BIOGEOCHEMICAL HOTSPOTS AROUND BARK-BEETLE KILLED TREES

Courtney M. Siegert, Heidi J. Renninger, A.A. Sasith Karunarathna, John J. Riggins, Natalie A. Clay, Juliet D. Tang, Nicole Hornslein, and Brent L. Chaney

Abstract—Disturbance-induced mortality events in forest ecosystems generate significant hotspots in biogeochemical cycles. These events occur sporadically across the landscape and contribute to large sources of error in terrestrial biosphere carbon models, which have yet to capture the full complexity of biotic and abiotic factors driving ecological processes in the terrestrial environment. The balance between production of stable soil organic matter and respiration from decomposing biomass greatly influences whether temperate forests remain modest carbon sinks or are transformed into carbon sources. In 2015, a field experiment to mimic pine beetle attack was established by girdling loblolly pine trees. Subsequent measurements of throughfall and stemflow for water quantity and quality, transpiration, stem respiration, soil respiration, and soil chemistry were used to quantify the extent of spatial and temporal impacts of tree mortality on carbon budgets. Enhanced fluxes from dying trees primed surrounding soils while decreased tree water use provided additional soil moisture to create biogeochemical hotspots, which could lead to accelerated carbon decomposition and mineralization rates.

INTRODUCTION

Disturbances in forest ecosystems can alter functions like productivity, respiration, and nutrient cycling. Common landscape-scale disturbances such as fire, drought, windstorms, and insect outbreaks increase coarse woody debris inputs and alter the hydrology and biogeochemical processes in forests (Harmon and others 1986, Mikkelson and others 2012). Disturbances vary in their spatio-temporal extent and can have significant impacts on terrestrial carbon (C) cycling, but they are commonly omitted from terrestrial C models.

A major disturbance agent in the Southeastern United States is the southern pine beetle (Dendroctonus frontalis). This native bark beetle infests many species of southern pine, but most commonly loblolly pine (Pinus taeda L.). Southern pine beetles are a particularly aggressive bark beetle species capable of quickly killing a vast number of trees. Outbreaks across the region are estimated to cost timber producers approximately $43 million annually, although some of these costs may be recovered through timely salvage logging operations (Pye and others 2011). Historically, these outbreaks occurred on 5–7 year cycles with a duration of 2–3 years, although recent observations indicate a decline in outbreak activity (Asaro and others 2017). Forest management and outbreak reduction and mitigation following bark beetle disturbance have received much attention, but ecosystem impacts like nutrient fluxes have received much less (Clarke and others 2016).

Both water quantity and quality are impacted by bark beetle-killed trees, although much of this research comes from the Western United States due to the extensive damage from mountain pine beetle (D. ponderosae) (Kurz and others 2008, Mikkelson and others 2012, Morehouse and others 2008). The cascade of changes observed by Brouillard and others (2016) include (1) the increase in streamflow due to a reduction in water uptake and transpiration coupled with decreased canopy interception and (2) the release of carbon from biomass decomposition which is returned to the atmosphere via heterotrophic respiration or leaching into surface waters, increasing dissolved organic carbon loads. Typically, this carbon is more aromatic and composed of complex and recalcitrant molecules. The negative charge of dissolved carbon enables the transport of nutrient-rich base
cations and heavy metals. This, coupled with the more aromatic and recalcitrant nature of these compounds, leads to increased costs of water treatment services downstream (Mikkelsen et al. 2012; Brouillard et al. 2016).

Water is the dominant pathway of nutrient removal in these ecosystems, and initial rainfall inputs likely contribute to watershed fluxes. Precipitation and nutrients move in distinct pathways through the forest canopy to the forest floor; this water is called throughfall and can impact hydrology and nutrient fluxes throughout a watershed. This pathway may or may not come into contact with tree surfaces, and if it does, residence time is usually minimal. Depending on canopy structure, throughfall can exhibit a large degree of spatial variability (Siegert et al. 2016). Stemflow is rainfall that has been captured by the forest canopy and funneled down woody surfaces to be deposited at the base of the tree. Stemflow has much longer residence times and therefore is an important pathway in nutrient cycling. Both of these pathways may become enriched with nutrients and other solutes via washoff of dry deposition from antecedent dry periods or canopy leaching. Precipitation characteristics including magnitude, duration, and intensity are also strong determinants of nutrient flux (Van Stan et al. 2016; Nanko et al. 2016; Siegert et al. 2017). Spots of southern pine beetle-killed trees likely impact these processes.

