DOE award number: DE-SC0006986

Name of Recipient: Michigan Technological University

Project Title: Interactive Effects of Climate Change and Decomposer Communities on the Stabilization of Wood-Derived Carbon Pools: Catalyst for a New Study

Name of Project Director/Principal Investigator: Sigrid Resh

Consortium/Teaming Members: Evan Kane; Dana Richter; Andrew Burton; Marty Jurgensen; Erik Lilleskov

November 17, 2014

Abstract (5000 characters)

Globally, forest soils store ~two-thirds as much carbon (C) as the atmosphere. Although wood makes up the majority of forest biomass, the importance of wood contributions to soil C pools is unknown. Even with recent advances in the mechanistic understanding of soil processes, integrative studies tracing C input pathways and biological fluxes within and from soils are lacking. Therefore, our research objectives were to assess the impact of different fungal decay pathways (i.e., white-rot versus brown-rot)—in interaction with wood quality, soil temperature, wood location (i.e., soil surface and buried in mineral soil), and soil texture—on the transformation of woody material into soil CO_2 efflux, dissolved organic carbon (DOC), and soil C pools. The use of ^{13}C-depleted woody biomass harvested from the Rhinelander, WI free-air carbon dioxide enrichment (Aspen-FACE) experiment affords the unique opportunity to distinguish the wood-derived C from other soil C fluxes and pools.

We established 168 treatment plots across six field sites (three sand and three loam textured soil). Treatment plots consisted of full-factorial design with the following treatments: 1. Wood chips from elevated CO_2, elevated $CO_2 + O_3$, or ambient atmosphere AspenFACE treatments; 2. Inoculated with white rot (*Bjerkandera adusta*) or brown rot (*Gloeophyllum sepiarium*) pure fungal cultures, or the original suite of endemic microbial community on the logs; and 3. Buried (15cm in soil as a proxy for coarse roots) or surface applied wood chips. We also created a warming treatment using open-topped, passive warming chambers on a subset of the above treatments. Control plots with no added wood ("no chip control") were incorporated into the research design.

Soils were sampled for initial $\delta^{13}C$ values, CN concentrations, and bulk density. A subset of plots were instrumented with lysimeters for sampling soil water and temperature data loggers for measuring soil temperatures. To determine the early pathways of decomposition, we measured soil surface CO_2 efflux, dissolved organic C (DOC), and $DO^{13}C$ approximately monthly over two growing seasons from a subsample of the research plots. To determine the portion of soil surface CO_2 efflux attributable to wood-derived C, we used Keeling plot techniques to estimate the associated $\delta^{13}C$ values of the soil CO_2 efflux. We measured the $\delta^{13}CO_2$ once during the peak of each growing season.

Initial values for soil $\delta^{13}C$ values and CN concentrations averaged across the six sites were -26.8‰ (standard error = 0.04), 2.46% (se = 0.11), and 0.15% (se = 0.01), respectively. The labeled wood chips from the Aspen FACE treatments had an average $\delta^{13}C$ value of -39.5‰ (se 0.10). The >12 ‰ isotopic difference between the soil and wood chip $\delta^{13}C$ values provides the basis for tracking the wood-derived C through the early stages of decomposition and subsequent storage in the soil.

Across our six research sites, average soil surface CO_2 efflux ranged from 1.04 to 2.00 g CO_2 m^{-2} h^{-1} for the first two growing seasons. No wood chip controls had an average soil surface CO_2 efflux of 0.67 g CO_2 m^{-2} h^{-1} or about half of that of the wood chip treatment plots. Wood-derived CO_2 efflux was higher for loam textured soils relative to sands (0.70 and 0.54 g CO_2 m^{-2} h^{-1}, respectively; $p = 0.045$)), for surface relative to buried wood chip treatments (0.92 and 0.39 g CO_2 m^{-2} h^{-1}, respectively; $p < 0.001$), for warmed relative to ambient temperature treatments (0.99 and 0.78 g CO_2 m^{-2} h^{-1}, respectively; 0.004), and for natural rot relative to brown and white rots (0.93, 0.82, and 0.78 g CO_2 m^{-2} h^{-1}, respectively; $p = 0.068$).

Our first two growing seasons of soil surface CO_2 efflux data show that wood chip location (i.e., surface vs. buried chip application) is very important, with surface chips loosing twice the wood-

derived CO_2. The DOC data support this trend for greater loss of ecosystem C from surface chips. This has strong implications for the importance of root and buried wood for ecosystem C retention. This strong chip location effect on wood-derived C loss was significantly modified by soil texture, soil temperature, decomposer communities, and wood quality as effected by potential future CO_2 and O_3 levels.

A. Content

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3. **Provide an executive summary, which includes a discussion of 1) how the research adds to the understanding of the area investigated; 2) the technical effectiveness and economic feasibility of the methods or techniques investigated or demonstrated; or 3) how the project is otherwise of benefit to the public. The discussion should be a minimum of one paragraph and written in terms understandable by an educated layman.**

Globally, forest soils store ~two-thirds as much carbon (C) as the atmosphere. Although wood makes up the majority of forest biomass, the importance of wood contributions to soil C pools is unknown. Even with recent advances in the mechanistic understanding of soil processes, integrative studies tracing C input pathways and biological fluxes within and from soils are lacking. Therefore, our research objectives were to assess the impact of different fungal decay pathways (i.e., white-rot versus brown-rot)—in interaction with wood quality, soil temperature, wood location (i.e., soil surface and buried in mineral soil), and soil texture—on the transformation of woody material into soil CO_2 efflux, dissolved organic carbon (DOC), and soil C pools. The use of ^{13}C-depleted woody biomass harvested from the Rhinelander, WI free-air carbon dioxide enrichment (Aspen-FACE) experiment affords the unique opportunity to distinguish the wood-derived C from other soil C fluxes and pools.

