



Tree Species Effects on Soil CO₂ and CH₄ Fluxes in a Mixed Temperate Forest

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ABSTRACT

Temperate forest soils are net sources of carbon dioxide (CO₂) and net sinks for methane (CH₄), the two greenhouse gases most responsible for contemporary global climate change. Both soil carbon fluxes are sensitive to their local tree communities due to the direct effects of tree traits as well as indirect effects of associated soil properties. We asked how tree species identity and diversity predicts the flux of CO₂ and CH₄ from soils, how the two net fluxes are related, and what tree and soil characteristics predict their magnitudes. In a mixed temperate forest in central Massachusetts, we established 49 plots containing either a single tree species or a combination of those species and measured growing season soil CO₂ and CH₄ fluxes for two years. We found generally greater soil CO₂ and CH₄ fluxes associated with deciduous tree species. CH₄ uptake rates were more sensitive to tree species than

were CO₂ fluxes. Tree species mixtures lead to predictable intermediate fluxes of CO₂, but mixtures resulted in lower than predicted CH₄ uptake. Soil CO₂ emission and CH₄ uptake were both positively related to total litter inputs. Soil CO₂ emission was additionally associated with warmer temperatures and a lower ratio of soil carbon to nitrogen; in contrast, CH₄ uptake was associated with lower soil moisture and a shallower organic horizon. Thus, tree species community composition may prove useful for predicting soil carbon fluxes, but much remains to be discovered about the mechanisms linking tree species to associated microbial and biogeochemical processes.

Key words: soil respiration; soil carbon efflux; methane uptake; methane oxidation; tree species; temperate forest; tree diversity.

HIGHLIGHTS

- Soil respiration and soil methane uptake differ beneath tree species
- Soil methane uptake is greater where there is more soil respiration
- Soil respiration and methane uptake are related to different environmental factors

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INTRODUCTION

The production and consumption of CO₂ and CH₄ in forest soils represent large global fluxes of greenhouse gases; however, estimates of both soil CO₂ (Warner and others 2019) and CH₄ fluxes contain considerable uncertainty (Kirschke and others 2013). Part of the uncertainty is due to the high variability of soil carbon fluxes at small spatial scales due to their complex drivers (Maestre and Cortina 2003). Global variation in soil carbon fluxes has long been known to be driven by climate (Raich and Potter 1995; Hursh and others 2017), but, recent work suggests that even at large spatial scales, land cover and plant community composition can play an equally important role (Huang and others 2020). Within forests, specifically, soil carbon fluxes may vary with tree species composition. In temperate forests, both soil respiration (Vesterdal and others 2012; Li and others 2017) and methane oxidation (Degelmann and others 2009; Fender and others 2013) rates may differ among stands dominated by different tree species, often with broadleaf species associated with greater fluxes than conifer species (Menyailo and Hungate 2003; Boroken and Beese 2006; Ullah and others 2008; Akburak and Makineci 2013). However, the mechanisms driving these stand level differences are complex and remain unclear.

Net soil gas efflux is ultimately the product of gross production and consumption pathways. Soil CO₂ emission (that is, net efflux, also referred to in this text as “soil respiration”) is the result of autotrophic and heterotrophic respiration; therefore, plants contribute directly to gross soil CO₂ production pathways by root respiration (Bond-Lamberty and others 2004). Methane uptake is primarily the product of gross microbial CH₄ production (methanogenesis) and consumption (methanotrophy or CH₄ oxidation (Le Mer and Roger 2001). In upland soils, methanogenesis may occur at depth or in anoxic microsites where water-filled pore space is high or oxygen and other terminal electron acceptors are depleted; however, aerobic methane oxidation results in the net uptake of CH₄ (Yavitt and others 1990; Adamsen and King 1993; Castro and others 1994; Megonigal and Guenther 2008).

