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Tree Stress and Mortality from Emerald Ash Borer Does Not Systematically Alter Short-Term Soil Carbon Flux in a Mixed Northeastern U.S. Forest

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Abstract: Invasive insect pests are a common disturbance in temperate forests, but their effects on belowground processes in these ecosystems are poorly understood. This study examined how aboveground disturbance might impact short-term soil carbon flux in a forest impacted by emerald ash borer (*Agrilus planipennis* Fairmaire) in central New Hampshire, USA. We anticipated changes to soil moisture and temperature resulting from tree mortality caused by emerald ash borer, with subsequent effects on rates of soil respiration and methane oxidation. We measured carbon dioxide emissions and methane uptake beneath trees before, during, and after infestation by emerald ash borer. In our study, emerald ash borer damage to nearby trees did not alter soil microclimate nor soil carbon fluxes. While surprising, the lack of change in soil microclimate conditions may have been a result of the sandy, well-drained soil in our study area and the diffuse spatial distribution of canopy ash trees and subsequent canopy light gaps after tree mortality. Overall, our results indicate that short-term changes in soil carbon flux following insect disturbances may be minimal, particularly in forests with well-drained soils and a mixed-species canopy.

Keywords: emerald ash borer; forest disturbance; *Fraxinus*; soil respiration; methane oxidation

1. Introduction

Invasive forest insects can create biotic disturbances that critically alter ecosystem processes and ecosystem services [1–3]. However, the impacts of invasive insects on the ecosystem carbon cycle remain uncertain, particularly for indirect impacts such as changes to soil carbon cycling [4,5]. Many invasive insects cause tree stress and mortality through wood-boring, phloem-feeding, or defoliation, which potentially changes plant carbon and nutrient allocation strategies and subsequent plant-soil feedbacks [6,7]. This study examined the short-term, two-year impact of the invasive emerald ash borer (EAB; *Agrilus planipennis* Fairmaire) on soil carbon dioxide (CO₂) and methane (CH₄) fluxes shortly following the first detection of EAB in a mixed deciduous forest in New Hampshire, USA.

EAB is an invasive wood-boring insect that kills trees of the genus *Fraxinus* by effectively girdling stem xylem tissue with larval feeding tunnels [8]. EAB was first identified in the state of New Hampshire, USA, in 2013. Mortality in *Fraxinus* trees infested with EAB is rapid and trees generally die 1–2 years after EAB activity is detected [9–11]. Initial stages of infestation are characterized by a visible thinning of the canopy [12]. Currently there are several management strategies under consideration, including preemptive and salvage logging, insecticide, and biological control; however, none of these have been particularly effective in slowing the spread of EAB [13]. Although losses to aboveground biomass due to EAB-induced mortality have been documented in

previous studies [14], impacts to the soil carbon processes beneath *Fraxinus* are less well understood. Given that forest soil carbon stocks are approximately twice the size of aboveground carbon stocks, it is critical that we understand how invasive pests impact this globally important carbon pool [15].

Previous studies have examined the complex feedbacks between biotic disturbances and soil carbon flux in other systems, which might provide a useful analog to EAB impacts for short-term changes in soil respiration. Defoliation studies in grasslands have found that moderate clipping or defoliation can increase [16], decrease [17,18], or have no significant effect [19,20] on soil CO₂ flux within a year of vegetation removal. In forest ecosystems, the effects of biotic disturbances are similarly variable, with evidence for higher [21,22], lower [23,24], or no net change [25] in soil CO₂ flux following disturbance. Higher soil respiration following defoliation may result from stress-induced root carbon exudation [26], higher soil temperature caused by canopy gaps, and higher soil moisture caused by reduced transpiration under defoliated trees [27]. However, insect disturbances that are severe enough to cause host mortality or significant loss of active tissues may also dampen soil carbon emissions by reducing root respiration [28]. All together, these factors suggest a complex set of mechanisms that could enhance, reduce, or produce no net change in soil CO₂ efflux. For many insect disturbances, these mechanisms are likely to change with time and infestation severity [29].

In addition to the effects of insect disturbance on soil CO₂ flux, these disturbances may alter rates of CH₄ oxidation in forest soils. Globally, methanotrophic bacteria in upland forest soils oxidize approximately 28 teragrams of CH₄ each year to CO₂ [30]. This CH₄ uptake reduces the radiative forcing of greenhouse gases in the atmosphere, as CH₄ has a stronger radiative forcing capacity than CO₂, and is an important component of the global CH₄ budget [31,32]. Rates of CH₄ uptake in soil may depend on several chemical and biological factors, including soil temperature, pH, texture, moisture, nutrient content, and the dynamics of the soil microbial community [33–37]. Previous studies that manipulated precipitation in forests have found lower CH₄ oxidation rates as soil moisture increased, likely due to a corresponding decrease in diffusivity [38,39]. However, to our knowledge, no previous studies have assessed potential changes in CH₄ oxidation in forest soils beneath trees experiencing biotic disturbance. Biotic disturbances may have important indirect effects on this component of the forest carbon cycle, particularly through changes in soil microclimate that follow tree mortality and tissue loss.

