulting shifts in initial fungal colonization. We designed a long-term experiment to examine the effects of initial fungal inoculation, temperature, soil texture, Free Air CO<sub>2</sub> Enrichment (FACE) wood type, and location of wood residue in proximity to mineral soil in a multi-factor experiment investigating the stabilization of wood-derived C in soils. Here, we examine the fate of wood-derived C from soils in the first two years following clear-cut harvest in aspen (*Populus tremuloides* Michx.) forests. We applied <sup>13</sup>C-depleted aspen wood in experimental clear-cuts and tracked the isotopic C signature in CO<sub>2</sub> and DOC fluxes, to address our over-arching hypothesis: fungal community priority effects control the fate of wood-derived C in soils during the first two years following disturbance. We further hypothesized that white-rot fungi would produce larger wood-derived C losses as soil CO<sub>2</sub> efflux and DOC than brown-rot fungi, that there would be greater wood-derived C losses when wood chips are located at the surface compared to buried wood chips, and that wood-derived C losses would be greater from open-top chamber (OTC) treatments compared to ambient temperature treatments. We expected more evidence of wood derived C in fluxes from the CO<sub>2</sub> and O<sub>3</sub> fumigated FACE wood type, based on preliminary data and a prior Aspen FACE study (e.g., Loya et al., 2003).

## 2. Methods

### 2.1. Experimental design

A total of 168 individual research plots with controls (1 m<sup>2</sup>) were created across six sites. The following treatments were applied in a full factorial 3 × 3 × 2 design, with site (n = 6) as the replication: initial fungal inoculation (brown rot, white rot, natural rot), FACE wood type (elevated CO<sub>2</sub>, elevated CO<sub>2</sub> + O<sub>3</sub>, ambient atmosphere), and location of wood residue (surface, buried) (108 plots + 2 no-wood control plots at each site = 120 total at ambient temperatures). Because three of the sites are loams and three are sands, we also measured the effect of soil texture on our measured parameters, as it has been well documented that soil texture can affect decomposition rates. An additional 48 plots had an elevated temperature treatment (Fig. S1 and below for details), for a total of 120 + 48 = 168 plots. All plots were randomly distributed around the cleared site, leaving at least 1 m between plots and a 10 m buffer around the edge of the cleared area. It was beyond the scope of this study to measure all response variables at all treatment plots, so we chose a subset at which to measure more intensive variables (soil CO<sub>2</sub> efflux and soil water chemistry; see below and Figs. S1, S2, S3 for more details).

All wood used in the decomposition study came from the Aspen FACE experiment at Rhinelander, WI (Dickson et al., 2000). The 12-year-old aspen trees were harvested between December 2009 and February 2010, and stored until used for this project. The Aspen FACE experiment exposed trees, during their growth from 1997 to 2009, to four atmospheric treatments. Three of the atmospheric treatments were the source of material for the FACE wood treatment: elevated CO<sub>2</sub>, elevated CO<sub>2</sub> + O<sub>3</sub>, and ambient atmospheric conditions. Treatments with elevated CO<sub>2</sub> and elevated CO<sub>2</sub> + O<sub>3</sub> were fumigated with 560 ppm CO<sub>2</sub> and 1.5 x ambient O<sub>3</sub>.

The Aspen FACE wood grown with ambient atmospheric conditions had an average δ<sup>13</sup>C signature of −27‰, very similar to the average δ<sup>13</sup>C signature of soil found at all of the research sites (−26.8‰; Table 1). In contrast, the Aspen FACE wood fumigated with elevated CO<sub>2</sub> and elevated CO<sub>2</sub> + O<sub>3</sub> had an average δ<sup>13</sup>C signature of −39‰, due to the addition of fossil fuel-derived CO<sub>2</sub> to create the elevated CO<sub>2</sub> atmosphere. This >12‰ depletion in <sup>13</sup>C in wood produced under the elevated CO<sub>2</sub> and elevated CO<sub>2</sub> + O<sub>3</sub> treatments provided the basis for measuring the movement of wood-derived C from soil as CO<sub>2</sub> efflux and in soil water as DOC, through the early stages of decomposition.

In 2011, the aspen tree stems were chipped with a 6 inch 27HP BC600XL Vermeer chipper (Vermeer Corporation, Pella, Iowa, USA), resulting in chips ranging in size from ~1 to 4 cm<sup>2</sup>. Chips were heat treated at 80 °C for 24 h in a lab drying oven for pasteurization to eliminate fungi, and then inoculated with wood decaying fungi. There were three inoculation treatments: pure cultures of white-rot fungi (*Bjerkandera adusta* (Willd.) P. Karst. (DR-447)), pure cultures of brown-rot fungi (*Gloeophyllum sepiarium* (Wulfen) P. Karst. (DR-436)), and “natural” rot, a suite of microbial species endemic to the aspen wood at the time of chipping. The white- and brown-rot fungi were chosen for their abundance in forested ecosystems and their tendency to decompose differing wood components (Boddy and Watkinson, 1995). A full characterization and identification of the natural rot species were beyond the scope of the study. For the pure species cultures, fungi were grown in 100 mm petri plates on 2% malt agar, mixed with autoclaved aspen wood chips from the ambient atmosphere FACE wood type, and transferred to 1 L jars of autoclaved primary inoculum chips. For the natural-rot treatments, unpasteurized ambient atmosphere FACE wood chips were placed directly into jars and treated the same as the pure inoculum. Jars were incubated at 25–27 °C for six weeks, allowing the chips to become completely ramified with hyphae. Approximately 14 kg (dry weight) batches of pasteurized wood chips were inoculated with one of the three cultures of fungal inoculum sources. Two liters of inoculum along with 2 L of distilled water were mixed into each 14 kg batch of wood chips in 20 gallon plastic totes (TuffNuff, J. Terence Thompson LLC., Hagerstown, MD, USA) to be used for each experimental plot. After inoculation, the totes were covered with tote lids and incubated for three months at ~18 °C. The efficacy of the inoculation treatments was verified by both re-isolation and by Fourier Transform Infrared (FTIR) spectroscopy, which examined the relative amounts of lignin or cellulose degraded over time (see section 2.5, below). For re-isolation, after inoculation and incubation but prior to putting chips in the field, three representative totes from brown rot and white rot treatments were examined and fungi were successfully re-isolated to determine that the inoculated decay fungi were present. This was done on benomyl-amended agar so that no molds grew.

Following incubation, the wood chips were deployed across six sites located in the Upper Peninsula of Michigan in the late summer of 2012. Each site (~1600 m<sup>2</sup>) was located on an aspen clear-cut that was performed in the summer of 2012. The six sites were stratified by their soil texture; three sites were coarse textured sands, and three were finer textured loams (Table 1). The sand sites (1–3) were Haplorthods and Fragiorthods, loam site 4 was a Glossudalf, and loam sites 5 and 6 were complexes of Haplorthods, Fragiouquods, and Humaquepts. Approximately 14 kg (dry weight basis) of chips were used for each 1 m<sup>2</sup> wood amended plot. Wood chips were either placed on the surface of the plots or buried 15 cm below the soil surface. Control plots without wood added (“no wood” controls) were included at each site. For the buried “no wood” control treatment, the soil was excavated to 15 cm and the soil was returned to the plot without wood. For the surface “no wood” control treatment, the plots were the same 1 m<sup>2</sup> area with no wood

**Table 1**  
Site descriptions. Soils from sites 1–3 had sand textures and sites 4–6 had loam textures. Average  $\delta^{13}\text{C}$  of soil, bulk density, and average soil surface temperature reported  $\pm$  standard errors.