Pathways of Tree Species Effects on Soil Carbon Fluxes

Tree species may influence soil carbon fluxes by multiple potential pathways: microclimatic effects, litter production and chemistry, and root and

belowground processes (Metcalf and others 2011). In turn, these drivers may influence soil physical and chemical properties, such as the carbon to nitrogen ratio (C:N) or the pH (Finzi and others 1998a, b; Prescott and Vesterdal 2013). Together, these factors may affect root respiration rates and the composition and activity of the soil microbial communities responsible for both CO₂ emission and CH₄ uptake, resulting in differences in soil carbon fluxes among tree species. In addition, the habitat preferences of different tree species may be associated with soil properties that influence soil carbon fluxes. Therefore, differences in soil carbon fluxes among stands of different tree species may result from either the direct effects of trees on gas flux rates or from indirect correlations with soil conditions that influence root and microbial activity.

Soil Microclimate

Temperature and moisture have long been known as key controls over soil respiration and microbial activity (Lloyd and Taylor 1994; Davidson and others 1998). Thus, if the soil microclimate varies beneath trees of different species, soil carbon fluxes are likely to respond. Differences in soil microclimate beneath particular tree species may be due to differences in phenology patterns and leaf habit. For example, greater light penetration through a deciduous canopy (particularly in spring or autumn) may cause higher soil temperature, and therefore higher soil respiration, relative to an evergreen canopy (Laganière and others 2012). Evidence for temperature effects on methanotrophy, on the other hand, tend to be mixed, with some evidence for only weak temperature dependence (Hanson and Hanson 1996; Warner and others 2018) and idiosyncratic seasonal patterns (Jang and others 2011; Shukla and others 2013), and others showing stronger temperature sensitivity (Ullah and Moore 2011; Ni and Groffman 2018). Soil moisture, however, is consistently the dominant control on CH₄ uptake, as the high-affinity methanotrophs in upland forest soils rely on diffusion of atmospheric CH₄ and O₂ through the soil, which is depressed when moisture fills some of the available pore space (Castro and others 1994; Bowden and others 1998). Therefore differences in soil moisture that are associated with tree species distributions (for example due to different water use efficiencies or root uptake depths) may link tree species with CH₄ oxidation rates (Menyailo and Hungate 2003).

Litter Production and Chemistry

Different tree species also have different traits with potential cascading effects for soil fluxes beneath them. For example, tree species have different leaf and root lifespans (Withington and others 2006; McCormack and others 2012), litter chemistry (Aber and others 1990; Hobbie and others 2010), and secondary metabolite contents (Talbot and Finzi 2008). Therefore, litter quantity and quality varies among species, with certain tree species producing more litter or litter that decomposes more quickly. Globally, litter quantity is an important predictor of soil CO₂ emission (Chen and others 2011), and litter quality is a crucial control on decomposition rate (Bradford and others 2016). Litter quantity and quality may also affect CH₄ uptake, as the thickness and composition of the litter layer may influence the diffusivity of CH₄ to methanotrophs (Ball and others 1997; Brumme and Borken 1999). Litter quality may also influence methane oxidation indirectly by altering the soil microbial community (Pancotto and others 2010).

Root and Belowground Processes

Tree species also differ in their belowground traits. While fine root respiration rates do not typically differ among temperate tree species (Paradiso and others 2019), tree species have different belowground allocation patterns, production rates and phenology (McCormack and others 2014; Abramoff and Finzi 2015). As approximately 30–60% of total soil respiration is autotrophic root respiration (Subke and others 2006), root biomass is typically positively correlated with total soil CO₂ emission (Zhou and others 2020). Roots and their associated mycorrhizal fungi may also contribute to soil carbon fluxes: for example, tree species associated with arbuscular mycorrhizal fungi may also exhibit greater soil respiration that that associated with ectomycorrhizal fungi (Wang and Wang 2018), although this is sometimes due to associated soil characteristics, rather than the fungi themselves (Lang and others 2020). Similarly, the presence of both an intact rhizosphere and the presence of ectomycorrhizal fungi (relative to no fungi) have been found to stimulate methane oxidation (Subke and others 2018). CH₄ uptake has also been found to increase with increasing fine root biomass, possibly due to greater soil porosity or because rhizodeposition indirectly promotes larger populations of methanotrophs (Fender and others 2013).