The two primary goals of this study were: (1) to identify patterns in soil CO₂ and CH₄ fluxes that related to EAB impact; and (2) to suggest mechanisms for changes in these fluxes under EAB infestation. Because different environmental parameters control soil CO₂ efflux and CH₄ uptake, we formulated independent sets of alternative hypotheses for each process. Our null expectation was that EAB disturbance does not produce a net change in CO₂ nor CH₄ flux, either because stress and mortality produce no effect on soil CO₂ or CH₄ flux or because multiple concurrent changes to the soil environment counteract each other such that no net effect is produced. In this study, we assessed two alternate hypotheses for the potential effects of EAB impact on either soil CO₂ flux or CH₄ uptake (Figure 1):

- CO₂ flux, H₁: Soils beneath EAB-impacted *Fraxinus americana* L. (*F. americana*) will have lower rates of CO₂ flux due to lower active root metabolism and exudation of labile carbon substrates compared to soil beneath visibly healthy trees.
- CO₂ flux, H₂: Soils beneath EAB-impacted *F. americana* will have higher rates of CO₂ flux due to an increase in dead root biomass available for decomposition, as well as an increase in soil temperature and moisture, as infestation results in large canopy gaps and reduced evapotranspiration.
- CH₄ uptake, H₁: Soils beneath EAB-impacted *F. americana* will have lower rates of CH₄ uptake due to an increase in soil moisture from decreased evapotranspiration.
- CH₄ uptake, H₂: Soils beneath EAB-impacted *F. americana* will have higher rates of CH₄ uptake due to an increase in soil temperature, as canopy gaps allow more sunlight to reach the forest floor.

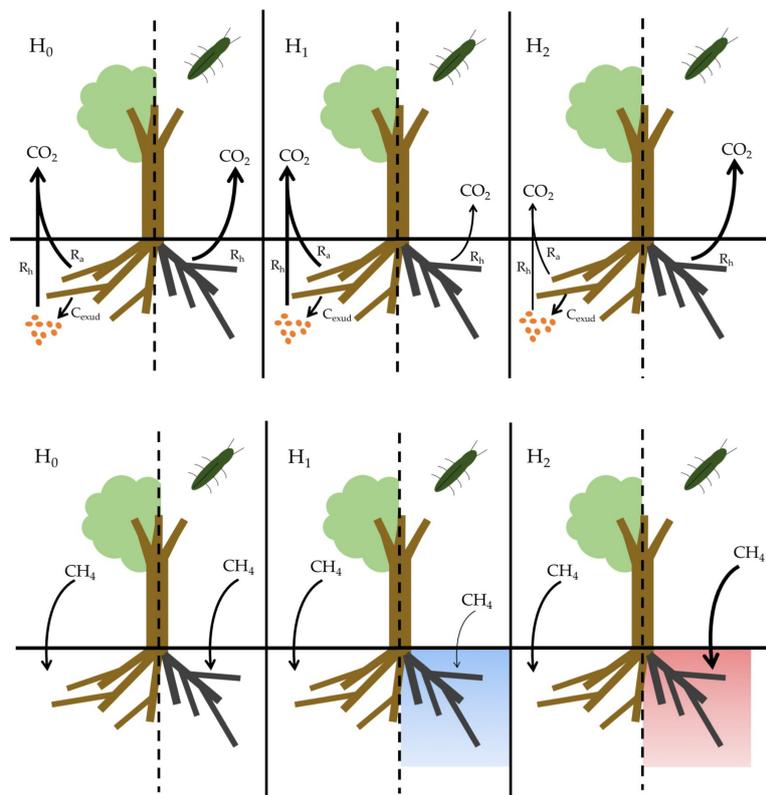


Figure 1. Null hypothesis (H₀) and two alternate hypotheses (H₁ and H₂) for the effect of tree stress induced by the emerald ash borer (EAB) on soil CO₂ flux and CH₄ uptake. H₀ poses that there is no net change on soil flux, including autotrophic (R_a) and heterotrophic (R_h) respiration, since processes that differ beneath live and dead *Fraxinus americana* L. might balance each other. Other alternate hypotheses suggest that either live or dead *F. americana* might have higher soil flux due to biological and physical changes to the soil beneath impacted trees.

2. Materials and Methods

2.1. Study Site

Our study site was located in Canterbury, NH, USA, in a mixed forest comprised of approximately 30% *Fraxinus americana* L., and also *Acer saccharum* Marsh., *Tsuga canadensis* L., and *Acer rubrum* L. The 1981–2010 climatological mean annual temperature at the nearest long-term weather station was 7.96 ± 0.58 C (mean \pm standard deviation) and the annual precipitation was 1020 ± 178 mm (Concord, NH weather station code GHCND:USW00014745, 21 km from the study site [40]). For climate variables during our study period, we used total monthly precipitation data and mean monthly temperature data from the same station as the 30-year climatology.

2.2. Study Design

At our study site, we classified *F. americana* trees into three categories based on visual canopy cover estimation: healthy, impacted, or dead. Trees were considered healthy if their canopy was fully leafed-out in midsummer, with no bare branches or visible signs of stress (i.e., epicormic branching). Trees were classified as impacted if their canopy showed evidence of thinning (Figure 2b) or epicormic branching. Trees designated as dead were examined for signs of EAB infestation (D-shaped exit holes; Figure 2a) and only trees killed by EAB were included in the study. On 28 April 2016, sixteen *F. americana* trees were selected across a range of classes impacted by EAB: three healthy, six impacted, and seven recently dead, and we measured tree diameter at 1.3 m from ground level (DBH, cm).

Trees were selected for our study within two separate tracts of about 100 m² area less than 1 km from each other, where *F. americana* were spaced about 5–20 m with other species typically occurring between *F. americana* individuals (Figure 2d,e). Eight additional *F. americana* trees, five healthy and three impacted, were added to the study on 8 August 2016 to achieve higher replication ($n = 24$ from this date onward), and we measured DBH for these additional replicates. On 16 June 2017, the impact classes of individual trees were re-evaluated.



Figure 2. Evidence of emerald ash borer (EAB) impact and relative locations of study trees: (a) D-shaped exit hole in trunk from an emerging adult beetle; (b) thinning canopy of an impacted tree; (c) bare canopy and conspicuous light gap surrounding EAB-killed trees; (d) study trees from Plot 1; and (e) Plot 2 on a 11 September 2017 true-color satellite image, where red indicates trees that were dead from the start of the study, orange indicates trees that died during the study, and yellow indicates trees that were stressed by the end of the study.