Site	Site location (Latitude, Longitude)	Soil depth	Average $\delta^{13}\text{C}$ (‰)	Bulk density ( $\text{g cm}^{-3}$ )	% Sand	% Silt	% Clay	Average soil surface temperature ( $^{\circ}\text{C}$ )
1	46.676560, –88.502110	0–15 cm	–27.2 (0.1)	1.18 (0.07)	83.8	13.1	3.1	17.79 (1.93)
2	46.676288, –88.529487		–26.6 (0.0)	1.16 (0.02)	75.8	22.1	2.1	16.87 (0.82)
3	46.666986, –88.530606		–27.4 (0.1)	1.23 (0.04)	82.9	17.0	0.1	16.15 (0.93)
4	46.743429, –88.677512		–27.1 (0.1)	0.90 (0.07)	34.8	47.1	18.1	18.90 (0.64)
5	47.101468, –88.560574		–27.2 (0.0)	0.95 (0.08)	68.8	24.1	7.1	18.01 (0.50)
6	47.098990, –88.558547		–27.2 (0.0)	0.93 (0.05)	71.8	23.0	5.2	16.40 (0.43)
1	same as above	15–30 cm	–25.9 (0.0)	1.07 (0.08)	88.9	9.0	2.1	13.97 (1.36)
2			–25.7 (0.0)	1.14 (0.06)	78.8	19.2	2.0	14.89 (0.62)
3			–26.6 (0.1)	1.06 (0.08)	88.9	10.0	1.1	14.21 (0.77)
4			–25.7 (0.1)	1.32 (0.15)	38.9	35.1	26.0	15.66 (0.48)
5			–27.3 (0.1)	1.08 (0.08)	74.8	22.1	3.1	15.42 (0.43)
6			–26.8 (0.0)	1.21 (0.12)	84.8	13.0	2.2	14.46 (0.42)

added to the surface. To explore any moisture artifacts associated with wood amendments at the surface or buried, we measured surface soil moisture in one campaign in 2013 during the peak growing season (Hydrosense TDR probe (20 cm); Campbell Scientific; Logan UT, USA). There were no significant differences in soil moisture in surface ( $0.185 \text{ g g}^{-1}$ ) or buried ( $0.189 \text{ g g}^{-1}$ ) chip plots ( $n = 145$ ,  $t = 0.37$ ,  $p = 0.71$ ).

A temperature treatment was also established on a subset of the plots (Fig. S1). Of the 168 plots, 120 plots were exposed to ambient temperatures and 48 plots were exposed to temperatures within OTCs (see Marion et al., 1997). The OTCs were created from Sun-Lite® HP fiberglass glazing panels constructed into a cone shape. The base diameter was 1 m with 60° sloping sides. For OTC “no wood” control treatments, an OTC was added to the unamended control plots. Soil temperature was monitored in a subsample of plots with and without OTCs (50 total) with i-button data loggers (model DS9108, Maxim Integrated, San Jose, CA). Temperature readings were taken every hour from three soil depths (soil surface, 7.5 cm and 15 cm below surface). We used the soil surface and 15 cm depths for our temperature analyses. To explore any moisture artifacts associated with the OTCs we measured surface soil moisture in 2013 as previously described. There were no significant differences in soil moisture in plots with and without OTCs ( $n = 165$ ,  $t = 1.97$ ,  $p = 0.46$ ).

## 2.2. Soil $\text{CO}_2$ efflux measurements

Soil surface  $\text{CO}_2$  efflux was measured on four occasions per growing season in 2013 and 2014. Measurements were taken from a subsample of the research plots (Figs. S1 and S2), using a PP Systems EGM-2 or EGM-4 infrared gas analyzer (IRGA) with an attached closed system chamber (surface area of  $78.5 \text{ cm}^2$ ; SRC-1; PP Systems, Amesbury, MA, USA). At each loam soil site, there were a total of 22 treatment plots and four “no wood” control plots measured for soil surface  $\text{CO}_2$  efflux (Fig. S2). At each sand soil site, there were a total of six treatment plots and two “no wood” control plots measured (Fig. S2). Each plot was equipped with three custom fit PVC collars (10.2 cm in diameter, inserted approximately 2 cm into plot soil surface)—collars were installed in 2012 and left in place for the duration of the study. Soil  $\text{CO}_2$  efflux was determined by the linear fit of  $\text{CO}_2$  concentration change over 120 s at each collar, with efflux reported as the average measurement across the three collars within each plot.

## 2.3. Soil water chemistry measurements

To analyze soil water chemistry and initial C losses as DOC,

lysimeters were included in a subset of plots in two of the loam textured sites (a total of 40 plots had lysimeters; Figs. S1 and S3). We focused on loam textured sites to ensure more reliable water yield with soil lysimeters than would be permissible with the coarser textured sand sites. In the spring of 2013, ceramic cup tension lysimeters (30 cm length; Soil Moisture Corp., Goleta, CA, USA) were inserted near the center of the plots. Soil water was extracted twice during the growing seasons of 2013 and 2014. On the day of collection, samples were filtered through a 0.45 micron filter prior to analysis of DOC (Shimadzu Scientific Instruments, Columbia, MD, USA), spectral absorbance ( $\lambda = 254, 365, 465$ , and  $665 \text{ nm}$ ) measured using a Spectra Max M2 multimode microplate reader (Molecular Devices Corporation, Sunnyvale, CA, USA), and total phenolics (hydrolyzed aromatic compounds relative to tannic acid standard, TanniVer reagent; Hach corporation, Loveland, CO, USA).

The remainder of the water samples were then freeze-dried and analyzed as a solid for the  $\delta^{13}\text{C}$  signature of DOC as previously described. Relative DOC aromaticity was determined by dividing absorbance at  $\lambda = 254 \text{ nm}$  by total DOC to calculate specific ultraviolet absorbance at 254 nm ( $\text{SUVA}_{254}$ ; Weishaar et al., 2003). The ratio of  $\lambda = 254 \text{ nm}$  to  $\lambda = 365 \text{ nm}$  was used as an indicator of molecular size of DOC (Lou and Xie, 2006), and the ratio of  $\lambda = 465 \text{ nm}$  to  $\lambda = 665 \text{ nm}$  was used as indicator of DOC humification (Worrall et al., 2002).

## 2.4. Laboratory incubations

Laboratory incubations of only wood chips were used to determine the pure  $\delta^{13}\text{C}$  signatures of the wood and wood-respired  $\text{CO}_2$  during decomposition (i.e., no soil contamination), which were used to calculate the wood-derived soil  $\text{CO}_2$  efflux and wood-derived DOC concentrations (see equations (1) and (2) in section 2.5 below). The incubations were also conducted in order to examine FACE wood type and fungal inoculation treatment effects on wood chip chemistry from pure wood chips in a controlled setting.

All wood chips for the laboratory incubations were stored in 2 L jars in a humidity-controlled setting at  $26 \pm 3 \text{ }^{\circ}\text{C}$  and  $80 \pm 10\%$  relative humidity beginning in March 2013. For determining the  $\delta^{13}\text{C}$  signature of wood, the wood chips were sampled six different times over the course of a year, ground in a Wiley mill, followed by a ball mill, and analyzed for the  $\delta^{13}\text{C}$  signature using a Costech Elemental Combustion system 4010 connected to a ThermoFinnigan Deltaplus Continuous Flow Stable Isotope Ratio Mass Spectrometer (IRMS; Costech Analytical Technologies Inc., Valencia, CA, USA; Thermo Fisher Scientific Inc., Waltham, MA, USA). IAEA, USGS,

and NIST certified isotopic standards were run at the beginning and end of each analytical sequence, and internal standards were run for every 10 samples. Values were reported on the Vienna PeeDee Belemnite (VPDB) scale for  $\delta^{13}\text{C}$ . The precision of the certified isotopic standards is typically 0.2–0.5‰.

To measure the  $\delta^{13}\text{C}$  signature of the pure wood respired  $\text{CO}_2$ , the jars were flushed with air scrubbed with soda lime for 5 min, and then sealed. The  $\text{CO}_2$  gas samples were extracted from the jars through septa at 5 time intervals (0, 30, 60, 90, 120 min). The  $\text{CO}_2$  sampling occurred six times (~2 months apart) over a one-year period. The samples were analyzed for  $\text{CO}_2$  concentrations (ppm) with a gas chromatograph (Agilent 6850 Gas Chromatograph with Thermoconductivity detector, Santa Clara, CA, USA) and for  $\delta^{13}\text{C}$  (‰) with a GasBench II (Thermo Fisher Scientific Inc., Waltham, MA, USA) connected to the ThermoFinnigan Deltaplus IRMS described above. The gas chromatograph was calibrated with a 1500 ppm  $\text{CO}_2$  gas standard. The inverse of the  $\text{CO}_2$  concentrations was plotted on an x-axis against the associated  $\delta^{13}\text{C}$  values on a y-axis to create Keeling plots (Keeling, 1958). The y-intercept of the resulting linear equation represents the true isotopic signature of the  $\text{CO}_2$  respired from the wood chips without the contamination of atmospheric  $\text{CO}_2$  (Table 2).