We hypothesized that bark beetle-killed trees undergoing mortality create biogeochemical hotspots in the vicinity of their trunk due to (1) increased soil moisture from reductions in plant water uptake and increased stemflow production, (2) enhanced canopy-derived inputs of carbon and nitrogen (N), and (3) increased microbial activity and root mortality.

**MATERIALS AND METHODS**

**Study Sites**

The study was conducted in a 60-year-old loblolly pine stand in central Mississippi (33.2639°N, 88.8884°W) (fig. 1A). The overstory basal area was 25.5 m² ha⁻¹ with 395 trees ha⁻¹. The midstory had a basal area of 29.4 m² ha⁻¹ with 1,664 trees ha⁻¹ dominated by sweetgum (*Liquidambar styraciflua* L.), red maple (*Acer rubrum* L.), winged elm (*Ulmus alata* Michx.), and oak species (*Quercus* spp.). The soil on this site is a somewhat poorly drained Urbo silt loam, with a depth to the water table of 30–35 cm and occasional flooding (Natural Resources Conservation Service 2015). Average temperatures in summer (June, July, and August) and winter (December, January, and February) are 26.5 °C and 6.9 °C, respectively (30-year mean: Arguez 2010). Total annual precipitation is 140.3 cm, which falls fairly evenly throughout the year with the lowest rainfall occurring in September (8.6 cm) (Arguez 2010).

**Study Design and Treatments**

To test our predictions, we established a field study in Summer 2015 and simulated a bark beetle infestation by girdling loblolly pine trees to sever phloem and cambium tissue (Davis et al. 2017). Fifteen canopy-dominant loblolly pine trees were selected around a centrally located data logger and randomly allocated to three different treatments: 1) five trees were girdled and received inoculations of bluestain fungus (*Ophiostoma minus*), 2) five trees were girdled and inoculated with agar as negative controls, and 3) five trees were not girdled and received no inoculations as controls (fig. 1B). Southern pine beetles are vectors of bluestain fungus, which is a non-decay fungus that colonizes the vascular system, and may accelerate death due to restrictions of plant water uptake, and preferentially attracts subterranean termites (Little et al. 2012; Clay et al. 2017).

**Measurements**

All trees were outfitted with stemflow collars constructed from 2.5 cm inner diameter polyethylene tubing cut longitudinally and sealed around the trunk of each tree above the girdling and inoculation site with aluminum nails and silicone caulk (fig. 1C). Collars drained into 20 L polyethylene bins. Stemflow volumetric flux was measured in the bins and homogenized samples were collected within 24 hours of rainfall events, filtered through a 0.45 μm membrane, and stored at 4 °C until chemical analysis. Dissolved organic carbon (DOC) was analyzed on a Hach DR5000 spectrometer, dissolved organic matter (DOM) absorbance characteristics were analyzed on a Lambda 850 spectrometer, and dissolved nitrogen species [total nitrogen (TN), organic nitrogen (ON), nitrate (NO₃⁻), and ammonium (NH₄⁺)] were analyzed on a Bran+Luebbe autoanalyzer. The spectral slope ratio (*S_R*) is a DOM absorption metric that describes the molecular weight of a sample and is calculated by:

\[
S_R = \frac{S_{275-295}}{S_{350-400}}
\]

where

\[S_{275-295} = \text{the slope of the absorption coefficient between wavelengths 275–295 nm}\]

\[S_{350-400} = \text{the slope of the absorption coefficient between wavelengths 350–400 nm}\] (Helms et al 2008).
The absorption coefficient ($a_{254}$) is another metric that describes the aromaticity of a compound and is calculated by:

$$a_{254} = \frac{2.303 \times A(\lambda)}{l}$$  \hspace{1cm} (2)

where

$A(\lambda)$ = the absorbance at 254 nm

$l$ = the cell path length of the instrument, set to 0.01 m

(Green and Blough 1994)

The specific UV absorbance (SUVA) also describes compound aromaticity and standardized for the concentration of DOC in the sample by:

$$SUVA_{254} = \frac{a_{254}}{[DOC]}$$  \hspace{1cm} (3)

where

$[DOC]$ = the concentration of dissolved organic carbon in the sample (Weishaar and others 2003).

Sample collection began in fall 2015 and continued through fall 2016 representing nine rainfall events large enough to generate stemflow. DOC and DOM analyses were conducted on a subset of sample dates on November 18, 2015, March 24, 2016, and June 6, 2016. On all 15 study trees, sapflow probes were installed to measure tree water use following the heat dissipation method (Granier 1987) (fig. 1C). Sapflow probes were connected to a CR1000 datalogger (Campbell Scientific Inc.) and powered by deep cycle batteries and a solar panel located in a nearby canopy gap. Measurements were recorded every 30 seconds and averaged over 30 minutes.