We established 168 treatment plots across six field sites (three sand and three loam textured soil). Treatment plots consisted of full-factorial design with the following treatments: 1. Wood chips from elevated CO_2, elevated CO_2 + O_3, or ambient atmosphere AspenFACE treatments; 2. Inoculated with white rot (*Bjerkandera adusta*) or brown rot (*Gloeophyllum sepiarium*) pure

fungal cultures, or the original suite of endemic microbial community on the logs; and 3. Buried (15cm in soil as a proxy for coarse roots) or surface applied wood chips. We also created a warming treatment using open-topped, passive warming chambers on a subset of the above treatments. Control plots with no added wood ("no chip control") were incorporated into the research design.

Soils were sampled for initial $\delta^{13}C$ values, CN concentrations, and bulk density. A subset of plots were instrumented with lysimeters for sampling soil water and temperature data loggers for measuring soil temperatures. To determine the early pathways of decomposition, we measured soil surface CO_2 efflux, dissolved organic C (DOC), and $DO^{13}C$ approximately monthly over two growing seasons from a subsample of the research plots. To determine the portion of soil surface CO_2 efflux attributable to wood-derived C, we used Keeling plot techniques to estimate the associated $\delta^{13}C$ values of the soil CO_2 efflux. We measured the $\delta^{13}CO_2$ once during the peak of each growing season.

Initial values for soil $\delta^{13}C$ values and CN concentrations averaged across the six sites were -26.8‰ (standard error = 0.04), 2.46% (se = 0.11), and 0.15% (se = 0.01), respectively. The labeled wood chips from the Aspen FACE treatments had an average $\delta^{13}C$ value of -39.5‰ (se 0.10). The >12 ‰ isotopic difference between the soil and wood chip $\delta^{13}C$ values provides the basis for tracking the wood-derived C through the early stages of decomposition and subsequent storage in the soil.

Across our six research sites, average soil surface CO_2 efflux ranged from 1.04 to 2.00 g CO_2 m^{-2} h^{-1} for the first two growing seasons. No wood chip controls had an average soil surface CO_2 efflux of 0.67 g CO_2 m^{-2} h^{-1} or about half of that of the wood chip treatment plots. Wood-derived CO_2 efflux was higher for loam textured soils relative to sands (0.70 and 0.54 g CO_2 m^{-2} h^{-1}, respectively; $p = 0.045$)), for surface relative to buried wood chip treatments (0.92 and 0.39 g CO_2 m^{-2} h^{-1}, respectively; $p < 0.001$), for warmed relative to ambient temperature treatments (0.99 and 0.78 g CO_2 m^{-2} h^{-1}, respectively; 0.004), and for natural rot relative to brown and white rots (0.93, 0.82, and 0.78 g CO_2 m^{-2} h^{-1}, respectively; $p = 0.068$).

Globally, woody debris is estimated to contain 73 petagrams (10^{15} g) of carbon, yet we still do not no how much of that makes it into the soil carbon pool. The long-term goal of this study is to address that question. In the shorter-term, this exploratory research has allowed the assessment of environmental and biological impacts on the fate of wood-derived C as it is transformed into CO_2 and dissolved organic carbon (DOC). Our first two growing seasons of soil surface CO_2 efflux data show that wood chip location (i.e., surface vs. buried chip application) is very important, with surface chips loosing twice the wood-derived CO_2. The DOC data support this trend for greater loss of ecosystem C from surface chips. This has strong implications for the importance of root and buried wood for ecosystem C retention. This strong chip location effect on wood-derived C loss was significantly modified by soil texture, soil temperature, decomposer communities, and wood quality as effected by potential future CO_2 and O_3 levels.

This exploratory research has laid the foundation for a longer-term study tracking the fate of wood-derived C into distinct soil C pools. This research will also provide a better understanding of the potential long-term impacts of wood residue removal (e.g., timber and biofuel extraction, salvage logging, or whole-tree harvest) on the formation and stability of soil C components.

4. Provide a comparison of the actual accomplishments with the goals and objectives of the project.

a. Project Goal: To catalyze long-term research examining the incorporation of wood-derived C into different SOC pools
Project Objectives: (1) To establish a wood decomposition study addressing the impact of different fungal decay pathways (i.e., white versus brown rot), soil texture, soil temperature, and initial contact with mineral fractions (i.e., buried versus surface placement of wood) on the transformation of this woody material; and (2) To track the early pathways of C decomposition.

Questions related to woody residue incorporation into SOC have been difficult to answer because woody material is biochemically difficult to differentiate from the SOC matrix, pedogenesis is inherently slow, and microbial communities are difficult to experimentally control in the field. We proposed to surmount these difficulties with a two-phase study consisting of short-term goals addressed in this exploratory research and long-term goals for future funding opportunities that will use the experimental design of this current research. Specifically, isotopically-labeled woody biomass harvested from the Rhinelander, WI free-air carbon dioxide enrichment (AspenFACE) experiment affords the unique opportunity to track the fate of wood-derived C as it is transformed into CO_2, dissolved organic carbon (DOC) from two distinctly different decomposition pathways. This research lays the foundation for a longer-term study tracking the fate of wood-derived C into distinct soil C pools.