## 2.5. Calculations for wood-derived soil $\text{CO}_2$ efflux and DOC

Keeling plots were used to estimate the  $\delta^{13}\text{C}$  signatures of the field measured soil  $\text{CO}_2$  efflux (Keeling, 1958). Keeling plots were performed once during peak growing season for two years, 2013 and 2014, on the same plots that were used for the total soil  $\text{CO}_2$  efflux measurements (Fig. S2). To collect the data for each Keeling plot, the IRGA was set to continuous  $\text{CO}_2$  monitoring, connected to the soil chamber, and gas samples were extracted from an in-line septum with a syringe and injected into a He flushed IRMS vial at 5 min increments (0, 5, 10, and 15 min). The  $\delta^{13}\text{C}$  signatures of each soil  $\text{CO}_2$  efflux gas sample were determined using the same IRMS system for  $\text{CO}_2$  described in section 2.4. These  $\delta^{13}\text{C}$  values were then paired with the inverse of  $\text{CO}_2$  concentrations to create Keeling plots as described earlier.

We calculated the percentage of wood-derived soil  $\text{CO}_2$  efflux ( $\%\text{wood}_{\text{CO}_2}$ ) of the total soil  $\text{CO}_2$  efflux by using a 2-endpoint mixing model (Del Gado et al., 2003):

$$\begin{aligned} \%\text{wood}_{\text{CO}_2} = & \left( \left( \delta^{13}\text{C}_{\text{soil efflux}} - \delta^{13}\text{C}_{\text{"no wood" control CO}_2} \right) / \right. \\ & \left. \times \left( \delta^{13}\text{C}_{\text{pure wood CO}_2} - \delta^{13}\text{C}_{\text{"no wood" control CO}_2} \right) \right) \\ & \times 100, \end{aligned} \quad (1)$$

where  $\delta^{13}\text{C}_{\text{soil efflux}}$  is the  $\delta^{13}\text{C}$  value of the soil  $\text{CO}_2$  efflux from Keeling plot calculations from each treatment plot,  $\delta^{13}\text{C}_{\text{"no wood" control CO}_2}$  is the  $\delta^{13}\text{C}$  of soil  $\text{CO}_2$  efflux from the corresponding "no wood" control plots (i.e., surface treatment with surface control, etc.), and the  $\delta^{13}\text{C}_{\text{pure wood CO}_2}$  is the  $\delta^{13}\text{C}$  of pure

wood  $\text{CO}_2$  efflux determined during laboratory incubations (Table 2).

The soil  $\text{CO}_2$  efflux data provided for each treatment summary included: 1. the average total soil  $\text{CO}_2$  efflux measured from each plot, as described above; 2. the percentage of wood-derived soil  $\text{CO}_2$  efflux ( $\%\text{wood}_{\text{CO}_2}$ ), calculated with equation (1); and 3. the average wood-derived soil  $\text{CO}_2$  efflux, derived by multiplying the  $\%\text{wood}_{\text{CO}_2}$  for each plot by the average total  $\text{CO}_2$  efflux measured from that plot.

We calculated the percentage of wood-derived DOC ( $\%\text{wood}_{\text{DOC}}$ ) of the total soil water DOC by using a 2-endpoint mixing model (Del Gado et al., 2003):

$$\begin{aligned} \%\text{wood}_{\text{DOC}} = & \left( \left( \delta^{13}\text{C}_{\text{DOC}} - \delta^{13}\text{C}_{\text{"no wood" control DOC}} \right) / \left( \delta^{13}\text{C}_{\text{pure wood}} - \right. \right. \\ & \left. \left. \delta^{13}\text{C}_{\text{"no wood" control DOC}} \right) \right) \times 100, \end{aligned} \quad (2)$$

where  $\delta^{13}\text{C}_{\text{DOC}}$  is the  $\delta^{13}\text{C}$  value of the DOC from each treatment plot,  $\delta^{13}\text{C}_{\text{"no wood" control DOC}}$  is the  $\delta^{13}\text{C}$  of DOC from the corresponding "no wood" control plots (i.e., surface treatment with surface control, etc.), and the  $\delta^{13}\text{C}_{\text{pure wood}}$  is the  $\delta^{13}\text{C}$  of pure wood of the appropriate treatments determined during laboratory incubations (Table 2).

The soil water DOC data provided for each treatment summary included: 1. the average total DOC measured from each plot, as described above; 2. the percentage of wood-derived DOC ( $\%\text{wood}_{\text{DOC}}$ ), calculated with equation (2); and 3. the average wood-derived DOC, derived by multiplying the  $\%\text{wood}_{\text{DOC}}$  for each plot by the average total DOC from that plot.

## 2.6. FTIR analysis

We used FTIR (Fourier Transform Infrared spectroscopy) analysis to determine if there were temporal changes in wood chip chemistry resulting from FACE wood type and fungal inoculation treatment interactions during decomposition of the laboratory incubations. The analysis used infrared light and adsorption wavelengths to identify classes of chemical compounds found in the wood chips. In March 2013, chipped wood samples used for FTIR were incubated in separate jars using the same design as for the laboratory incubations described above. One set of jars was sampled at  $T_0$ , and one set was incubated for an additional year. The wood chips that were analyzed represented a full factorial design of the three FACE wood types (i.e., ambient,  $+\text{CO}_2$ , and  $+\text{CO}_2 + \text{O}_3$ )  $\times$  3 fungal inoculation treatments (i.e., brown-, white-, and natural-rot) for a total of 9 treatments  $\times$  2 dates = 18 samples, with no replication. The wood chip samples were oven dried at 65 °C to constant mass, ground with a Wiley mill and ball mill (SPEX CertiPrep 8000-series), and analyzed using a Varian 800 Scimitar series FTIR (Varian, Inc, Palo Alto, CA, USA) equipped with a diffuse reflectance accessory (Pike Technologies, Madison, WI, USA).

The FTIR output was used to determine the proportion of lignin to other carbohydrates. These proportions were compared across the two incubation times (i.e.,  $T_0$  and 1 year). Absorbance intensities for lignin bands were compared against carbohydrate

**Table 2**  
Average pure wood  $\delta^{13}\text{C}$  and pure wood  $\delta^{13}\text{CO}_2$  values  $\pm$  standard errors from laboratory incubations taken from six sampling dates over one year.

FACE wood/inoculation treatments	Pure wood $\delta^{13}\text{C}$ value	Pure wood $\delta^{13}\text{CO}_2$ value
$+\text{CO}_2$ /brown-rot	-38.05 (0.38)	-38.94 (0.52)
$+\text{CO}_2$ /white-rot	-38.16 (0.20)	-41.09 (0.38)
$+\text{CO}_2$ /natural-rot	-38.71 (0.35)	-38.95 (0.82)
$+\text{CO}_2 + \text{O}_3$ /brown-rot	-39.02 (0.30)	-42.20 (1.31)
$+\text{CO}_2 + \text{O}_3$ /white-rot	-38.42 (0.32)	-41.95 (1.34)
$+\text{CO}_2 + \text{O}_3$ /natural-rot	-38.99 (0.25)	-40.25 (1.90)

bands to determine if the relative proportion of lignin:carbohydrates decreased or increased as decomposition progressed, following the methods of Pandey and Pitman, 2003. The lignin reference peak used was  $1505\text{ cm}^{-1}$ . While other peaks also account for lignin, this reference peak arises only from the aromatic skeletal vibration (C=C) in lignin, with no contributions from carbohydrates. Four different carbohydrate reference peaks were used:  $1738\text{ cm}^{-1}$  representing the unconjugated C=O in xylans (specifically attributed to hemicellulose),  $1375\text{ cm}^{-1}$  representing the C-H deformation in cellulose and hemicellulose,  $1158\text{ cm}^{-1}$  representing the C-O-C vibration in cellulose and hemicellulose, and  $898\text{ cm}^{-1}$  representing the C-H deformation in cellulose. These carbohydrate reference peaks were chosen because they arise solely from carbohydrates, with no contributions from lignin. Decreases or no change (i.e., in the case that lignin and the carbohydrates are degrading equally) in the relative peak height ratio of lignin ( $1738\text{ cm}^{-1}$ ) to the averaged four carbohydrate reference peaks (i.e., lignin:average carbohydrate ratio) throughout decomposition show evidence for lignin degradation.

Proportions were also compared for the deformation (demethylation) of lignin. While brown-rot fungi cannot decompose lignin, they can alter the chemical structure of lignin by removing methoxy groups, which would affect the chemical absorbance intensities in the FTIR output. Peaks representing the C-H deformation of lignin,  $1462\text{ cm}^{-1}$  and  $1425\text{ cm}^{-1}$ , were compared against the reference lignin peak,  $1505\text{ cm}^{-1}$ . Increases in this ratio throughout decomposition show evidence for the demethylation of lignin.