Soil elemental composition and respiration were measured around individual study trees. A circular grid with concentric rings of three 0.5-m intervals split into six sample quadrants was established around each tree (fig. 1C). Soil samples were taken at the initiation of the study in fall 2015 and again in fall 2016 with a 2.5-cm soil auger in the A and B horizons (5 cm and 10 cm, respectively). Two sample points were randomly selected in each of the three distance intervals radiating away from the tree boles. Different random points were used for each measurement date. These samples were dried at room temperature, ground to pass through a 0.1-mm sieve, oven dried at 105 °C for 24 hours, and then stored in air tight Whirl-pak bags. Carbon and nitrogen were determined on a Costech 4010 ECS CHNO elemental analyzer.

In each of the three concentric distance quadrants, a 20-cm inner diameter polychlorinated vinyl tube cut to a length of 10 cm was installed permanently into the soil profile (fig. 1C). Installation locations were randomly generated, but these locations remained fixed.
throughout the study to limit disturbance of the soil and root profile. Respiration was measured monthly with a LI8100A Soil Automated Flux Analyzer (Li-Cor Inc) outfitted with a 20-cm survey chamber along with volumetric water content and soil temperature. Respiration data were adjusted based on the average measured depth of each PVC collar. Monthly measurements began in spring 2016 and continued throughout winter 2016/17 for as long as respiration was still measurable.

Data Analysis and Statistics
A two-way factorial design analysis of variance (ANOVA) was used to identify differences in sapflow, stemflow volume, stemflow chemistry, and soil respiration as each responded to girdling treatments, sampling date, and the interacting effects between the two treatments. For soil chemistry, an n-way ANOVA was performed to determine the response of soil nutrient concentrations to interactions between tree treatments, distance from tree bole, and depth in soil profile for individual sampling dates, and additionally between sampling dates in 2015 and 2016. All statistical analyses were performed at α = 0.05 unless otherwise indicated in the text.

RESULTS AND DISCUSSION
By fall 2016, eight of the girdled trees were completely dead and had lost all their needles, and the remaining two girdled trees had only one cohort of needles remaining. All five control trees were healthy and had fully foliated canopies.

Mortality Impacts on Canopy Hydrology
Throughout the study, the sapflow data indicated no differences between trees inoculated with bluestain fungi versus those inoculated with agar as controls, leading us to assume that the bluestain inoculations were unsuccessful. As such, the results presented here separate the treatment trees into control (i.e., non-girdled) and girdled trees.

During the first month of data collection, girdled pines displayed significantly higher average daily sapflow than control pines (p <0.0001; fig. 2A). Sapflow rates were not statistically different between control and girdled pines throughout the following fall months. Control pines began to exhibit significantly higher sapflow rates in January of 2016 (p <0.001; fig. 2A), and continued to have at least 25 percent higher average daily sapflow for each month of the study with the exception of June of 2016, where sapflow did not significantly differ between the two treatments (p >0.050). Sapflow of girdled pines decreased at least twofold when comparing the same fall months in 2015 to 2016 (fig. 2A).

Cumulative stemflow volume standardized for basal area (L m⁻²) throughout the entire study was not significantly different between treatments (p-value = 0.937), although some differences were observed for individual storms (fig. 2B: November 9, 2015: p-value = 0.063 and December 2, 2015: p-value = 0.010). Control trees generated an average total stemflow of 560 L m⁻² in contrast to girdled trees which generated 415 L m⁻².

From June 2016 through December 2016 an extreme drought plagued the study region so no data points for canopy hydrology were obtained in this period.