Soil Physical and Chemical Attributes

Additional soil physical and chemical attributes are also often found beneath different tree species, both as a result of the above processes and due to the different habitat preferences of different trees. For example, soil pH, nutrient content, and organic horizon depth often differ among stands dominated by different species, likely due at least in part to differences in plant traits (Finzi and others 1998a, b; Prescott and Vesterdal 2013). In turn, these soil physical and chemical attributes may drive soil carbon fluxes: for example, CH₄ uptake is negatively correlated with organic horizon mass (Borken and Beese 2006), which is often greater in conifer stands (Finzi and others 1998b). However, while tree species certainly affect soils via the above pathways (for example, differences in microclimate, litter, and roots) soils also vary due to differences in topography, mineralogy, and land use. Therefore, disentangling the effects of tree species on soil carbon fluxes can be difficult in natural systems.

Tree species Mixture Effects

Finally, much of what we know about tree species' effects on soil carbon fluxes comes from studies that compare stands dominated by a single tree species. However, several of the pathways by which tree species influence soil carbon fluxes could result in non-additive interactions when multiple species occur together. For example, mixed species stands may exhibit niche complementarity resulting in greater than expected root biomass due to greater soil volume filling (Brassard and others 2013). Similarly, litter production may be greater in more diverse stands (Scherer-Lorenzen and others 2007; Capellesso and others 2016). Tree species mixtures are also sometimes associated with higher soil carbon stocks (Schleuß and others 2014; Dawud and others 2016). However, in other cases tree species identity and mixture composition may be more important than diversity. For example, the presence of certain tree species might drive the distribution of soil microbial and heterotrophic organisms, regardless of the local tree diversity (Cesarz and others 2013; Eissfeller and others 2013; Scheibe and others 2015; Schwarz and others 2015). Litter mixtures may result in either additive (Hoorens and others 2010) or synergistic (Gartner and Cardon 2004) interactions, depending on whether more diverse substrates may promote niche complementarity among decomposers. In addition, species combinations can have negative

effects on ecosystem functioning, particularly when a dominant species is associated with higher levels of functioning (Creed and others 2009). Together, this suggests that soil carbon fluxes could respond to species mixtures additively, such that fluxes beneath mixtures are predicted by the component species, or non-additively, such that fluxes beneath tree species mixtures might be higher or lower than expected based on individual species flux values.

In this study we examined how tree species identity and diversity affect net fluxes of CO₂ and CH₄ from temperate forest soils. We first ask how soil CO₂ and CH₄ fluxes differ beneath tree species that represent a variety of plant functional traits and that commonly co-occur in the northeastern United States: red oak (*Quercus rubra*), red maple (*Acer rubrum*), eastern hemlock (*Tsuga canadensis*), and white pine (*Pinus strobus*). To minimize large landscape differences that may be present among stands dominated by different species, we used meter-scale variation in tree species distributions. We tested for covariation in soil CO₂ and CH₄ fluxes, as predicted if conditions promoting greater microbial activity overall lead to greater fluxes of both gases. We tested whether CO₂ and CH₄ fluxes respond additively or non-additively to tree species mixtures by comparing fluxes predicted based on single species plots to observed fluxes from plots beneath tree species mixtures. Finally, we examined the relative importance of the various hypothesized pathways by which tree species identity and diversity may affect the soil carbon fluxes: leaf litter quantity and quality, soil temperature and moisture, root biomass, and soil chemical and physical attributes (Figure 1).