## 2.7. Statistical analyses

A general linear mixed-effects model with repeated measures was used to examine wood chip location, FACE wood type, temperature, soil texture, fungal inoculation, and interactions of location with texture and of fungal inoculation with temperature as fixed effects on soil  $\text{CO}_2$  efflux, wood-derived soil  $\text{CO}_2$  efflux, and the percentage of wood-derived soil  $\text{CO}_2$  efflux using SAS 9.4 (SAS Institute Inc., Cary, NC, USA). Higher level interactions including wood chip location, texture, FACE wood type, and temperature were not possible because, as detailed above, this was not designed as a full-factorial experiment (Fig. S2), but lower level interactions among location, temperature, and inoculation were tested for the subset of plots for which they were in factorial combination. Interactions that were not significant for any of the response variables were omitted from tables presented. The repeated measures component was represented by sampling date. Each treatment (i.e., soil texture, wood chip location, FACE wood type, and fungal inoculation) was considered a categorical predictor variable.

A general linear mixed-effects model with repeated measures was used to examine fungal inoculation, temperature, and the interaction of inoculation with temperature as fixed effects on total DOC, wood-derived DOC, and the average percentage of wood-derived DOC. A reduced suite of fixed effects was investigated for DOC, because lysimeters were only in a subset of plots and were only at one depth, precluding comparisons with the wood location treatment (Fig. S3). Since all lysimeters did not yield water on all sampling dates, we had  $n = 67$  for DOC analysis. Plot level replications were represented as random variables.

Data distributions were examined, and any requisite transformations for data not meeting assumptions of normality were evaluated with Shapiro-Wilk  $W$  and Kolmogorov-Smirnov statistics. A log transformation was used on the average total soil  $\text{CO}_2$  efflux, the wood-derived soil  $\text{CO}_2$  efflux, and average tannin content. The average percentage of wood-derived soil  $\text{CO}_2$  efflux, the wood-derived DOC, average SUVA<sub>254</sub>, and the ratios of  $\lambda = 254\text{ nm}$  to  $\lambda = 365\text{ nm}$  and  $\lambda = 465\text{ nm}$  to  $\lambda = 665\text{ nm}$  needed no

transformation. An inverse transformation was used on the average total soil water DOC and average tannin. A square root transformation was used on the average percentage of wood-derived DOC. Type III tests of fixed effects and post-hoc comparisons of least-squared means tests of  $\text{CO}_2$  efflux and DOC employed the Tukey–Kramer adjustment and were considered significant at  $\alpha = 0.05$ . Analysis of covariance was used to ascertain differences in the relationship between DOC and  $\text{CO}_2$  efflux as a function of inoculation treatment. Differences in mean DOC quality metrics were tested with t-tests. All effects were considered significant at  $\alpha = 0.05$ .

A regression analysis using an ANOVA mixed model was used to determine the effects of the OTCs on mean, minimum, and maximum daily soil temperature (R Core Team, 2013). The temperature data were restricted to May through August and two time intervals, 10:00 a.m. to 4:00 p.m. and 5:00 p.m. to 9 a.m. The OTC and ambient temperature treatments were treated as random independent variables with site and day as random effects for the whole data or only site as a random effect for everyday mean/max/min.

## 3. Results

### 3.1. Inoculation efficacy using FTIR

At the onset of the laboratory incubations there was no significant difference in the lignin:average carbohydrates ratio between fungal inoculation treatments (Fig. 1). After 12 months of decomposition the lignin:average carbohydrate ratio increased from  $0.50 \pm 0.01$  to  $0.53 \pm 0.02$  in wood inoculated with brown-rot and from  $0.51 \pm 0.01$  to  $0.52 \pm 0.01$  in wood inoculated with natural-rot. The lignin:average carbohydrate ratio decreased from  $0.49 \pm 0.01$  to  $0.48 \pm 0.01$  in wood inoculated with white-rot. The lignin:average carbohydrate ratio was significantly lower in wood inoculated with white-rot compared with wood inoculated with brown-rot for all FACE wood treatments ( $p = 0.001$ ; Fig. 1).

### 3.2. Soil $\text{CO}_2$ efflux

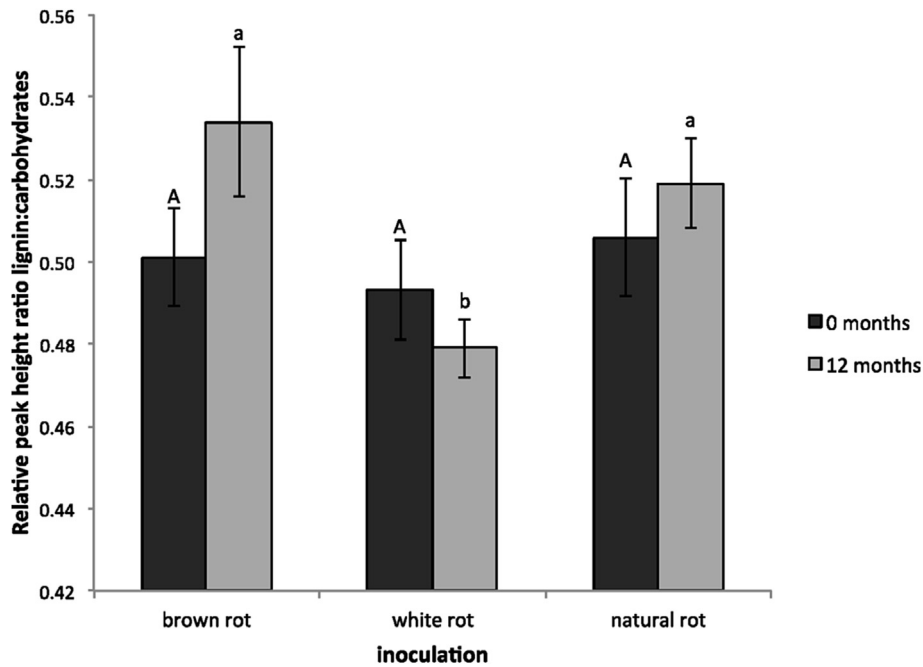
Average soil  $\text{CO}_2$  efflux ranged from seasonal lows of  $0.28\text{--}1.60\text{ g CO}_2\text{ m}^{-2}\text{ h}^{-1}$  in the spring and fall to highs of  $1.77\text{--}2.92\text{ g CO}_2\text{ m}^{-2}\text{ h}^{-1}$  in the mid growing seasons of 2013 and 2014, with no significant difference in the mean growing season rates between 2013 and 2014. The wood-derived efflux was about half the total soil  $\text{CO}_2$  efflux, ranging from means of  $0.13\text{--}1.77\text{ g CO}_2\text{ m}^{-2}\text{ h}^{-1}$ . The average total soil  $\text{CO}_2$  efflux from treatments with wood chips was significantly larger than from “no wood” control plots ( $1.71 \pm 0.05$  versus  $0.67 \pm 0.04$ ;  $p < 0.001$ ). There was no significant difference in the average total soil  $\text{CO}_2$  efflux between “no wood” control surface plots and “no wood” control buried plots ( $0.70 \pm 0.04$  versus  $0.64 \pm 0.06$ ; Fig. 2).