Mortality Impacts on Canopy Biogeochemistry
Stemflow DOC concentrations were much greater than those observed in rainfall and throughfall (p = 0.013), but were not significantly different between treatments (p-value = 0.811) (fig. 3A). In the three storms analyzed for DOC, control trees produced an average total of 818.8 kg ha⁻¹ compared to girdled trees which produced an average total of 646.9 kg ha⁻¹ DOC over the three storms analyzed. Stemflow carbon had a higher molecular weight than rainfall or throughfall as indicated by the smaller S_R (fig. 3B) and was higher in aromaticity as indicated by the larger a254 (fig. 3C), but when standardized for carbon concentration was not different (fig. 3D). Furthermore, there were no differences in DOM absorbance metrics between treatments (S_R: p = 0.080; a254: p = 0.407; SUVA p = 0.607) although only three storm events have been analyzed thus far (fig. 4). Nitrogen concentrations in stemflow were significantly different by rainfall event (NO₃⁻: p <0.001; NH₄⁺, p <0.001; ON: p <0.001) (fig. 4). Cumulatively, the control trees lost more nitrogen in stemflow flux in all forms (NO₃⁻: 9.19 vs. 4.42 kg ha⁻¹; NH₄⁺: 46.34 vs. 29.40 kg ha⁻¹; ON: 88.53 vs. 56.84 kg ha⁻¹; TN: 144.06 vs. 90.47 kg ha⁻¹). The large degree of inter-storm variability in nitrogen flux led to insignificant differences between treatments, although girdled trees were beginning to show larger fluxes at the conclusion of the fall 2016 drought.

Mortality Impacts on Soil Properties
Initially, soils surrounding girdled trees had higher C:N ratios in fall 2015 compared to treatment trees (12.7 vs. 11.7), but in fall 2016 the trend had reversed (12.1 vs 12.8). In both fall 2015 and 2016, these differences were significant between treatments and between depths, while distance from tree bole was also significant in fall 2016 (table 1). In fall 2015, there were no differences in soil carbon concentrations between treatments, although carbon concentrations decreased with depth (table 1). In fall 2016, carbon concentrations in soils surrounding control trees averaged 0.72 mg C mg⁻¹ soil versus 0.66 mg C mg⁻¹ soil around girdled trees, with
Figure 2—(A) Average monthly sapflow from control and girdled study trees. Differences between treatments were observed beginning in December 2015 and remained significant for the remainder of the study. (B) Stemflow (SF) hydrologic flux from study trees for individual rainfall events. Means + standard errors are given in both graphs.

Figure 3—Boxplots of dissolved organic matter characteristics for (A) dissolved organic carbon concentrations; (B) spectral slope ratio; (C) specific UV absorbance; and (D) absorption coefficient for rain, throughfall (TF), and stemflow (SF) from study trees. Significant differences between sample sources are denoted by different letters.
Comparing the two years, there was a significant interaction effect between treatment and date (table 1), with carbon concentrations remaining stable around girdled trees (0.65 mg C mg$^{-1}$ soil) but increased around control trees (0.59 to 0.72 mg C mg$^{-1}$ soil). Similarly, no differences in nitrogen concentrations were observed in fall 2015 between treatments, only between depths, with less nitrogen deeper in the soil profile (table 1). In 2016, distance was also a significant factor along with depth (table 1). Comparing the two measurement years there were still no indications of differences in nitrogen concentrations between treatments (table 1) suggesting that the changes observed in the C:N ratios were driven by changes in soil carbon concentrations and not by nitrogen concentrations. Lastly, while soil respiration decreased from growing season to dormant season in 2016 ($p < 0.00$) and was marginally higher closer to stems than away ($p = 0.091$), there were no significant differences between treatments ($p = 0.154$) (fig. 5).

Figure 4 — Barplots of dissolved nitrogen characteristics for (A) nitrate-nitrogen; (B) ammonium-nitrogen; and (C) organic nitrogen for rain, throughfall (TF), and stemflow (SF) from study trees during individual rainfall events. Means + standard errors are given.

**CONCLUSIONS**

In this study, we documented differences in hydrology, canopy-derived biogeochemical fluxes and soil properties following a simulated bark beetle outbreak in a loblolly pine stand. Treatment differences in sapflow and tree water uptake were evident within the first 6 months of study initiation demonstrating the successful girdling efforts to mimic a bark beetle infestation and subsequent mortality. The decreases in stemflow quantity in girdled trees towards the end of the study was surprising and contradictory to our hypothesis, in which the removal of canopy needles would reduce canopy interception and promote stemflow generation (Siegert and Levia 2014). The tree crowns in this loblolly pine plantation were quite small, and removal of intercepting surfaces likely decreased the potential for those crowns to capture rainwater which would then be diverted to stemflow. Additional storms are required to tease out these complex interactions given the variable nature of stemflow generation to storm characteristics.
Table 1—Results of n-way factorial analysis of variance on soil biogeochemical properties including carbon concentrations (C), nitrogen concentrations (N), and the ratio of the two (C:N)