b. Actual Project Accomplishments:
(1) Transported (from Wisconsin AspenFACE site to Michigan Tech) and chipped over 2700 kg of wood;
(2) Heat treated wood chips to 80C to sterilize chips with respect to fungal colonies
(3) Created white rot (*Bjerkandera adusta*) and brown rot (*Gleoephyllum sepiarium*) fungal cultures to inoculate wood chip treatments and stored samples of wood chips to reinoculate wood chips for a natural rot treatment consisting of the endemic suite of species on the wood when transported from the AspenFACE site;
(4) Inoculated and incubated wood chip treatments for 3 months to ensure colonization;
(5) Cleared six sites (1600 m^2 each) of all woody stems in the upper peninsula of Michigan;
(6) In the summer of 2012, established 168 treatment plots (1 m^2) across six field sites (three sand and three loam textured soil) with Aspen-FACE wood chips and the following treatments:
- Wood chips from elevated CO_2, elevated $CO_2 + O_3$, or ambient atmosphere Aspen-FACE treatments;
- Inoculated with white rot (*Bjerkandera adusta*), brown rot (*Gloeophyllum sepiarium*) fungal cultures, or the original suite of endemic microbial community on the logs;
- Buried (15cm in soil) or surface applied wood chips; and
- Ambient temperature or warming with open-topped, passive warming chambers (subset of plots)

(7) Cored soils to 30 cm in 0-15 cm and 15-30 cm segments and analyzed soils for initial stable carbon isotope values and CN concentrations;
(8) Archived freeze-dried initial soil samples for future soil microbial analysis to show changes in soil microbial communities;
(9) Included 2 mesh decomposition bags of chips to examine mass loss and microbial community changes in each plot for future retrievals;
(10) Instrumented subset of treatment plots with lysimeters, temperature data loggers, and open-topped, passive warming chambers;
(11) Initiated laboratory incubations to test the effect of wood quality and fungal inoculation on CO_2 flux rates and obtain pure isotopic signatures of wood and wood respired CO_2 through early decomposition;
(12) Measured soil $^{13}CO_2$ efflux, $DO^{13}C$ over two growing seasons; and
(13) Collected first round of wood chip decomposition bags in spring of 2014 (2 years after field deployment) for mass loss and fungal community analysis.

The actual accomplishments of this project have met the objectives of this exploratory grant and will allow continued research toward the long-term project goal of examining the incorporation of wood-derived C into different SOC pools

5. Summarize project activities for the entire period of funding, including original hypotheses, approaches used, problems encountered and departure from planned methodology, and an assessment of their impact on the project results. Include, if applicable, facts, figures, analyses, and assumptions used during the life of the project to support the conclusions.

Project activities summary, approaches used, problems encountered, departure from planned methodology, and an assessment of their impact on the project results

In the summer of 2012, we established 168 treatment plots (1 m^2) across six field sites (three sand and three loam textured soil; Figure 1) in the upper peninsula of Michigan with Aspen-FACE wood chips and the following treatments: (1) Wood chips from elevated CO_2, elevated CO_2 + O_3, or ambient atmosphere Aspen-FACE treatments; (2) Inoculated with white rot (*Bjerkandera adusta*), brown rot (*Gloeophyllum sepiarium*) fungal cultures, or the original suite of endemic microbial community on the logs; (3) Buried (15cm in soil) or surface applied wood chips; and (4) Ambient temperature or warming with open-topped, passive warming chambers (subset of plots). We applied approximately 12 kg (dry wt.) of wood chips per 1 m^2 plot.

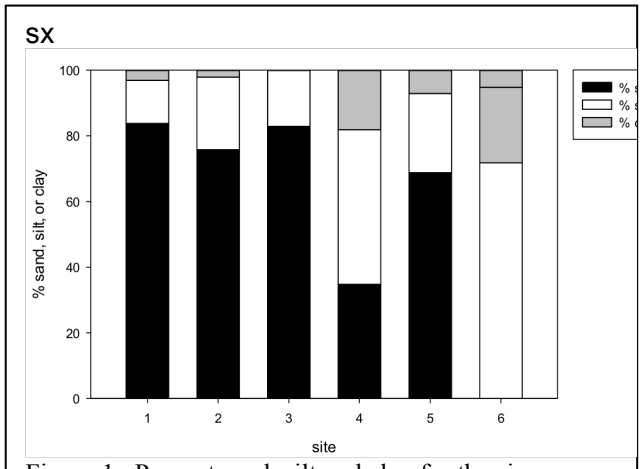

Figure 1. Percent sand, silt and clay for the six research sites. Sites 1-3 were classified as sands. Sites 4-6 were classified as loams.

We had originally proposed having a full-

factorial design with all treatments, which would have required 240 total experimental plots including no wood controls (40 plots per site x 6 sites). However, we reduced the plot total to 168 by decreasing the number of warmed plots (Table 1), while leaving the other treatments as a full-factorial design (Table 2). Reducing the number of warmed treatments allowed the project to be more manageable in terms of set-up and monitoring, decreased the amount of labeled Aspen-FACE wood needed, and decreased the cost of materials for open-topped chambers. Our warming experimental design still allowed a strategic testing for warming effects on soil CO_2 efflux for: fungal inoculations, wood chip location, wood quality, and soil texture treatments. All other treatment comparisons for long-term effects of wood on soil organic carbon and microbial communities are as originally proposed.

Table 1. Research design for warmed treatments. The number of treatment factors are provided for each warmed treatment by soil texture.

Warmed Treatments	Research Design by Soil Texture				Warmed Plot TOTAL
	Loam		Sand		
	Warmed	No wood control	Warmed	No wood control	
Inoculation	3 (white, brown, natural rot)	na	1 (natural rot)	na	
Wood location	2 (buried, surface)	2 (buried, surface)	2 (buried, surface)	na	
Wood quality	2 (ambient, $+CO_2$)	na	1 ($+CO_2$)	na	
Plot SUBTOTAL	12 plots	2 plots	2 plots	na	
Replication	3 sites	3 sites	3 sites	3 sites	
Plot TOTAL	**36 plots**	**6 plots**	**6 plots**	**na**	**48 plots**

Table 2. Research design for ambient temperature treatments. The number of treatment factors are provided for each ambient temperature treatment by soil texture.