#### 3.2.1. Wood location

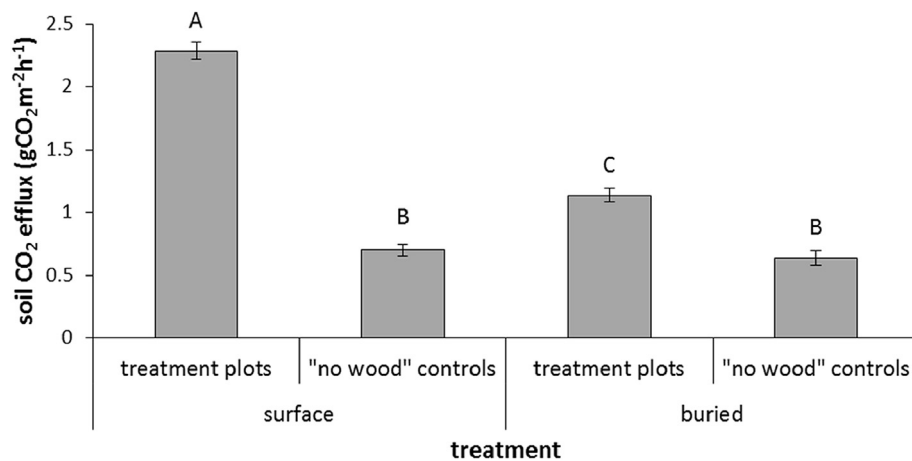
Wood location had a significant effect on soil  $\text{CO}_2$  efflux (Table 3). The average total soil  $\text{CO}_2$  efflux for surface wood chip treatments was twice that of buried wood chip treatments ( $2.28 \pm 0.07\text{ g CO}_2\text{ m}^{-2}\text{ h}^{-1}$  versus  $1.14 \pm 0.05\text{ g CO}_2\text{ m}^{-2}\text{ h}^{-1}$ , respectively;  $p < 0.001$ ; Fig. 2). The wood-derived soil  $\text{CO}_2$  efflux was also significantly higher from surface wood chip versus buried wood chip treatments ( $p < 0.001$ ; Fig. 3). The percentage of wood-derived soil  $\text{CO}_2$  efflux also differed significantly by wood location (Table 3), with percentage wood-derived soil  $\text{CO}_2$  efflux of  $53 \pm 2\%$  for surface wood chip treatments and  $43 \pm 2\%$  for buried wood chip treatments.

#### 3.2.2. Temperature

Soil temperature was marginally affected by the OTCs. In 2013,



**Fig. 1.** Relative peak height ratio of the lignin reference peak intensity ( $1505\text{ cm}^{-1}$ ) to the average of carbohydrate reference peak intensities ( $1738\text{ cm}^{-1}$ , hemicellulose;  $1375\text{ cm}^{-1}$ , cellulose and hemicellulose;  $1158\text{ cm}^{-1}$ , cellulose and hemicellulose; and  $898\text{ cm}^{-1}$ , cellulose) at 0 months and after 12 months of decomposition by brown-rot, white-rot, and natural-rots. After 12 months of decomposition, white-rot had a significantly lower proportion of lignin:average carbohydrates compared to brown- and natural-rots ( $p = 0.001$ ). Uppercase letters represent significant differences between proportions of lignin:average carbohydrates at 0 months and lowercase letters represent significant differences ( $p < 0.05$ ) between proportion of lignin:carbohydrates after 12 months of decomposition.

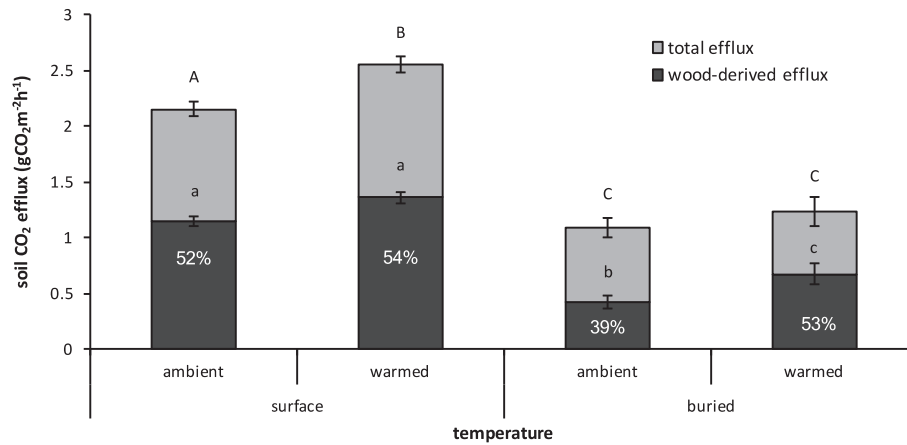


**Fig. 2.** Average total soil  $\text{CO}_2$  efflux rates from experimental and “no wood” control surface plots compared with experimental and “no wood” control buried plots. Different letters represent significant differences ( $p < 0.05$ ) between average total soil  $\text{CO}_2$  efflux rates.

**Table 3**

Type III test of fixed effects for total soil  $\text{CO}_2$  efflux rates ( $\text{g CO}_2\text{ m}^{-2}\text{ h}^{-1}$ ), the percentage of wood-derived soil  $\text{CO}_2$  efflux, and the wood-derived soil  $\text{CO}_2$  efflux with wood location (loc), FACE wood type (face), temperature (temp), soil texture (text), and fungal inoculation (inoc) as factors. “No wood” control values were left out of the final statistical analyses.

Effect	total soil $\text{CO}_2$ efflux				% wood-derived soil $\text{CO}_2$ efflux				wood-derived soil $\text{CO}_2$ efflux			
	Num DF	Den DF	F Value	Pr > F	Num DF	Den DF	F Value	Pr > F	Num DF	Den DF	F Value	Pr > F
loc	1	589	149.62	<0.0001	1	494	16.53	<0.0001	1	484	126.83	<0.0001
face	2	589	5.55	0.004	1	494	14.69	<0.0001	1	484	7.99	0.005
temp	1	589	4.73	0.030	1	494	3.32	0.069	1	484	9.37	0.002
text	1	589	0.33	0.563	1	494	12.83	<0.0001	1	484	0.22	0.639
inoc	2	589	0.56	0.573	2	494	7.02	0.001	2	484	2.35	0.097
loc x text	1	589	1.17	0.280	1	494	5.69	0.018	1	484	0.18	0.673
inoc x temp	2	589	2.63	0.073	2	494	3.47	0.032	2	484	3.21	0.041



**Fig. 3.** Average total soil CO<sub>2</sub> efflux rates and the wood-derived portion of soil CO<sub>2</sub> efflux differed significantly by temperature treatment ( $p = 0.007$ ,  $p < 0.001$ , respectively). The average total soil CO<sub>2</sub> efflux rate difference was only significant when wood chips were located on the surface and not when wood chips were buried ( $p = 0.011$ ). Percentages are the average percentage of wood-derived soil CO<sub>2</sub> efflux. These percentages differed significantly between ambient temperature and OTC treatments ( $p < 0.001$ ), as did the wood-derived portion of CO<sub>2</sub>, but only for the buried chip treatments. Uppercase letters represent significant differences ( $p < 0.05$ ) between average total soil CO<sub>2</sub> efflux and lowercase letters represent significant differences ( $p < 0.05$ ) between the wood-derived soil CO<sub>2</sub> efflux.

daytime (i.e., between 10 a.m. and 4 p.m.) soil temperatures at the surface and at 15 cm were 0.4 and 0.5 °C warmer, respectively, for the OTC than the ambient temperature treatments. In 2014, the OTC treatments cooled the daytime soil surface and 15 cm depth by 1.2 and 0.2 °C, respectively, relative to the ambient temperature treatments. The general cooling effect in 2014 was likely due to tree regrowth and shading of the OTC treatments. Average nighttime (i.e., between 5 p.m. and 9 a.m.) minimum soil temperatures at the surface showed the most consistent warming response from OTC compared with ambient temperature treatments in both 2013 and 2014 (0.9 and 0.6 °C,  $p < 0.0001$ ).

Despite the small change in soil temperatures, the OTC treatment had a significant effect on soil CO<sub>2</sub> efflux (Table 3). Treatments at ambient temperatures had lower average total soil CO<sub>2</sub> efflux than those of OTC treatments ( $1.62 \pm 0.06$  g CO<sub>2</sub> m<sup>-2</sup> h<sup>-1</sup> versus  $1.90 \pm 0.09$  g CO<sub>2</sub> m<sup>-2</sup> h<sup>-1</sup>, respectively;  $p = 0.03$  Fig. 3). The wood-derived soil CO<sub>2</sub> efflux and the percentage of wood-derived soil CO<sub>2</sub> efflux were higher for the OTC treatments for buried wood chips compared with those of the ambient temperature treatments ( $p < 0.002$  and  $p < 0.001$ ; Fig. 3). There was no significant difference between the wood-derived soil CO<sub>2</sub> efflux or percentage of wood-derived soil CO<sub>2</sub> efflux from the OTC compared to the ambient temperature treatments for surface wood chip treatments.

### 3.2.3. Fungal inoculation

The fungal inoculation treatment had no significant effect on total soil CO<sub>2</sub> efflux or wood-derived soil CO<sub>2</sub> efflux, but did affect the percentage of wood-derived soil CO<sub>2</sub> efflux (Table 3). The percentage of wood-derived soil CO<sub>2</sub> efflux was significantly higher from treatments with wood chips inoculated with natural-rot, as compared to treatments with wood chips inoculated with white-rot ( $52 \pm 3\%$  versus  $45 \pm 3\%$ ;  $p = 0.001$ ). The average percentage of wood-derived soil CO<sub>2</sub> efflux from brown-rot treatments was  $47 \pm 3\%$  and did not significantly differ from white- or natural-rot.