<table>
<thead>
<tr>
<th></th>
<th>Fall 2015</th>
<th>Fall 2016</th>
<th>Fall 2015 vs. fall 2016</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C:N C N C:N C N C:N C N</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment</td>
<td>0.035 0.106 0.763 0.015 0.041 0.205</td>
<td>0.880 0.790 0.768</td>
<td></td>
</tr>
<tr>
<td>Distance</td>
<td>0.121 0.095 0.363 0.006 0.004 0.011</td>
<td>0.057 0.035 0.075</td>
<td></td>
</tr>
<tr>
<td>Depth</td>
<td>&lt;0.001 &lt;0.001 &lt;0.001 &lt;0.001 &lt;0.001</td>
<td>&lt;0.001 &lt;0.001 &lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Date</td>
<td>- - - - - -</td>
<td>- - - - - -</td>
<td></td>
</tr>
<tr>
<td>Treatment x Distance</td>
<td>0.284 0.421 0.137 0.951 0.991 0.913</td>
<td>0.661 0.546 0.171</td>
<td></td>
</tr>
<tr>
<td>Treatment x Depth</td>
<td>0.554 0.439 0.588 0.415 0.331 0.272</td>
<td>0.376 0.230 0.230</td>
<td></td>
</tr>
<tr>
<td>Treatment x Date</td>
<td>- - - - - -</td>
<td>- - - - - -</td>
<td>&lt;0.001 0.007 0.218</td>
</tr>
<tr>
<td>Distance x Depth</td>
<td>0.945 0.559 0.285 0.374 0.421 0.578</td>
<td>0.655 0.771 0.919</td>
<td></td>
</tr>
<tr>
<td>Distance x Date</td>
<td>- - - - - -</td>
<td>- - - - - -</td>
<td>0.023 0.020 0.079</td>
</tr>
<tr>
<td>Depth x Date</td>
<td>- - - - - -</td>
<td>- - - - - -</td>
<td>0.326 0.693 0.996</td>
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<tr>
<td>Treatment x Distance x Depth</td>
<td>0.487 0.242 0.241 0.574 0.586 0.841</td>
<td>0.477 0.436 0.615</td>
<td></td>
</tr>
<tr>
<td>Treatment x Date x Date</td>
<td>- - - - - -</td>
<td>- - - - - -</td>
<td>0.875 0.959 0.704</td>
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<tr>
<td>Treatment x Distance x Date</td>
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<td>- - - - - -</td>
<td>0.253 0.654 0.602</td>
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<tr>
<td>Depth x Date</td>
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<td>- - - - - -</td>
<td>0.516 0.181 0.158</td>
</tr>
<tr>
<td>Treatment x Depth x Date</td>
<td>- - - - - -</td>
<td>- - - - - -</td>
<td>0.590 0.556 0.728</td>
</tr>
</tbody>
</table>

Treatment: control vs. girdled.
Distance from tree bole: 0.5, 1.0, and 1.5 m.
Depth in soil horizon: A horizon: 0-5 cm; B horizon: 5-10 cm.
Date of sample: fall 2015 and fall 2016.
Bolded values are statistically significant at α = 0.05.

(Keim and others 2006, Staelens and others 2008, Van Stan and others 2011). Although mortality was clearly underway in girdled trees, no differences were observed in DOC, DOM quality, or N concentrations in stemflow as hypothesized (Bade and others 2015, Frost and Levia 2014). The inopportune drought that occurred when girdled trees were finally succumbing to mortality represents a significant missed window into canopy biogeochemical processes. Without rainfall during this period, it was impossible to capture these changes, but recovery post-drought will likely provide further insights. In the soils, C:N ratios were greater under control trees than under girdled trees, which was driven by an increase in carbon around control trees and not a decrease around girdled trees. Here, it is likely that mortality of competitors (i.e., girdled trees) opened up additional growing space and resources which control trees were able to capture and increase belowground biomass growth and subsequent fine root turnover, contributing to increased soil carbon (Rasse and others 2005). While this process was not supported by the soil respiration data, it is possible that heterotrophic respiration from decomposition of belowground biomass on girdled trees was balanced by increased autotrophic respiration from increased belowground biomass production on control trees. In summary, the hydrologic and biogeochemical response of trees undergoing mortality from bark beetle infestations is complex and in real-world circumstances can be complicated further by climatic variability such as drought. In 18 months,
we documented changes in canopy-derived hydrologic and biogeochemical fluxes, tree water use, and soil biogeochemical processes although no clear trends were yet apparent. As tree mortality and decomposition of above- and belowground biomass continue, these processes will likely become clearer.

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LITERATURE CITED