METHODS

This study took place in the Prospect Hill tract of Harvard Forest in Central MA (42.530° N, 72.190° W, 300 m elevation above sea level) during the growing seasons of 2016 and 2017. This forest has a mean annual temperature of 7.1 °C and mean annual precipitation of 1066 mm. Soils are primarily well-drained Typic dystrudepts of the Canton series. This upland stand comprises mixed hardwood and coniferous trees of varying ages, with the canopy primarily composed of 80- and 100-year-old trees. However, the stand also includes numerous older trees, particularly *Tsuga canadensis*, which have been growing in this area for hundreds of years (Jenkins and others 2008). In May of 2016, 49 plots were selected within a 4-hectare area of Prospect Hill. This area is dominated by *Tsuga canadensis* (31% of basal area), *Quercus rubra* (31%

of basal area), *Pinus strobus* (16% of basal area) and *Acer rubrum* (13% of basal area). Plots were selected to capture variability in terms of the species identity of the surrounding three trees. This approach has previously been used to assess the effects of local tree species identity and diversity on soil properties (Langenbruch and others 2012). Briefly, clusters of three trees were selected that contained one or more of the 4 dominant species. All trees had a diameter at breast height greater than 10 cm (mean diameter at breast height for all tree was 29.4 cm). Plots were defined as the interior area of the triangle created by the cluster of three trees. Clusters contained either 1, 2 or 3 tree species, and were chosen such that plots with multiple species always contained the two dominant species, *T. canadensis* and *Q. rubra*. Thus, the single species plots included *A. rubrum* ($n = 5$), *P. strobus* ($n = 5$), *Q. rubra* ($n = 8$), and *T. canadensis* ($n = 8$). Mixed plots ($n = 25$) included plots with *Q. rubra* and *T. canadensis* ($n = 8$), *Q. rubra*, *T. canadensis*, and *A. rubrum* ($n = 7$), and *Q. rubra*, *T. canadensis*, and *P. strobus* ($n = 8$).

Field Methods

Flux Measurements

At the approximate centroid of each plot, we installed polyvinyl chloride (PVC) soil flux collars (10.16 cm diameter) that remained in place for the duration of the study. Plots varied in size, such that collars were on average 0.9–3.6 m from their constituent trees; however, plot size (as measured by mean distance from the collar to the three trees) did not vary by plot type ($F_{4,44} = 1.67$, $p = 0.18$). While this plot design was intended to primarily capture the effects of the three nearest trees, other neighboring vegetation, including other nearby trees (whose roots can extend up to tens of meters) and understory plants, may also have influenced the soil carbon fluxes in the collars. However, the collars were placed such that they did not include any vegetation inside their surface, and the understory in this closed canopy stand is sparse.

Collars were installed to an approximate depth of 7 cm, and we measured the depth of each collar at 4 points to calculate the total volume of the collar once installed. After installation, we waited 3 weeks before taking the first flux measurements on June 1, 2016 to allow for settling and to minimize capturing any disturbance effects on C flux rates due to collar installation (Davidson and others 2002).

Soil CO₂ and CH₄ fluxes were measured at each soil flux collar approximately every two weeks