The interaction between fungal inoculation and temperature was significant (Table 3). The average wood-derived soil CO<sub>2</sub> efflux and the average percentage of wood-derived soil CO<sub>2</sub> efflux were significantly greater from OTC treatments compared with that of ambient temperature only when wood chips were inoculated with brown-rot ( $p = 0.05$ ), but not when wood chips were inoculated with white- or natural-rot.

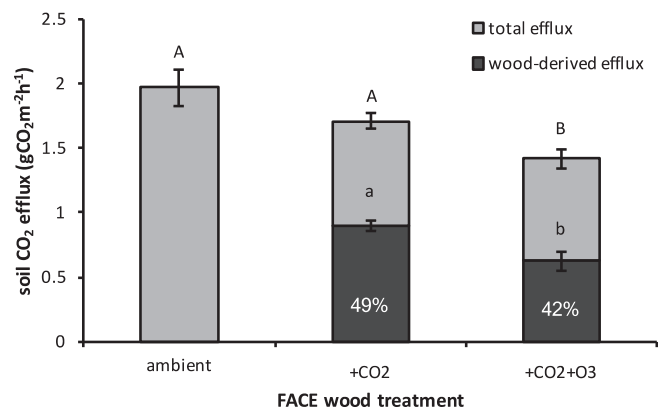
### 3.2.4. FACE wood type

The FACE wood treatment had a significant effect on soil CO<sub>2</sub> efflux (Table 3). The average total soil CO<sub>2</sub> efflux from elevated CO<sub>2</sub> + O<sub>3</sub> FACE wood type was significantly lower than the average total soil CO<sub>2</sub> efflux from both the ambient and elevated CO<sub>2</sub> FACE wood types ( $1.37 \pm 0.12$  g CO<sub>2</sub> m<sup>-2</sup> h<sup>-1</sup> versus  $1.97 \pm 0.14$  g CO<sub>2</sub> m<sup>-2</sup> h<sup>-1</sup> and  $1.71 \pm 0.06$  g CO<sub>2</sub> m<sup>-2</sup> h<sup>-1</sup>,  $p = 0.008$  and  $p = 0.007$ , respectively; Fig. 4). Similarly, the wood-derived soil CO<sub>2</sub> efflux and average percentage of wood-derived soil CO<sub>2</sub> efflux were significantly lower from the elevated CO<sub>2</sub> + O<sub>3</sub> compared to the elevated CO<sub>2</sub> FACE wood type ( $p = 0.002$  and  $p = 0.004$ , respectively; Fig. 4).

FACE wood types did not statistically differ from each other for the individual lignin, carbohydrate peaks, or the lignin:average carbohydrates ratio using FTIR analysis. The lack of data supporting a chemical difference in wood quality is discussed in section 4.5.

### 3.2.5. Soil texture

Soil texture did not have a statistically significant effect on total



**Fig. 4.** Average total soil CO<sub>2</sub> efflux rates from the ambient and the elevated CO<sub>2</sub> FACE wood types differed significantly from the elevated CO<sub>2</sub> + O<sub>3</sub> FACE wood type ( $p < 0.001$  and  $p = 0.007$ , respectively). Percentages are the average percentage of wood-derived soil CO<sub>2</sub> efflux. The wood-derived soil CO<sub>2</sub> efflux and the average percentage of wood-derived soil CO<sub>2</sub> efflux were significantly greater from the elevated CO<sub>2</sub> FACE wood type compared with the elevated CO<sub>2</sub> + O<sub>3</sub> wood type ( $p = 0.002$  and  $p = 0.004$ , respectively). Uppercase letters represent significant differences ( $p < 0.05$ ) between average total soil CO<sub>2</sub> efflux rates and lowercase letters represent significant differences between the wood-derived soil CO<sub>2</sub> efflux.



soil CO<sub>2</sub> efflux (Table 3). However, the percentage of soil CO<sub>2</sub> efflux from wood was significantly higher for loam versus sand textured soil (50 ± 2% versus 44 ± 3%;  $p < 0.001$ ). The interaction of soil texture and wood location was significant for the percentage of wood-derived soil CO<sub>2</sub> efflux (Table 3), with the percentage of wood-derived soil CO<sub>2</sub> efflux being greater for loam-textured soils compared with sandy soils only when wood chips were buried. There was no significant difference between average total soil CO<sub>2</sub> efflux from loam and sand soils for surface wood chip treatments (2.27 ± 0.08 g CO<sub>2</sub> m<sup>-2</sup> h<sup>-1</sup> versus 2.33 ± 0.15 g CO<sub>2</sub> m<sup>-2</sup> h<sup>-1</sup>).

### 3.3. Soil water DOC

#### 3.3.1. Fungal inoculation

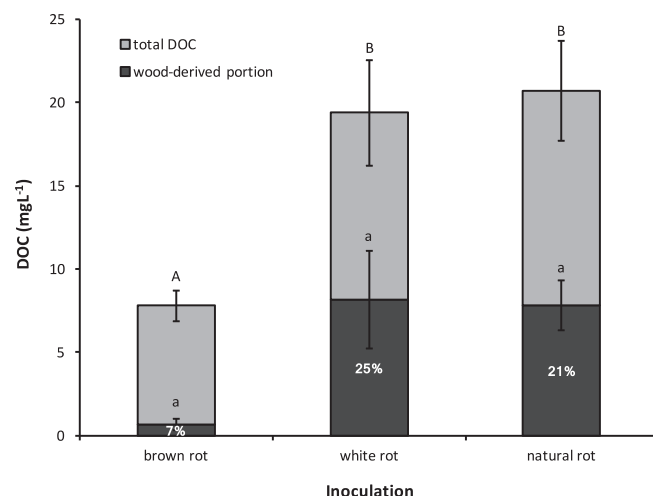
The total DOC concentrations from lysimeters ranged from 3.84 to 71.40 mg L<sup>-1</sup> in the 2013 and 2014 growing seasons. The wood-derived DOC averaged about half the total DOC, ranging from 0.01 to 38.80 mg L<sup>-1</sup>. The fungal inoculation treatment had a significant effect on total DOC and percentage of wood-derived DOC (Table 4). The average total DOC content for brown-rot treatments was lower than those for white-rot and natural-rot treatments (7.80 ± 0.92 mg L<sup>-1</sup> versus 19.37 ± 3.19 mg L<sup>-1</sup> and 20.69 ± 2.97 mg L<sup>-1</sup>, respectively;  $p = 0.005$ ; Fig. 5). The average percentage of wood-derived DOC was lower from treatments where wood chips were inoculated with brown-rot compared to either natural- or white-rot (7 ± 4% versus 31 ± 4% and 25 ± 6%, respectively;  $p = 0.01$ ; Fig. 5).

The interaction between fungal inoculation and temperature treatment was significant (Table 4). DOC concentrations were lower from brown-rot compared with white-rot from the ambient temperature treatments (7.75 ± 1.17 mg L<sup>-1</sup> versus 26.71 ± 4.05 mg L<sup>-1</sup>;  $p = 0.002$ ), but not from the OTC treatment. Natural-rot treatments had higher average DOC than white- or brown-rot for the OTC treatments, but not the ambient temperature treatments (24.14 mg L<sup>-1</sup> ± 4.64 versus 8.37 ± 1.17 mg L<sup>-1</sup> and 7.85 ± 1.50 mg L<sup>-1</sup>, respectively;  $p = 0.027$  and  $p = 0.008$ ). Seasonal changes in soil DOC concentrations were correlated with soil CO<sub>2</sub> efflux rates in the white-rot and natural-rot treatments, but not in the brown-rot treatments (Fig. 6).