On the same dates listed above when trees were selected (either 16 June or 8 August 2016), we installed 10.16 cm diameter polyvinyl chloride (PVC) soil flux collars that remained in place for the duration of the study, following standard soil flux measurement protocol [41]. Respiration collars were installed 1.5 m from the base of the tree in a randomly chosen direction, to a depth of approximately 7 cm to capture aggregate changes in both microbial and root respiration. We waited one month to measure CO₂ and CH₄ fluxes at the soil collars to allow for belowground acclimation to the installation disturbance. We measured the height of each collar at the four polar coordinates and took the mean of those four measurements as the collar height in cm. To validate soil flux models at different spots beneath the same trees, we installed a second collar at half the trees within the study ($n = 12$). The second collars were installed on four healthy, four impacted, and four dead trees, as evaluated in 2016.

We mapped the location of all study trees with a GPS unit (Garmin, Olathe, KS, USA). We plotted our study tree coordinates on a ~2 m resolution WorldView satellite image collected on 11 September 2017 in Google Earth (Google Earth Pro, v. 7.3.0.3832, Mountain View, CA, USA) to

visualize the mosaic composition of the forest at our study site (Figure 2). By visually locating the approximate canopy extent of trees that were either dead from the start of the study or died during the study, we used the area calculator in Google Earth on the 11 September 2017 satellite image to roughly estimate the canopy gap size created by individual ash mortality.

2.3. Chamber Measurements and Flux Calculations

Soil CO₂ and CH₄ fluxes were measured at each collar during twelve sampling events: once per month from June to November 2016 (six sampling dates) and May to October 2017 (six sampling dates). Measurements were collected across all collars on each sampling date between approximately 09:00–12:00. Previous studies have demonstrated that instantaneous soil carbon flux in the late-morning is a good approximation of the daily mean soil CO₂ flux at a northeastern U.S. temperate forest site [42], thus avoiding potential biases in the effect of measurement time on soil flux [43]. During each chamber measurement, soil temperature was measured with a digital thermometer inserted 10 cm below the soil surface adjacent to the chamber. Integrated soil moisture was measured with a time domain reflectometer. We used a Decagon G3 Soil Moisture and Conductivity Sensor (Decagon Devices, Pullman, WA, USA, ±0.03 m³ m⁻³ accuracy) to measure soil moisture across the top 10 cm of soil on 2016 sampling dates and on 19 May 2017, and integrated soil moisture and conductivity across the top 12 cm of soil was measured with an H2 HydroSense II (Campbell Scientific, Logan, UT, USA, ±0.03 m³ m⁻³ accuracy) on the remaining five 2017 sampling dates.

During each soil CO₂ and CH₄ flux sampling event, we connected a portable greenhouse gas analyzer to the PVC respiration collar for a period of two minutes. On 2016 sampling dates, a Los Gatos Research (LGR) Ultraportable Greenhouse Gas Analyzer (Los Gatos Research, Los Gatos, CA, USA) cavity ring-down spectrometer was used to measure the CO₂, CH₄, and H₂O concentration inside the PVC chamber at 0.2 Hz temporal resolution (0.3 ppm CO₂ precision and 2 ppb CH₄ precision). On 2017 sampling dates, a Picarro GasScouter G4301 Gas Concentration Analyzer (Picarro Inc., Santa Clara, CA, USA) cavity ring-down spectrometer was used to measure the CO₂, CH₄, and H₂O concentration inside the PVC chamber at 1 Hz temporal resolution (0.4 ppm CO₂ precision and 3 ppb CH₄ precision). We used values of dry CO₂ and CH₄ concentration data (in ppm) that were corrected by the internal software for each greenhouse gas analyzer for CO₂ and CH₄ flux calculations.

We calculated soil CO₂ and CH₄ flux by fitting a linear regression model to the concentration data collected in the center 70 s of the two-minute measurement period to reduce potential errors associated with securing and removing the chamber top, which is a reasonable measurement interval for high-resolution soil flux chamber measurements [44]. We estimated the flux as the slope of a linear fit between concentration and time during each chamber measurement and converted units from ppm (provided by the analyzer) to the mass flux in μmol m⁻² s⁻¹ using the measured chamber height and surface area, and temperature and pressure at the time of measurement.

2.4. Soil Characteristics

After the last sampling event at the study site, we harvested the soil within each replicate collar to 7 cm depth to measure root biomass, soil texture, and soil pH. We sieved soils to 1 mm, separating roots from particulates, dried roots from each soil collar at 45 C for 72 h, and weighed dried roots within each replicate. We measured soil pH with an electrode in a 2:1 deionized water-to-soil suspension (Thermo Scientific, Waltham, MA, USA). We measured soil texture for a well-mixed 2 mL subsample of the remaining mineral soil fraction (<1 mm) within each soil collar. To measure soil texture, we dispersed each 2 mL subsample in 10 mL sodium hexametaphosphate and then placed each subsample on a shaking table for 24 h prior to measurement on a laser diffraction particle size analyzer (LS 13-320, Beckman Coulter, Brea, CA, USA). Output from the particle size analyzer was classified into volume fraction of clay, silt, and sand for each sample according to United States Department of Agriculture (USDA) particle size classes [45]. These soil variables—root biomass, pH, and fraction sand content—were used as covariates in the mixed models.

2.5. Statistical Methods

We nested all of our statistical analyses within each tree in the study ($n = 24$). For trees that had two replicate collars, we randomly selected one collar to be in the model fit dataset (training data) and the second replicate collar to be in a model validation dataset. To test our hypotheses that the level of EAB impact altered soil moisture and temperature (Figure 1), we created two mixed effects models with either soil moisture or temperature data as the response variable. For the soil moisture model, we fit two linear fixed effects for total monthly rainfall and the fraction of soil sand content and a fixed effect for EAB impact status (healthy, impacted, or dead). Additionally, we fit random effects for each replicate tree to reflect the non-independence of the repeated measurements and a second set of random effects for sampling month. For the temperature model, we fit a fixed effect for EAB impact status, and random effects for replicate tree and for month sampled.