Ambient Temperature Treatments	Research Design by Soil Texture				Ambient Temperature Plot TOTAL
	Loam		Sand		
	Experimental	No wood control	Experimental	No wood control	
Inoculation	3 (white, brown, natural rot)	na	3 (white, brown, natural rot)	na	
Wood location	2 (buried, surface)	2 (buried, surface)	2 (buried, surface)	2 (buried, surface)	
Wood quality	3 (ambient, $+CO_2$, $+CO_2+O_3$)	na	3 (ambient, $+CO_2$, $+CO_2+O_3$)	na	
Plot SUBTOTAL	18 plots	2 plots	18 plots	2 plots	
Replication	3 sites	3 sites	3 sites	3 sites	
Plot TOTAL	**54 plots**	**6 plots**	**54 plots**	**6 plots**	**120 plots**

As the field sites and plots were being prepared we took initial soil samples from around the border of every plot (Table 3). We cored soils to 30 cm in 0-15 cm and 15-30 cm segments using a punch corer (2.5 cm diameter). We pooled 3 cores per depth per plot. Soil samples were homogenized in resealable freezer bags, subsampled for microbial community analysis, and stored in a cooler until transport back to lab. Samples for microbial analyses were immediately

frozen with dry ice and were kept frozen until freeze-dried. In the lab, bulk soils were dried to 100C, sieved, ground, and analyzed for initial stable carbon isotope values and CN concentrations (Costech Elemental Combustion System 4010 connected to a Thermo Finnigan ConfloIII Interface and Deltaplus Continuous Flow-Stable Isotope Ratio Mass Spectrometer). We also archived freeze-dried initial soil samples for future soil microbial analysis to show changes in soil microbial communities.

The labeled wood chips from the Aspen FACE treatments had an average $\delta^{13}C$ value of -39.5‰ (se 0.10). The >12 ‰ isotopic difference between the soil (Table 3) and wood chip $\delta^{13}C$ values provides the basis for tracking the wood-derived C through the early stages of decomposition and subsequent storage in the soil.

Rock-free bulk densities were measured from 2 pits dug at each site using a double cylinder bulk density sampler with a volume of 70 cm^3 (Table 3). Bulk densities for each site represent the average of 3 bulk density cores per depth per pit (= 6 samples per depth per site). Bulk density depths were 5-10 cm and 20-25 cm, which are the central depths for the 0-15 and 15-30 soil sampling depths. Bulk density samples were dried at 100 °C, and sieved to remove large roots and rocks. The mass of rock-free soil and rocks were recorded, and the bulk density core volume was adjusted to a rock-free core volume using a predetermined equation (rock volume = 0.2367 + 0.3962 * rock mass (g)).

Table 3. Site averages for soil $\delta^{13}C$, bulk density, C and N values by soil depth. Standard errors are in parentheses.

site	soil depth	average $\delta^{13}C$ (se) (‰)	bulk density (se) (g/cm3)	average C (se) (g m^{-2})	average N (se) (g m^{-2})
1	0-15 cm	-27.2 (0.1)	1.18 (0.07)	2964.92 (168.77)	150.90 (7.97)
2		-26.6 (0.0)	1.16 (0.02)	4409.14 (218.94	286.50 (13.88)
3		-27.4 (0.1)	1.23 (0.04)	3351.14 (223.72)	150.23 (9.39)
4		-27.1 (0.1)	0.90 (0.07)	3155.44 (217.28)	176.13 (7.92)
5		-27.2 (0.0)	0.95 (0.08)	9729.96 (613.61)	603.12 (28.26)
6		-27.2 (0.0	0.93(0.05)	5285.15 (266.86)	338.79 (18.16)
1	15-30 cm	-25.9 (0.0)	1.07 (0.08)	1515.34 (67.88)	76.22 (2.86)
2		-25.7 (0.0)	1.14 (0.06)	2838.07 (138.39)	161.47 (7.98)
3		-26.6 (0.1)	1.06 (0.08)	1662.47 (119.72)	85.88 (5.11)
4		-25.7 (0.1)	1.32 (0.15)	1172.38 (72.22)	93.09 (4.82)
5		-27.3 (0.1)	1.08 (0.08)	5701.71 (508. 56)	369.43 (27.56)
6		-26.8 (0.0)	1.21 (0.12)	2535.62 (236.97)	173.95 (14.50)

Instead of using wood decomposition blocks as proposed, we deployed two mesh decomposition bags with chips in each plot to examine mass loss and microbial community changes. We collected the first round of mesh decomposition bags in spring of 2014 (2 years after field deployment) for mass loss and fungal community analysis. Bags are being kept frozen until freeze-drying and mass loss and microbial community analysis with future funding cycles. The advantage of the decomposition bags versus wood blocks is that the bags are the same material (wood chips) and same treatments (inoculation, wood quality, and wood location) as the treatment plots, so mass loss and microbial communities will be more applicable to our experimental design.

To investigate changes in soil water chemistry as a function of initial decomposer effects, we installed 40 lysimeters in 2 loam textured sites (Table 4). Shallow lysimeters (30 cm) were installed in the spring of 2013 (Soil Moisture corp.). Samples have been collected twice per season over 2 growing seasons and filtered (0.45 micron) prior to analysis for DOC, aromaticity (specific ultra-violet absorbance (at 254 nm)), and total phenolics (hydroxylated aromatic compounds relative to tannic acid standard; Hach corporation TanniVer reagent). Splits of samples have been freeze-dried for δ^{13}DOC analysis. Soil water data are still being processed; however preliminary data from 2013 shows some trends for DOC in chip location and inoculation (Figure 2). Surface chips produced more DOC relative to buried chips, and white and natural rot chips produced more DOC relative to brown rot chips. Tannin content and aromaticity followed the same trend shown for DOC, with surface values twice that of buried chips, and white and natural rot values more than two times that of brown rot.