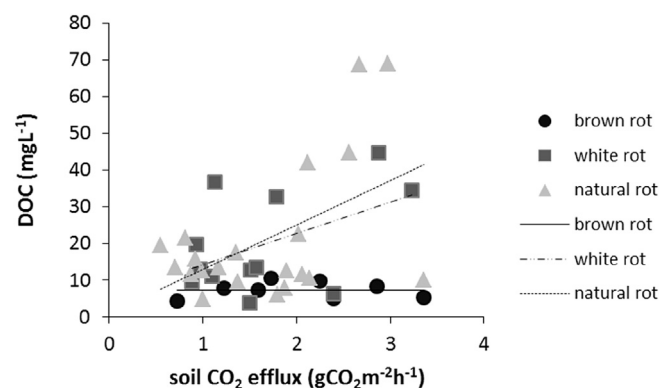
The fungal inoculation treatment also had a significant effect on the composition of DOC. The DOC from brown-rot inoculation treatments showed significantly less tannin content than both white- and natural-rot inoculation treatments (1.37 ± 0.29 mg L<sup>-1</sup> versus 2.47 ± 0.47 mg L<sup>-1</sup> and 3.04 ± 0.62 mg L<sup>-1</sup>, respectively;  $p = 0.057$  and  $p = 0.023$ ; Fig. 7a) and less humification than that from white-rot inoculation treatments ( $\lambda=465:665$  ratio: 1.67 ± 0.88 versus 6.31 ± 1.01;  $p = 0.024$ ; Fig. 7b).

#### 3.3.2. FACE wood type

The FACE wood treatment had a significant effect on DOC characteristics. The average SUVA<sub>254</sub> (measure of aromaticity) of soil water from the elevated CO<sub>2</sub> FACE wood type was higher than the average SUVA<sub>254</sub> from the ambient FACE wood type (4.95 ± 0.51 L mg<sup>-1</sup> m<sup>-1</sup> versus 2.16 ± 0.42 L mg<sup>-1</sup> m<sup>-1</sup>;  $p = 0.013$ ). DOC from the elevated CO<sub>2</sub> FACE wood type also showed



**Fig. 5.** Average total soil water DOC differed significantly between inoculation treatments. Average total DOC from treatments containing wood chips inoculated with natural-rot and white-rot were significantly greater than treatments with wood chips inoculated with brown-rot ( $p = 0.005$  and  $p = 0.01$ , respectively). Percentages are the average percentage of wood-derived DOC. These percentages were significantly higher from both natural-rot and white-rot inoculation treatments compared to brown-rot inoculation treatments ( $p = 0.01$ ). Uppercase letters represent significant differences ( $p < 0.05$ ) between average total DOC and lowercase letters represent significant differences ( $p < 0.05$ ) between the wood-derived DOC.



**Fig. 6.** The relationships between total soil CO<sub>2</sub> efflux rates and total DOC concentrations were significantly affected by fungal inoculation of brown-rot, white-rot, and natural-rot (Analysis of Covariance,  $F$ -value = 4.14,  $p = 0.01$ ).

significantly more humification than that of the ambient FACE wood type ( $\lambda=465:665$  ratio; 4.90 ± 0.70 versus 1.71 ± 1.09;  $p = 0.050$ ). There was no significant difference between FACE wood types in total DOC, molecular size ( $\lambda=254:365$  ratio), or total phenolics.

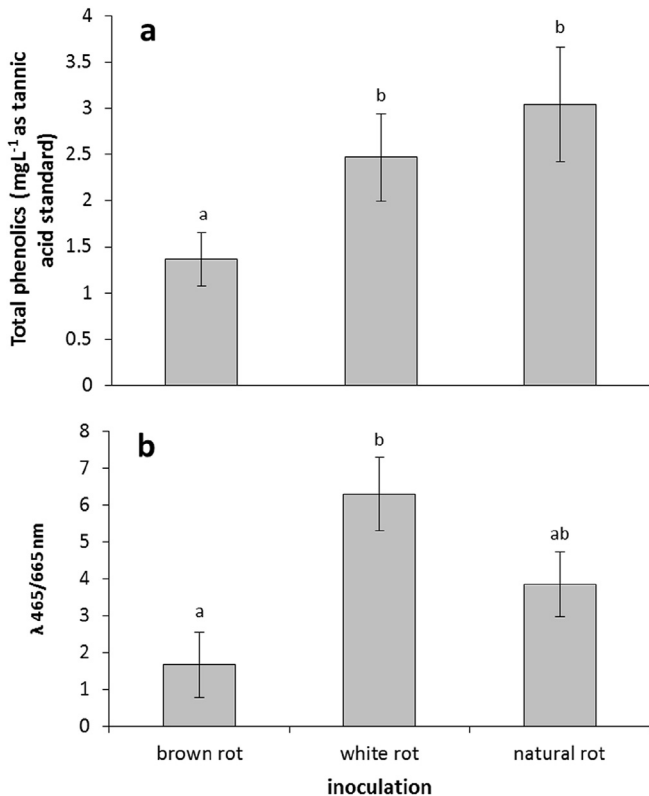
## 4. Discussion

Our two most important and/or novel findings are that the

**Table 4**

Type III test of fixed effects for total DOC, the percentage of wood-derived DOC, and the wood-derived DOC with fungal inoculation (inoc) and temperature (temp) as factors.

Effect	total DOC				% wood-derived DOC				wood-derived DOC			
	Num DF	Den DF	F Value	Pr > F	Num DF	Den DF	F Value	Pr > F	Num DF	Den DF	F Value	Pr > F
inoc	2	67	9.42	0.0002	2	30	5.1	0.0124	2	30	1.85	0.1748
temp	1	67	2.18	0.1448	1	30	0.99	0.3284	1	30	0.04	0.8335
inoc x temp	2	67	7.09	0.0016	2	30	1.89	0.1686	2	30	2.62	0.0897



**Fig. 7.** Total soil water phenolics (a) and average degree of humification (b) were significantly different between inoculation treatments. Treatments inoculated with brown-rot had less total phenolics than both white-rot and natural-rot inoculation treatments ( $p = 0.057$  and  $p = 0.023$ , respectively). Treatments with brown-rot also showed less humification than those inoculated with white-rot ( $p = 0.024$ ). Different letters represent significant differences between inoculation treatments.

buried aspen stemwood chips decomposed much less rapidly than surface wood, and that initial fungal inoculum affected the amount and quality of the DOC leaching out of the decomposing woody materials. Both have significant implications for either fundamental or applied problems in soil science.

#### 4.1. Wood location

Surface wood chip treatments had significantly higher wood-derived soil CO<sub>2</sub> efflux than those from buried wood chip treatments, in agreement with earlier work and in support of our original hypothesis (Jorgensen and Wells, 1973; Singh and Gupta, 1977). While the importance of location of wood in terms of CO<sub>2</sub> fluxes was documented, we did not consider the biochemistry of root versus stem wood. Huang and Spohn (2015) demonstrated that root C is decomposed and mineralized at a slower rate compared to other litter C. The slower decomposition rates, at least in these early stages of root decay, can be attributed to lower litter quality and less labile components in roots compared to foliage (Palviainen et al., 2004; Cotrufo et al., 2009; Hansson et al., 2010). A unique aspect of our study is that by only varying location in the soil while controlling for wood chemistry, we can conclude that both root and buried wood contributions to SOC pools are at least partially explained by their vertical stratification in the soil. The implications of this for management suggest that wood residues from harvesting—slash materials on the soil surface and roots and stump materials in the soil—matter in the stabilization of soil C in the years following disturbance. Efforts are underway to quantify the transfer of surface and buried wood SOM into discrete soil C pools over time.

#### 4.2. Fungal inoculation

Fungal inoculation type significantly affected the percentage of wood-derived CO<sub>2</sub> efflux and DOC components. These data support our hypothesis that priority effects related to initial fungal colonization of woody residues significantly determine the rate of decomposition, and the rate and quality of the water soluble products of decomposition generated in the soil. These factors determine the stabilization of wood-derived C—either remaining as wood or evading as CO<sub>2</sub>—or through the reactivity of illuvial DOC. Wood-derived DOC content was greater from white-rot compared to brown-rot treatments. DOC in the soil water from treatments inoculated with brown-rot appeared to be less decomposed than DOC from treatments inoculated with white-rot, because there was less total DOC, and DOC exhibited a lower degree of humification. Also, treatments inoculated with brown-rot had less total phenolics present, likely because less phenolic material was released from the decomposition of wood residues. The lower accumulation of these water-soluble products of decomposition in treatments with brown-rot could be indicative of less lignin degradation (Kalbitz et al., 2006). The lack of a relationship between DOC concentrations and soil CO<sub>2</sub> efflux in the brown-rot treatments could also indicate a disconnect between pore water composition and mineralization processes. Together, these data indicate that white-rot fungi might be more important in the generation of wood-derived illuvial C in soils, whereas brown-rot fungi are more instrumental in stabilizing wood residues on the soil surface, which exhibit degraded polysaccharides and a lignin skeleton (“cubic” rot decay residues).