To test our hypotheses that EAB presence influenced soil CO₂ and CH₄ flux, we constructed two additional sets of Bayesian linear mixed models with either soil CO₂ or CH₄ flux as the response variable. In both models, we fit separate random effects for measurement month and replicate (i.e., each tree) to reflect our repeated measures design. Our fixed effects for the CO₂ flux model were: (1) soil temperature and (2) soil moisture at the time of flux measurement; (3) monthly total rainfall; (4) DBH of the nearest *F. americana* tree; (5) root biomass of a 7-cm-deep core collected within the chamber at the conclusion of the experiment; (6) fraction of sand content; and (7) soil pH, with each measured from the same core.

Our fixed effects for the CH₄ uptake model were: (1) soil temperature and (2) soil moisture at the time of flux measurement; (3) monthly total rainfall; (4) fraction of soil sand content measured from the same core; and (5) soil pH. The CO₂ flux and CH₄ uptake models are each represented as Equation (1):

$$y_i = M_{t,i} + T_{j,i} + \beta_k X_k + \varepsilon_i \quad (1)$$

where y_i is each CO₂ flux or CH₄ flux measurement and $i = 380$ chamber flux measurements, M_t is a random month effect fit across all measurements within $t = 7$ study months, T_j is a random tree replicate effect fit across all measurements within $j = 24$ study trees, β_k is a set of linear regression coefficients for the X_k fixed effects variables, and ε_i is the residual variance. In our CO₂ flux model, $k = 7$ and $k = 5$ in the CH₄ uptake model, where X_k in each case represents the fixed effects described above. The random effects M_t and T_j were each modeled as normally distributed with means of zero and respective variances σ_M and σ_T , with uninformative priors on the variance ($\sigma_M = \sigma_T = 10^6$). We included a monthly random effect in addition to the fixed effects of soil temperature and moisture to account for potential seasonal changes in tree physiology (e.g., root carbon exudation, root elongation), which may have affected the soil carbon flux measurements.

We modeled CO₂ flux as $y_i \sim \text{lognormal}(\mu_i, \sigma_e)$, where y_i is each flux measurement, μ_i is the process mean fit as the sum of the modeled fixed and random effects, and σ_e is the measurement and process variance. Since all measured values of CH₄ flux in this study were negative, we took the absolute value of CH₄ flux (to conceptually represent the CH₄ uptake flux) and modeled the absolute value of CH₄ flux as $y_i \sim N(\mu_i, \sigma_e)$, where σ_e represents the measurement variance and i is each observation. Fitting the model with the absolute value of the CH₄ flux allowed for more congruent comparison between changes in CO₂ flux and CH₄ uptake.

We fit the mixed effects models in a Bayesian statistical framework with the RStan package [46]. All models were fit with standard Bayesian Markov chain Monte Carlo (MCMC) sampling techniques, with 100,000 iterations and four MCMC chains per model. For all fitted parameters, effective sample size exceeded 80,000 and $R_{\text{hat}} = 1$, indicating MCMC chain convergence and reliable estimates of posterior probability density [47]. This analysis was conducted entirely in R [48]. The code and raw data to fully reproduce the analysis within this paper is available at [49] and additionally as the EAB_soilflux.zip file included as Supplementary Materials. The code for this analysis relied on several R packages to facilitate data cleaning, processing, and visualization [50–55].

3. Results

3.1. *F. americana* Decline over Time

F. americana quickly declined during the study period. From May 2016 through October 2017, there was rapid progression of stress and mortality within *F. americana* as determined by visual survey methods: all trees that were healthy in 2016 had been impacted by 2017, leaving no trees in the “healthy” category for 2017 at this site (Figure 3). The mean canopy gap size created by individual ash mortality was 28 m² (full range of estimated trees 8.5–48 m²), which, if assuming that the gap is a circle, translated to a mean gap diameter of 2.9 m (full range 1.7–3.9 m). Despite widespread ash decline, the non-dominance of ash at this site meant that much of the forest remained intact by the end of the study (Figure 2d,e).

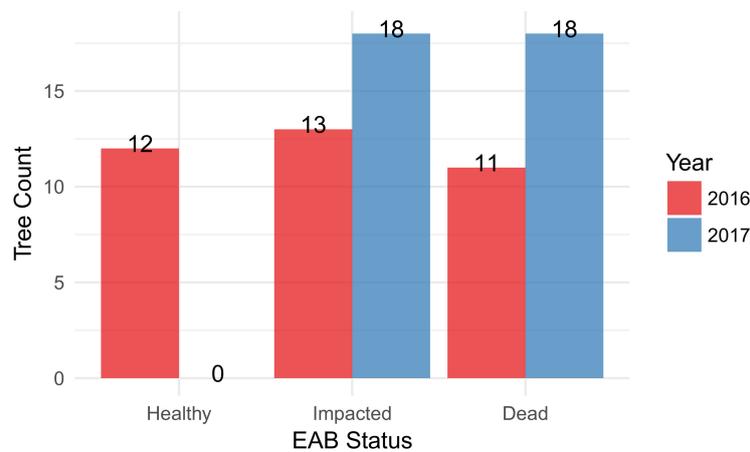


Figure 3. Emerald Ash Borer (EAB) impact classes of *F. americana* in July 2016 and 2017 from visual surveys. EAB impacts, estimated by visual surveys well after leaf-out, rapidly progressed within this forest stand from 2016 to 2017, where no *F. americana* remained in the Healthy class in 2017.