Table 4. Plots instrumented with lysimeters for soil water collections.

Soil Water Sampling Treatments	Research Design by Soil Texture		Soil Water Sampling Plot TOTAL
	Loam soil texture		
	Experimental	No Wood Control	
Inoculation	3 (white, brown, natural rot)	na	
Wood Location	2 (surface, buried)	2 (surface, buried)	
Wood Quality	2 (ambient, +CO_2)	na	
Temperature	2 (ambient, warmed)	2 (ambient, warmed)	
Plot SUBTOTAL	16 plots	4 plots	
Replication	2 sites	2 sites	
Plot TOTAL	**32 plots**	**8 plots**	**40 plots**

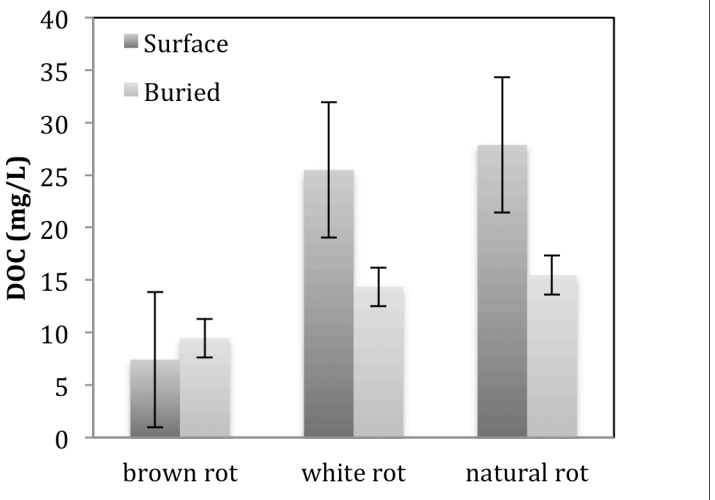

Figure 2. Averaged dissolved organic carbon (DOC) values by chip location and inoculation for 2013 growing season.

Temperature data loggers (iButton; Maxim Integrated) were placed in a subset of the research plots (Table 5). The temperature data loggers provide a temperature profile at 0, 7.5, and 15 cm

soil depths and allow the quantification of temperature differences between ambient and warmed treatments. The plots warmed with OTCs increased the average soil surface temperature by about 1 °C ($p = 0.094$; Figure 3A) and the average soil temperature at 15 cm by about 0.5 °C (p-value was not significant; Figure 3B).

Table 5. Plots instrumented with temperature data loggers.

Temperature Data Logger Treatments	Research Design by Soil Texture				Temperature Data Logger Plot TOTAL
	Loam		Sand		
	Experimental	No Wood Control	Experimental	No Wood Control	
Inoculation	1 (natural rot)	na	1 (natural rot)	na	
Wood Location	2 (surface, buried)	2 (surface, buried)	2 (surface, buried)	na	
Wood Quality	2 (ambient, $+CO_2$)	na	1 ($+CO_2$)	na	
Temperature	2 (ambient, warmed)	2 (ambient, warmed)	2 (ambient, warmed)	na	
Plot SUBTOTAL	8 Plots	4 Plots	4 Plots	na	
Replication	3 sites	3 sites	3 sites	na	
Plot TOTAL	**24 Plots**	**12 Plots**	**12 Plots**	**0 Plots**	**48 Plots**

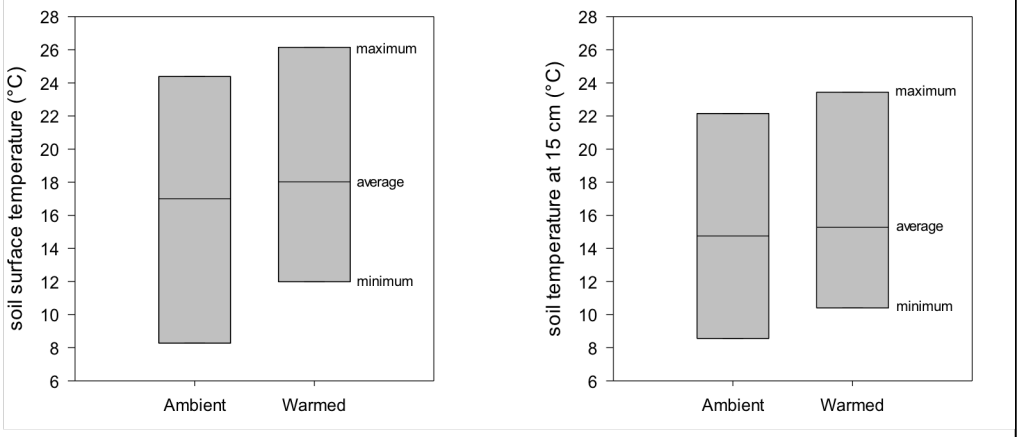

Figure 3. Minimum, average, and maximum soil surface (A) and 15 cm soil depth (B) temperatures for the ambient and warmed plots.

Laboratory incubations were initiated in March 2013 to test the effect of wood quality and fungal inoculation on CO_2 flux rates from the wood chips and obtain pure isotopic signatures of wood and wood respired CO_2 during early decomposition. The pure wood isotopic signatures are used in a two-endpoint mixing model to determine the wood-derived proportion of soil respired CO_2 and soil organic carbon (Table 6). We also conducted FTIR analyses on the wood, using supplementary funding, to assess the change in carbon compounds during the incubation.

Table 6. Pure wood $\delta^{13}CO_2$ values from pure wood chip laboratory incubations taken from an average of six sampling dates over a one-year incubation period.