The lignin:average carbohydrate ratio increased in wood decomposed by brown-rot and decreased in wood decomposed by white-rot during laboratory incubations. Since white-rot fungi are able to fully decompose lignin, unlike brown-rot, it is to be expected that lignin would be removed from wood decomposed by white-rot fungi and not in wood decomposed by brown-rot fungi. Pandey and Nagveni (2007) and Xu et al. (2013) found similar proportional lignin increases and decreases from both brown-rot and white-rot, respectively. Whether the laboratory incubation results are born out in the soil under field conditions awaits future assessment.

#### 4.3. CO<sub>2</sub> efflux

Our measurements of average soil CO<sub>2</sub> efflux, ranging from 0.28 to 2.92 g CO<sub>2</sub> m<sup>-2</sup> h<sup>-1</sup>, are similar to other soil CO<sub>2</sub> efflux reported for northern forest soils in general, ranging from 0.02 to 7.26 g CO<sub>2</sub> m<sup>-2</sup> h<sup>-1</sup> (Bowden et al., 1993; Raich and Schlesinger, 1992; Singh and Gupta, 1977). However, our average soil CO<sub>2</sub> efflux are higher than those reported for other aspen forests during the growing season (i.e. 0.09–0.90 g CO<sub>2</sub> m<sup>-2</sup> h<sup>-1</sup>; King et al., 2001; Wang et al., 2006; Pregitzer et al., 2008). Our “no wood” control plots had a lower range of soil CO<sub>2</sub> efflux (0.07–1.63 g CO<sub>2</sub> m<sup>-2</sup> h<sup>-1</sup>) compared to our treatment plots, providing soil CO<sub>2</sub> efflux rates more comparable to those from the other aspen forest soils. The higher soil CO<sub>2</sub> efflux from treatment plots resulted from the decomposition of additional wood chip C. Others have seen similar trends of increased soil CO<sub>2</sub> efflux with the addition of wood and litter (Sulzman et al., 2005; Crow et al., 2009; Forrester et al., 2015).

#### 4.4. Temperature

Although the OTC effect on soil temperature was small, the wood-derived soil CO<sub>2</sub> efflux was higher in the OTC compared to ambient temperature treatments. It is well documented that temperature has a positive effect on decomposition rates (Kirschbaum,

1995; Chen et al., 2013). However, it has also been documented that OTCs can have variable effects on soil temperature and are dependent on solar irradiance (Bokhorst et al., 2013). The differences in OTC effects on soil temperature between 2013 and 2014 were probably a result of shading in 2014 caused by sapling regrowth after clearing in 2012. OTCs are also known to cause other changes in microclimate such as higher humidity (Marion et al., 1997). While OTC artifact effects on soil moisture were not detected during peak growing season in this study, there could have been differences in moisture contents in OTC plots in the spring or fall seasons. As such, we cannot definitively conclude that the OTC effects on soil CO<sub>2</sub> efflux were due to temperature.

#### 4.5. FACE wood type

Average total and wood-derived soil CO<sub>2</sub> efflux rates were lower in elevated CO<sub>2</sub> + O<sub>3</sub> FACE wood type relative to the other FACE wood types. Furthermore, soil water DOC from the elevated CO<sub>2</sub> FACE wood type showed higher aromaticity (higher SUVA<sub>254</sub>) and humification index values compared with that of the ambient FACE wood type. The measurable differences in the FACE wood treatment impacts on soil C fluxes in the first two years of our study are notable given the inconsistent results in assessments of differences in wood properties of wood grown at FACE sites. For instance, statistically significant alterations in the chemical composition of leaf and woody tissues grown under elevated CO<sub>2</sub> and/or O<sub>3</sub> have been observed (Kostiainen et al., 2004, 2006; Liu et al., 2005; Parsons et al., 2008; Liu et al., 2009). However, at Aspen FACE, the effects of elevated CO<sub>2</sub> and/or O<sub>3</sub> on plant tissue biochemical composition varied among sampling dates, especially for wood, with consistent main effects of elevated CO<sub>2</sub> or elevated O<sub>3</sub> on wood chemical properties generally being absent (Kaakinen et al., 2004; Kostiainen et al., 2008). Unfortunately, our sampling design did not permit a comparison of soil water DOC for the elevated CO<sub>2</sub> + O<sub>3</sub> FACE wood type. Future study of the water-soluble products from the decomposition of this FACE wood type in soil is certainly warranted.

For the current study, we could not detect any differences in the intrinsic wood properties of the FACE wood types. Our FTIR analysis lacked significant differences in structural lignin and carbohydrates. Subsequently, we tested for differences between FACE wood types for non-structural carbohydrates. Because the trees from all treatments for the current study were harvested in the winter, we hypothesized that the elevated CO<sub>2</sub> + O<sub>3</sub> FACE wood type would have less non-structural C (sugar and starch) in the wood than the ambient wood type owing to more stress imposed by the fumigation. However, we observed no significant differences in non-structural carbohydrates between the FACE wood types (phenol extraction,  $p > 0.1$ ; unpublished data). Previous work has suggested that tissue chemistry responses to elevated CO<sub>2</sub> were likely due to competitive interactions between aspen and birch when grown in mixed stands (Liu et al., 2005; Talhelm et al., 2009), but we did not distinguish mono-specific aspen from aspen grown with birch in this study; this likely contributes to the variation in initial wood character observed (e.g., Fig. 1). Nonetheless, decreased soil CO<sub>2</sub> efflux in the elevated CO<sub>2</sub> + O<sub>3</sub> FACE wood type (Fig. 4) suggests a limitation to decomposition may be present with this global change scenario, which is contrary to an earlier study (Loya et al. 2003). Further study of the exact intrinsic wood properties responsible for this is certainly warranted.

#### 4.6. Soil texture

Both fine and coarse textured soils had similar wood-derived soil CO<sub>2</sub> efflux. The percentage of wood-derived soil CO<sub>2</sub> efflux

was significantly larger in the loam soils, but when applied to the average total soil CO<sub>2</sub> efflux, it was not significant. Overall, soil texture had very little impact on decomposition in this study. This is likely because litter quality tends to be more important for controlling decomposition rates in the early stages of decomposition, whereas organo-mineral interactions and physical C protection within soil aggregates become more important for controlling decomposition rates in the later stages of decomposition (von Lützwow et al., 2006). Future sampling efforts of the solid phases of soil density separates (and, in particular, the spodic and Bt horizons) will shed more light on the effects of texture on the stabilization of the products of wood decomposition in soil.

## 5. Conclusions & future directions

A key finding is that initial fungal inoculum had an effect on wood-derived C losses. Decomposer community composition is an important factor in the early stages of decomposition both in terms of fluxes and quality of soluble decomposition products, and therefore needs to be incorporated into both C loss models and C storage models. While it is unlikely (although not impossible) that forests will ever be managed for fungal decomposer community, it is important to understand how functional groups of wood-decomposing fungi affect wood C losses, and their potential for driving different pathways of soil organic matter formation.

Another key finding of this study was the importance of wood location effects. The buried wood treatments had smaller amounts of wood-derived C losses than surface wood treatments. This indicates the importance of buried wood, such as lignified coarse roots and tree stems, for overall stabilization and storage of SOC. Coarse wood decomposition and its contribution to C losses and C stabilization need to be studied more extensively and added to overall C models. Also, forest management operations need to be cognizant of the importance of wood C, especially buried wood C, when making decisions about removing biomass from forest ecosystems.

Factors associated with global change also influenced C losses in this study. Wood grown in elevated CO<sub>2</sub> + O<sub>3</sub> resulted in both decreased wood-derived CO<sub>2</sub> efflux and percentage of wood-derived CO<sub>2</sub> efflux compared with wood grown in elevated CO<sub>2</sub> only. While we were not able to identify the specific intrinsic properties of the wood responsible for these changes in wood decomposability, these data suggest that elevated CO<sub>2</sub> + O<sub>3</sub> could create a substrate more conducive to soil C stabilization in the future.

Future work will track wood-derived C into different soil organic matter fractions. By using density separations, we can determine if and where the traceable wood C is being stabilized in forest soils. Future work using Illumina sequencing of rDNA barcodes for fungal species in the wood chips after a longer timeframe (2–5 years) will shed light as to exactly how decomposer communities change over time in response to our initial fungal inoculation treatments, and how community dynamics affect the amount of wood-derived C in more stable (dense fraction) soil C pools.

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## Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.soilbio.2017.08.028>.

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