3.2. Soil Characteristics and Soil Microclimate Trends

Soil pH was between 4.05 and 5.43 at this site and sand content was high, ranging from 0.92 to 0.99 volumetric particle fraction (Table 1). Air temperature during the growing season was significantly higher than the upper quartile of the climatological monthly means for July, August, and September 2016 and September and October 2017 (Figure 4a). May 2017 was below the lower quartile for the climatological mean monthly temperature.

Table 1. Fixed regression parameters were estimated separately for CO₂ flux and CH₄ uptake Bayesian linear mixed models. Bolded regression parameters reflect posterior estimates with more than 90% of the posterior probability density different from zero, and italicized parameter estimates reflect posterior distributions where >50% of the probability density bridges zero. DBH: diameter at breast height (1.3 m from ground level).

Parameter	Variable Range	CO ₂ Param Median	CO ₂ 95% Interval	CH ₄ Param Median	CH ₄ 95% Interval
Intercept	n/a	−7.65	−15.64–0.53	−4.25	−21.0–12.2
Soil Temperature	7–20 °C	0.06	0.03–0.08	<i>0.03</i>	<i>−0.03–0.08</i>
Soil Moisture	0.009–0.530 m ³ m ^{−3}	0.21	−0.35–0.76	−6.00	−7.99–−3.91
Monthly Rainfall	5.64–19.9 cm	−0.02	−0.03–0.00	<i>−0.01</i>	<i>−0.06–0.03</i>
Fraction Sand	0.92–0.99	7.74	0.60–14.6	12.63	−1.45–27.11
pH	4.05–5.43	<i>0.09</i>	<i>−0.34–0.55</i>	−0.83	−1.73–0.08
DBH	24.7–66.5 cm	<i>0.01</i>	<i>−0.01–0.01</i>	n/a	n/a
Root Biomass	0.67–7.55 g	−0.06	−0.14–0.03	n/a	n/a

Strong differences existed in monthly precipitation between 2016 and 2017 in May and June, where precipitation in 2016 was about half that of 2017 in each of those two months (Figure 4b). Although monthly total precipitation had high variability in those months, instantaneous soil moisture measurements collected at the time of soil flux measurement were unrelated to total monthly precipitation (Figure S1), likely due to the high sand content of soils at this site leading to strong drainage capacity (Table 1). Given that monthly precipitation and soil moisture were not correlated, we included both variables as fixed effects within the mixed models, since conceptually they might represent different processes. For example, soil moisture inhibits diffusion locally and total monthly precipitation could enhance tree productivity and root carbon exudation on longer timescales, impacting soil flux through different mechanisms.

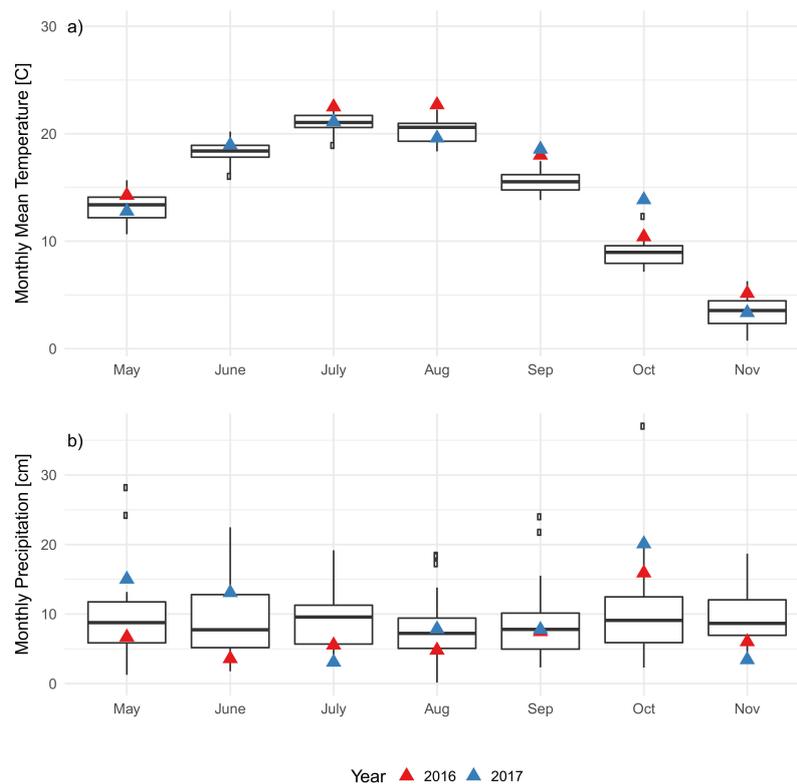


Figure 4. Monthly Mean Temperature and Total Precipitation in 2016 and 2017: (a) Monthly mean temperature was significantly higher than the 75% quartile range of the 30-year climatology for July, August, September, and November in 2016, and for September and October in 2017. (b) Monthly total precipitation was much higher in 2017 than in 2016 for May, June, and October, and the values for 2017 in those months were also higher than the 75% quartile of the 30-year climatology for total precipitation (box plot).

3.3. EAB Impact on Soil Microclimate and Gas Flux

We evaluated the mixed effects models fit to the repeated soil moisture and soil temperature measurements to determine whether soil microclimate was impacted by EAB infestation. In the model fit to measured soil moisture, the posterior probability density for the coefficient for total monthly rainfall was not significantly different from zero, but the coefficient for fraction soil sand content was negative (median = -1.69 , 95% interval = -2.63 – -0.79 ; Table S1). The random effects fit to EAB impact status (healthy, impacted, or dead) were not significantly different from zero (Table S1). For the model fit to the repeated soil temperature measurements, the random effects fit to EAB impact status were also not significantly different from each other (Table S1). For both the soil temperature and

soil moisture models, the parameter estimates for the EAB status effects led us to conclude that EAB impact was not a reliable predictor of soil microclimate at this site.