Wood quality/inoculation treatment	Pure wood $\delta^{13}CO_2$ value
$+CO_2$/natural rot	-38.95
$+CO_2$/brown rot	-39.87
$+CO_2$/white rot	-41.09
$+CO_2 + O_3$/natural rot	-40.25
$+CO_2 + O_3$/brown rot	-42.20
$+CO_2 + O_3$/white rot	-41.95

We measured soil surface CO_2 efflux approximately monthly over two growing seasons from a subsample of the research plots (Table 7). Soil CO_2 efflux is being measured using a portable infrared gas analyzer (IRGA; PP Systems EGM-4 with SRC) from three PVC collars (~10 cm diameter) per plot. The collars are inserted ~ 2 cm into the plot surface and left year round. Plot soil CO_2 efflux values are the average of measured soil CO_2 efflux from the three collars.

We are using Keeling plot techniques to estimate the associated $\delta^{13}C$ values of the soil CO_2 efflux. Briefly, a connector with septa has been put in line with the IRGA. As the IRGA monitors the CO_2 concentration, a gas sample is drawn with a syringe and injected into a 6 mL, He flushed IRMS vial. Four samples are collected per plot at times 0, 5, 10, and 15 minutes. The $\delta^{13}C$ values of soil CO_2 efflux are measured using a GasBenchII (Thermo Fisher Scientific) coupled with the ThermoFinnigan Delta^{plus} isotope ratio mass spectrometer. A Keeling plot is created with the four samples with $1/[CO_2]$ plotted on the x-axis and the associated $\delta^{13}C$ values on the y-axis. The y-intercept of the linear fit equation is the $\delta^{13}C$ value of the soil CO_2 efflux corrected for atmospheric contamination from initial placement of the respiration chamber. The percent of the soil CO_2 efflux from FACE wood respiration (percent wood) is calculated using a two end member mixing model (e.g., Del Galdo et al. 2003):

$$\%_{wood} = \frac{\delta_{soil\ efflux} - \delta_{no\ wood\ control}}{\delta_{pure\ wood} - \delta_{no\ wood\ control}} \times 100, \qquad \text{(equ. 1)}$$

where $\%_{wood}$ is the percent of soil CO_2 efflux derived from wood, $\delta_{soil\ efflux}$ is the $\delta^{13}C$ of our measured soil surface CO_2 efflux for each plot, $\delta_{no\ wood\ control}$ is the $\delta^{13}C$ of the soil CO_2 efflux from no wood control plots, and the $\delta_{pure\ wood}$ is the $\delta^{13}C$ of the CO_2 efflux from pure FACE wood measured during laboratory incubations (Table 6). Given the expense of Keeling plots (i.e., four IRMS samples per plot), we only measured the $\delta^{13}CO_2$ during the peak of each growing season. To get the wood-derived portion of total soil surface CO_2 efflux (wood flux), we multiplied the soil CO_2 efflux by the $\%_{wood}$.

Across our six research sites, average soil surface CO_2 efflux ranged from 1.04 to 2.00 with a minimum of 0.06 and maximum of 9.21 g CO_2 m^{-2} h^{-1}. Total soil CO_2 efflux and wood flux differed significantly by soil texture (loam vs. sand; $p = 0.033$ and $p = 0.045$, respectively; Figure 4), wood chip location (surface vs. buried; $p < 0.001$ for both total and wood flux; Figure 5), temperature (ambient vs. warming; $p = 0.004$ for both total and wood flux; Figure 5); and wood quality (grown in ambient atmosphere, elevated CO_2, or elevated $CO_2 + O_3$; $p = 006$ and $p = 0.007$, respectively; Figure 6). In addition, wood flux differed by inoculation (brown, white, or natural rot; $p = 0.068$; Figure 7).

Table 7. Plots used for measuring soil CO_2 efflux and $\delta^{13}CO_2$.

Soil CO_2 efflux and $\delta^{13}CO_2$ Treatments	Research Design by Soil Texture				Soil CO_2 efflux and $\delta^{13}CO_2$ Plot TOTAL
	Loam		Sand		
	Experimental	No Wood Control	Experimental	No Wood Control	
Inoculation	3 (white, brown, natural rot)	na	3 (white, brown, natural rot)	na	
Wood Location	2 (surface, buried)	2 (surface, buried)	2 (surface, buried)	2 (surface, buried)	
Wood Quality	3 (ambient, $+CO_2$, $+CO_2+O_3$) *	na	1 ($+CO_2$)	na	
Temperature	2 (ambient, elevated) **	2 (ambient, elevated)	na	na	
Plot SUBTOTAL	22 plots	4 plots	6 plots	2 plots	
Replication	3 sites	3 sites	3 sites	3 sites	
Plot TOTAL	**66 plots**	**12 plots**	**18 plots**	**6 plots**	**102 plots**

*In fine texture soils, only natural rot inoculations were measured for plots with ambient wood quality
**In fine texture soils, only ambient temperatures were measured for plots with $+CO_2+O_3$ wood quality

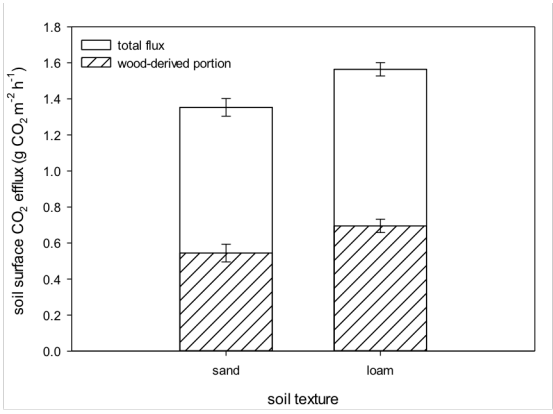

Figure 4. Average total soil surface CO_2 efflux and the wood-derived portion differed significantly by sand (3 sites) versus loam (3 sites) soil texture ($p = 0.033$ and $p = 0.045$, respectively).