The linear regression flux models for CO₂ flux and CH₄ uptake that were measured at individual collars had a mean standard error of 0.028 $\mu\text{mol m}^{-2} \text{s}^{-1}$ for CO₂ flux (0.026 $\mu\text{mol m}^{-2} \text{s}^{-1}$ for the LGR analyzer in 2016 and 0.030 $\mu\text{mol m}^{-2} \text{s}^{-1}$ for the Picarro analyzer in 2017) and 0.042 $\text{nmol m}^{-2} \text{s}^{-1}$ for CH₄ flux (0.035 $\text{nmol m}^{-2} \text{s}^{-1}$ for the LGR analyzer in 2016 and 0.047 $\text{nmol m}^{-2} \text{s}^{-1}$ for the Picarro analyzer in 2017). Since these errors represent less than one percent of the total measured flux magnitude, we did not explicitly incorporate the error from this flux calculation in subsequent analysis. In both 2016 and 2017, CO₂ flux followed a seasonal pattern with the peak annual flux in August 2016 and July 2017 (Figure 5a). CH₄ flux also had a strong seasonal pattern, with peak CH₄ uptake lagging the peak in CO₂ flux, in September 2016 and August 2017. Peak CH₄ uptake during mid-summer was much higher in 2016 compared to 2017, and fluxes for 2016 and 2017 were significantly different in July, August, and September (Figure 5b; paired *t*-test, $p < 0.05$).

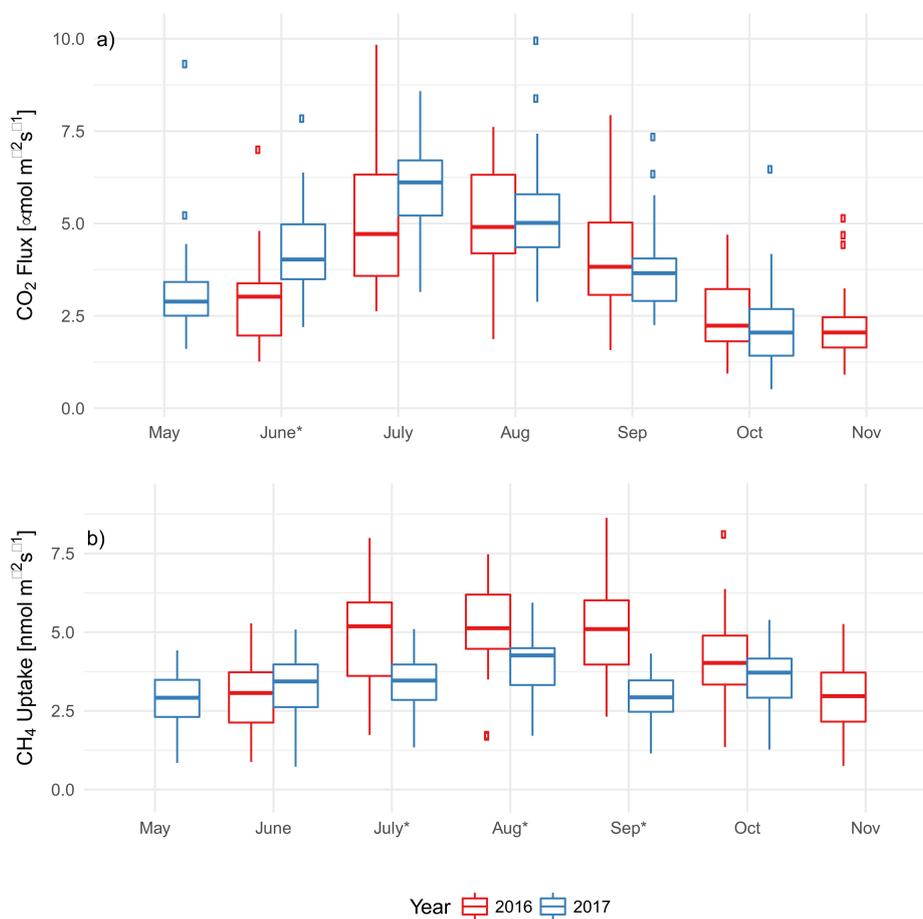


Figure 5. Seasonal patterns in CO₂ flux and CH₄ uptake: (a) monthly soil CO₂ flux and (b) monthly soil CH₄ uptake aggregated across all replicates both followed a clear seasonal pattern, with the highest efflux in mid-summer. For CO₂ flux, the measurements collected in 2016 and 2017 were significantly different only during June and July (paired *t*-test, $p < 0.05$) and the CH₄ uptakes in 2016 and 2017 were significantly different during July, August, and September (paired *t*-test, $p < 0.05$).

To evaluate potential changes in soil flux with *F. americana* decline, we used the three EAB impact categories (healthy, impacted, and dead) evaluated in 2016 and 2017 to assign two groups that described the trajectories of ash decline: trees that experienced EAB stress and mortality during the experiment (Impacted) and trees that were already dead at the start of the experiment (Dead).

These two transitional categories allowed us to compare changes in soil CO₂ flux and CH₄ uptake as *F. americana* declined. Patterns in the CO₂ flux and CH₄ uptake difference between 2016 and 2017 organized by these two classes were not significantly different, and did not exhibit any discernible trends across the sampling dates (Figure 6). It is important to note that this visual summary of the mean patterns in CO₂ flux and CH₄ uptake by category does not incorporate the covariate effects that also varied between the two years, which are additionally important drivers of CO₂ flux and CH₄ uptake.

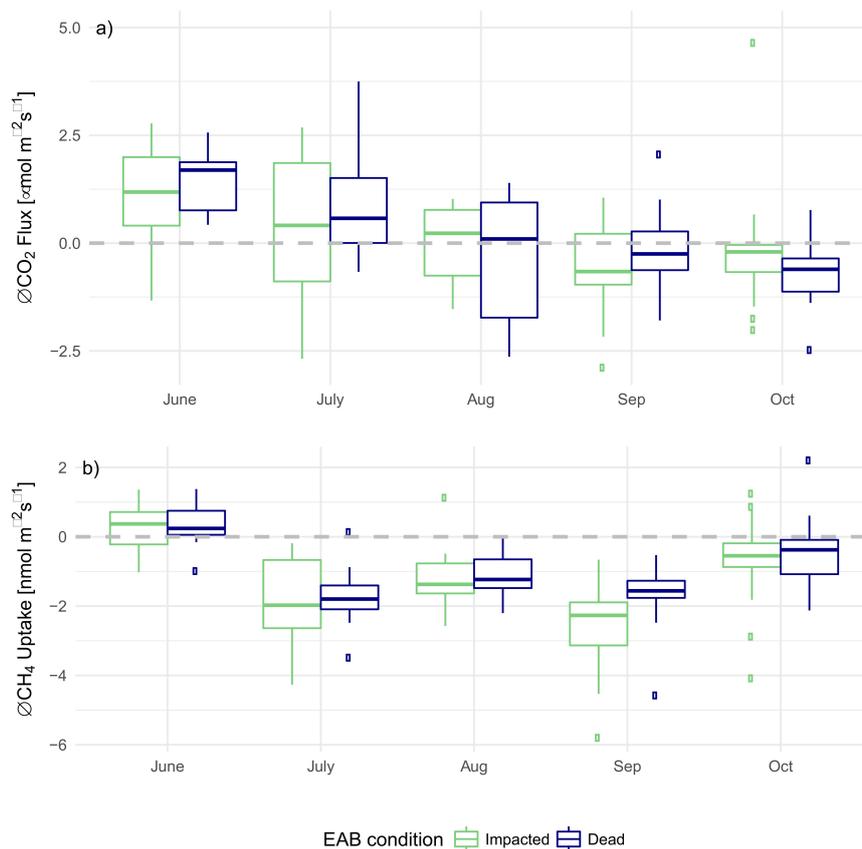


Figure 6. Monthly CO₂ flux and CH₄ uptake differences in 2016 and 2017 organized by trees that were stressed by emerald ash borer (EAB) during the experiment (Impacted) or already dead at the start of the experiment (Dead): (a) monthly soil CO₂ flux 2016–2017 differences and (b) monthly soil CH₄ uptake 2016–2017 differences had no consistent trends. Whether a tree was impacted by EAB during the experiment or dead from the start of the experiment did not have a significant impact on soil CO₂ flux or CH₄ uptake differences between 2016 and 2017.

3.4. Modeled Predictors of CO₂ Flux and CH₄ Uptake

In the mixed effects model fit to CO₂ flux data, the modeled regression coefficient for temperature was positive (Table 1). The regression coefficient for soil moisture was also positive, but the coefficient for monthly rainfall was negative. Fraction sand content had a large, but uncertain, positive effect on soil CO₂ flux. The posterior distributions of the regression coefficients for DBH of the nearest tree, root biomass, and soil pH were all not significantly different from zero for the CO₂ flux model.

In the mixed effects model fit to the CH₄ uptake data, the regression coefficient for soil temperature was not significantly different from zero (Table 1). The coefficients for soil moisture and monthly rainfall were both negative (although only the soil moisture coefficient is significantly different from zero), in contrast to the CO₂ flux model where the moisture coefficient was positive and the rainfall coefficient was negative. In the CH₄ uptake model, the coefficient for the fraction sand content was

positive but had large uncertainty bounds, as was the case for fraction sand regression coefficient in the CO₂ flux model. The coefficient for soil pH was negative for the CH₄ uptake model, in contrast to the CO₂ flux model where the pH coefficient was not significantly different from zero (Table 1).

Random month effects fit for both the CO₂ flux and CH₄ uptake models broadly paralleled trends apparent within Figure 5, with the largest positive effects in August and September for both CO₂ flux and CH₄ uptake, but with more variability in the order of the remainder of the months (Table S2). Because the random month effects are fit together with soil temperature, moisture, and rainfall, these effects represent patterns that move beyond the relationship with these other fixed factors, and could yield insight into which types of phenological signals might be important for soil processes. Random tree replicate effects were significant, but did not follow patterns that were associated with EAB impact class (Figure S2). Distribution statistics for all estimated parameters in both the CO₂ flux and CH₄ uptake models are provided in Table S2. We used the soil flux measurements that were replicated at half the trees ($n = 12$) and found that modeled CO₂ flux and CH₄ uptake generally corresponded (Figure S3). However, there remained large variability in the correspondence between measured and modeled soil flux (Figure S3). We caution that this is not necessarily a reliable predictive model, likely due to large variability among soil flux collars, even from within the same tree.

4. Discussion

4.1. Impact of EAB on Microclimate

We hypothesized that EAB-induced tree mortality would influence soil microclimate and subsequently alter CO₂ flux and CH₄ uptake in soil beneath affected trees. In our system, tree mortality and stress by EAB did not change soil microclimate conditions in a systematic direction. In previous studies of forest pests, outbreaks are often associated with increases in soil moisture, as transpiration of infested trees declines [3,24,56]. However, the fact that soils at our study site had high sand content in combination with the decoupling of monthly precipitation and soil moisture (Figure S1) indicated that our site was well drained, so that changes to plant transpiration from EAB damage might not be strong enough to influence overall soil moisture. Similarly, while previous studies indicate that forest insect disturbance may increase soil temperature [27], this was not the case in our study. We suspect this is due to the small relative size of the canopy gaps created by dead and dying *F. americana* (see Figure 2c). Indeed, previous studies on the effects of canopy gaps on soil temperature and moisture have found mixed results, but generally see greater impact with larger gaps [57].