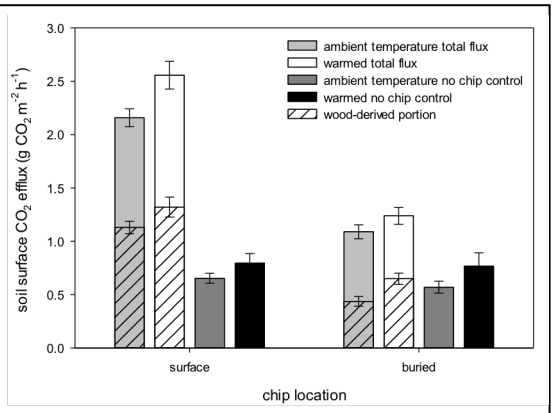

Figure 5. Average total soil surface CO_2 efflux and the wood-derived portion differed significantly by location ($p < 0.001$ for total and wood flux) and temperature ($p = 0.004$ for total and wood flux) for the treatment plots excluding no chip control plots. No chip control plots differed by temperature ($p = 0.025$) but not by location.

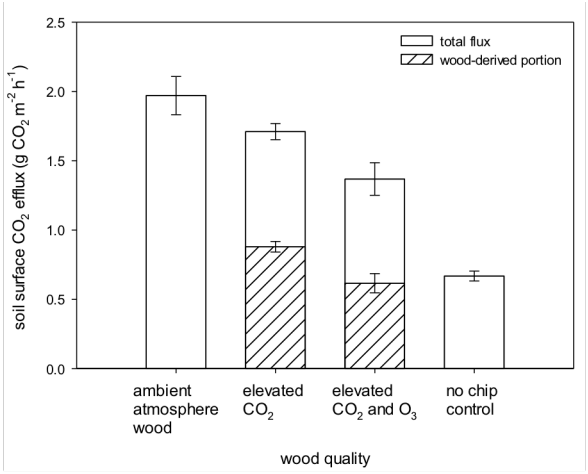

Figure 6. Average total soil surface CO_2 efflux and the wood-derived portion differed significantly by wood quality ($p = 0.006$ and $p = 0.007$, respectively), where differences in the $\delta^{13}C$ of the wood allowed the comparison.

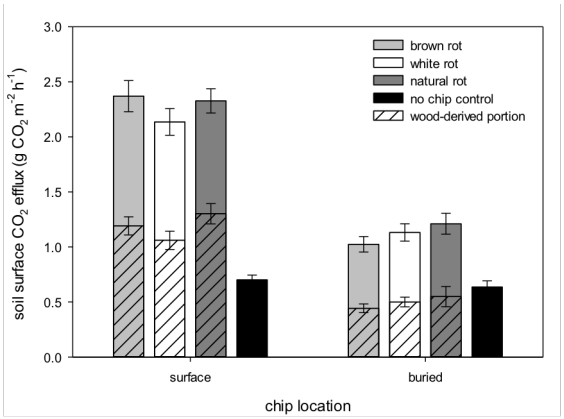

Figure 7. First two growing seasons (2103-2014) of soil CO_2 efflux showing significant differences between surface and buried chip treatments for total flux ($p < 0.001$) and the wood-derived portion ($p < 0.001$). Fungal inoculation differed for the wood-derived portion of soil CO_2 efflux ($p = 0.068$).

The percent wood (eqn. 1) is a rate independent assessment of how much wood is being decomposed. The percent wood differed significantly with soil texture ($p < 0.001$; loam > sand), location ($p < 0.001$; surface > buried), temperature ($p < 0.001$; warmed > ambient; Figure 8), the interaction of location x temperature ($p = 0.003$; Figure 9), wood quality ($p = 0.007$; elevated CO_2 > elevated $CO_2 + O_3$), inoculation ($p < 0.001$; natural rot > brown rot = white rot), and the interaction of inoculation x temperature ($p = 0.80$; Figure 10).

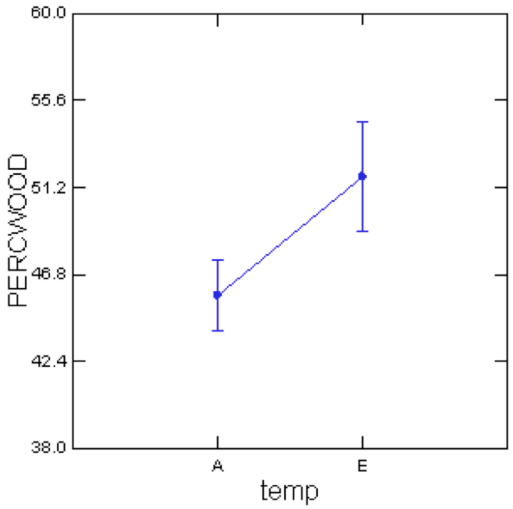

Figure 8. Percent of wood-derived CO2 was significantly higher for the elevated (E) temperature treatments compared with the ambient temperature (A) treatments ($p < 0.001$).

Original hypotheses

Though these hypotheses were written primarily for the later phases of this project where we plan to assess the degree of wood stabilization in SOC, our initial measurements of soil surface CO_2 efflux gives an indication of how much wood carbon is leaving the soil as CO_2. Therefore, by inference we have a measure of how much wood-derived C remains on site.