4.2. Impact of EAB on CO₂ Flux

Our findings agreed with previous studies that soil temperature and moisture overwhelmingly control soil CO₂ flux [58]. As we did not find a discernible effect of EAB infestation on these microclimate factors, we are therefore unsurprised that CO₂ flux also does not appear to be influenced by EAB impact. Another possibility is that our sample size was not adequate to detect a possible effect of EAB on soil flux. In studies that did find an effect of biotic disturbances on soil CO₂ flux [24], the impacted tree species was highly monodominant. In this study, the impacted species composed approximately one-third of the canopy. This difference is particularly important when considering the mechanisms suggested by our models: given that the variables that significantly influence soil CO₂ flux included climate and microclimate variables, but did not include root biomass, we would not expect that a diffuse infestation would immediately influence CO₂ flux if the infestation does not affect the soil microclimate. In our system, canopy gaps beneath affected trees were relatively small and reductions in transpiration by beetle-killed trees may have been balanced by increased water use of nearby trees. Thus, non-ash tree species unaffected by EAB may have buffered the effects of ash mortality on soil microclimate, and thus on soil CO₂ flux.

4.3. Impact of EAB on CH₄ Uptake

To our knowledge, this was the first study to assess the potential effects of tree mortality due to insect infestation on soil CH₄ uptake. In our mixed effects model, CH₄ uptake was strongly inhibited by soil moisture, monthly precipitation, and greatly enhanced by the fraction of sand content in the soil. This agrees with many previous studies that suggest that physical limits to atmosphere-gas diffusion might play a more important role than other soil carbon cycling components of CH₄ uptake [35–37,59]. While our data did not indicate a relationship between EAB-induced mortality and changes in soil moisture, this may be due to the mixed composition of our study site, and potential effects might be stronger in stands dominated by *Fraxinus* spp., for example, in riparian areas dominated by *Fraxinus nigra* Marsh. and/or *Fraxinus pennsylvanica* Marsh. The lack of *F. americana* dominance likely also dampened a potential temperature response feedback for CH₄ uptake at our site, and areas with more complete canopy mortality will likely experience much higher soil temperatures following EAB infestation.

Our findings suggested that the effect of insect disturbances on carbon dynamics in forests may depend on both edaphic and vegetative properties, and an understanding of these characteristics will complement future studies aiming to connect insect disturbances with ecosystem carbon storage. Sites where soils are poorly drained might experience a stronger effect of EAB infestation on soil carbon flux; for example, in stands of *Fraxinus* species that grow in riparian areas. Pre- and post-disturbance forest compositions are also likely to affect the magnitude of insect invasions on ecosystem carbon dynamics. In northern temperate forests, *F. americana* represents a small component of standing tree biomass, and the physiological response of co-dominant tree species in stands affected by EAB may reduce the overall effect of ash mortality on soil carbon fluxes. In less diverse systems where ash represents a greater fraction of forest biomass, the effects of EAB on the forest carbon balance are stronger [14,60]. However, the influence of EAB on forest carbon balance extends far beyond the period of active infestation. Our results support the idea that short-term impacts of insect disturbance on temperate forests are likely dwarfed by the effects of longer-term compositional changes in the tree community and transfer of *F. americana* snags into the soil pool, which are known to play a critical role in future carbon dynamics of disturbed forests [56,61]. If so, the impact of EAB on forest carbon dynamics will vary widely on a regional and local scale as different species replace ash in affected stands. A predictive framework for these compositional changes will be critical for understanding how EAB may alter forest carbon stocks into the future.

Our results may be relevant when considering management options for forests impacted by EAB. Early management strategies in the U.S. included attempts to use preemptive and salvage logging as a control measure in locations with confirmed EAB infestation [8,62]. While most of these initial attempts were abandoned as regional control strategies, salvage logging, both before and after EAB detection, remains a management strategy in some places [8,62]. Unlike other management techniques, such as insecticide or biological control, preemptive and salvage logging results in the immediate formation of canopy gaps at the site of the removed *Fraxinus* trees. Our results suggest that changes in microclimate, which is likely linked to the size and distribution of canopy gaps [57], are the critical factor in determining soil carbon flux responses to tree mortality at this scale. Therefore, this management strategy in particular may influence soil carbon fluxes if canopy gaps created by tree removal are large enough to change soil moisture or temperature.

5. Conclusions

As biotic disturbances become more common in temperate forests [63], there is a need to understand the consequences of these events for ecosystem carbon dynamics. Our results demonstrated that the short-term effects of EAB-induced ash mortality on soil carbon dynamics may be minimal in some ecosystems. In our study, EAB infestation did not lead to changes in soil microclimate conditions, possibly owing to coarse soil texture and sufficient drainage, and to the diffuse nature of the infested trees in this mixed forest. EAB-induced tree mortality created no change in CO₂ efflux

or CH₄ uptake from soil beneath affected trees. In ecosystems with poorly drained soils or higher proportions of *Fraxinus* in the canopy, changes to soil moisture or temperature following widespread *Fraxinus* mortality may be sufficient to alter these fluxes. Our findings emphasized that soil properties may mediate the effect of tree-killing insects on ecosystem carbon fluxes and should be considered when assessing how invasive pests may alter forest carbon balance following disturbance.

Supplementary Materials: The following are available online at www.mdpi.com/1999-4907/9/1/37/s1, Figure S1: Soil Moisture and Monthly Precipitation, Figure S2: Posterior distributions of random tree effects; Figure S3: Model-data correspondence for replicate tree validation data; Table S1: model posterior distribution statistics for soil microclimate models, Table S2: model posterior distribution statistics for soil CO₂ flux and CH₄ uptake models.

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