H1: Decomposition dominated by white rot fungi will lead to initially more rapid organic C inputs into the mineral soil when compared with brown rot fungi. In contrast, decomposition dominated by brown rot fungi will lead to slower early phase organic C

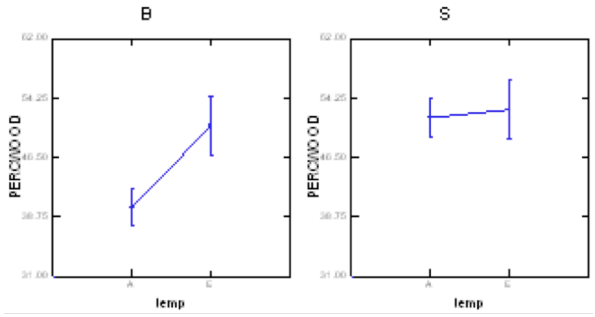

Figure 9. The response of the percent of wood-derived CO_2 to temperature differed depending on location of the wood. If the wood chips were buried (B), then there was a large response to elevated (E) temperature due to warming treatments compared with the ambient (A) temperatures. Surface (S) chips did not show a response to temperature

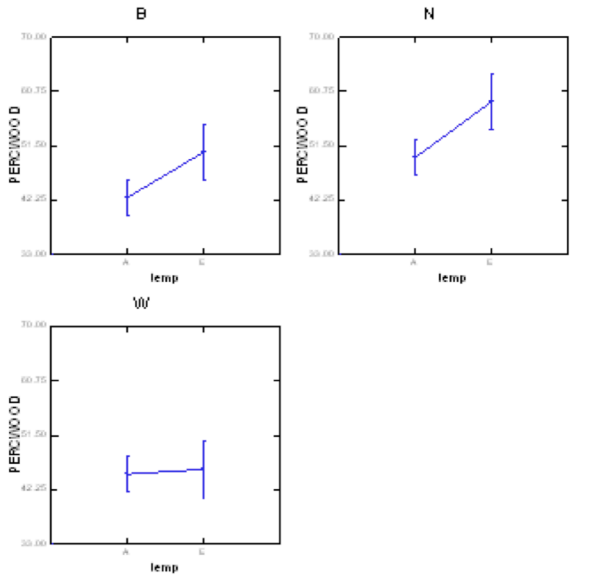

Figure 10. The response of the percent of wood-derived CO_2 to temperature differed depending on the inoculation treatment. Brown (B) and natural (N) rots showed an increase in percent of wood-derived CO_2 with elevated (E) temperature, whereas, white (W) did not show a temperature response

inputs into mineral soil, but greater residual recalcitrant carbon in the forest floor, and greater long-term input into the mineral soil.

Our results from the 2013 and 2014 growing seasons indicate that total flux does not differ between the inoculation treatments. However, the percent wood and the portion wood-derived soil surface CO_2 efflux is significantly different for fungal inoculation treatments ($p < 0.001$ and $p = 0.068$, respectively). Natural rot had a higher percent wood and higher wood efflux than brown or white rot (Figure 7). This could indicate that natural rot will result in less residual C in the mineral soil than that for white or brown rot, because more C is being lost as CO_2. Alternatively, it could indicate that there are more decomposition products being created with the faster decomposition by natural rot, which could result in greater stabilization of the residual C as those decomposition products interact with the mineral soil matrix. Given that natural rot is the only inoculation showing significant differences in percent wood and wood flux of CO_2, this hypothesis cannot be answered with the data collected from this exploratory grant.

H2: Increased temperatures will lead to surface drying which will decrease wood decomposition rates at the surface of the soil, but lead to increased decomposition in buried wood. This will accelerate flux of new C into SOC pools from buried wood more than surface wood.

Percent wood and wood CO_2 efflux are higher for the surface (40% and 0.92 g CO_2 m^{-2} h^{-1}, respectively) compared with the buried wood chip treatments (33% and 0.39 g CO_2 m^{-2} h^{-1}, respectively). These data indicate higher decomposition rates for surface chip applications, not supporting the first part of H2 (decreased decomposition for the surface wood). This is likely a response to warmer temperatures at the soil surface, as percent wood and wood CO_2 efflux was higher for the warmed compared with ambient temperature treatments (Figure 8). This warming effect on wood CO_2 efflux was driven by the effect of warming on the buried chip

treatments (Figure 9), supporting the second part of H2 (warming of buried chips causing increased decomposition). The third part of H2 (accelerated flux of new C into SOC pools) cannot yet be assessed by the data collected with this exploratory grant.

H3: Less wood-derived C will be stabilized in plots amended with wood grown in elevated CO2 + O3 relative to wood grown in ambient conditions, on a per C input basis.

Percent wood and wood CO_2 efflux were higher for the elevated CO_2 wood chips (48% and 0.88 g CO_2 m^{-2} h^{-1}, respectively) relative to the elevated CO_2 + O_3 chips (42% and 0.61 g CO_2 m^{-2} h^{-1}, respectively). This could indicate that the elevated CO_2 wood chips will result in less residual C in the mineral soil than that for elevated CO_2 + O_3, because more C is being lost as CO_2. Alternatively, it could indicate that there are more decomposition products being created with the faster decomposition of elevated CO_2 chips, which could result in greater stabilization of the residual C as those decomposition products interact with the mineral soil matrix. Therefore, H3 has not yet been answered by the data collected during this project.

B. Identify products developed under the award and technology transfer activities, such as:

a. Publications (list journal name, volume, issue), conference papers, or other public releases of results. If not provided previously, attach or send copies of any public releases to the DOE Program Manager identified in Block 15 of the Assistance Agreement Cover Page;

None yet

b. Web site or other Internet sites that reflect the results of this project;

None yet

c. Networks or collaborations fostered;

Collaborations fostered with USDA Forest Service researcher, Dr. Erik Lilleskov, to elaborate on fungal community changes following initial fungal inoculations.

d. Technologies/Techniques;

None

e. Inventions/Patent Applications, licensing agreements; and

None

f. Other products, such as data or databases, physical collections, audio or video, software or netware, models, educational aid or curricula, instruments or equipment.

None