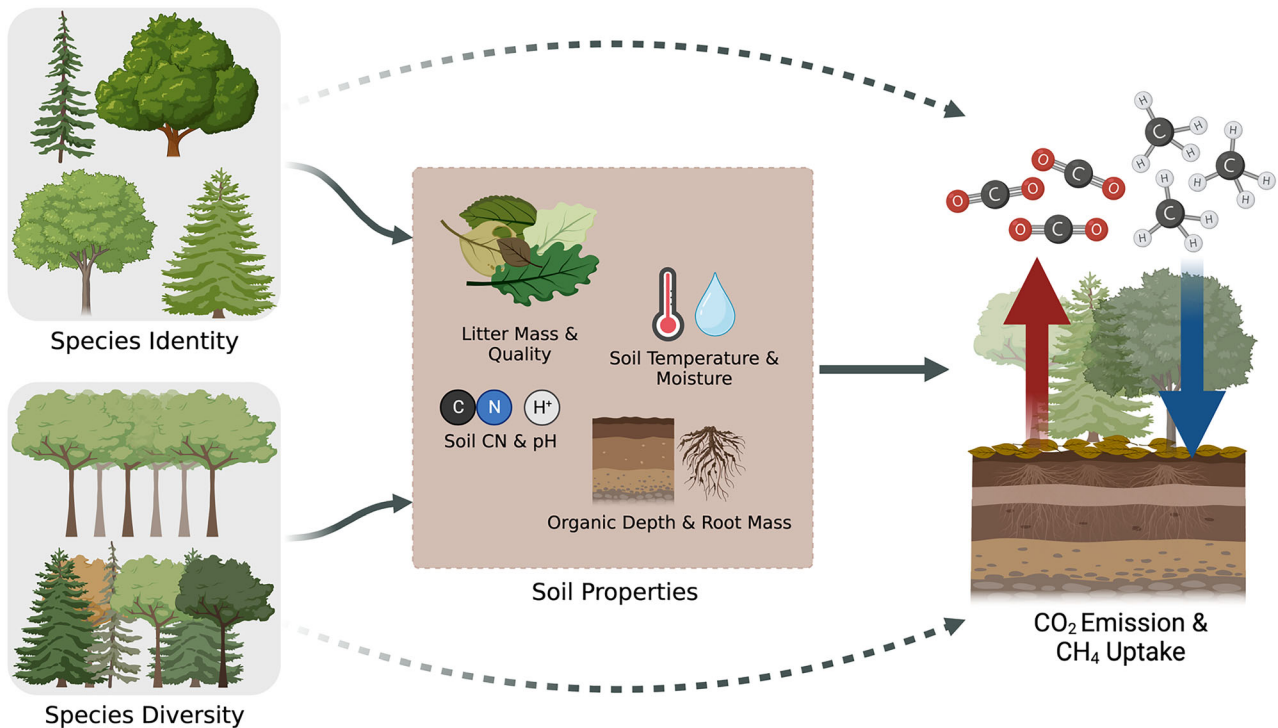


Figure 1. Tree species identity may influence soil CO₂ emissions (red arrow) and CH₄ uptake (blue arrow) either due to their traits (for example, litter mass and quality) and their effects on other environmental drivers (such as soil microclimate, chemistry, organic horizon depth, or rooting mass), as represented by the solid black arrow pathways, or due to additional drivers unmeasured in this study, as represented by the dashed arrows. The intermediate drivers may also be sensitive to tree species diversity: for example, litter mass and quality and rooting mass may exhibit overyielding or species mixtures effects that could result in higher or lower than predicted fluxes beneath species mixtures. Created with BioRender.com.

from June 2016 to Nov 2016 and April 2017 to Nov 2017 using a standard, static chamber method that estimates fluxes as a change in concentration over time (Davidson and others 2002). We chose to only sample during the growing season because accurately measuring gas fluxes beneath snowpack can be very difficult, and soil microbial activity is highest during that time. However, we acknowledge that there can be significant soil carbon fluxes during winter and emphasize that this study is intended to compare the relative, spatial variability in the magnitude of these fluxes and how that was related to tree species distributions rather than capture an annual soil carbon flux budget.

During each sampling date, measurements of CO₂ and CH₄ were collected between 10:00–14:00, which has been shown to provide a good approximation of daily mean soil CO₂ flux at this site (Davidson and others 1998). We used a Los Gatos Research (LGR) Ultraportable Greenhouse Gas Analyzer (Los Gatos Research, Los Gatos, CA, USA) Off-Axis Integrated Cavity Output Spectrometer attached to a PVC soil flux collar via a gas-tight lid to measure the CO₂ and CH₄ concentration inside

each collar at 0.2 Hz temporal resolution for two minutes (0.3 ppm CO₂ precision and 2 ppb CH₄ precision), resulting in 24 concentration measurements for each calculated flux. Fluxes were visually monitored during measurement for approximate linearity: this was indicated when CO₂ concentrations increased steadily while simultaneously CH₄ concentrations decreased steadily (Supplemental Figure S1). The change in these concentrations during the measurement period (typically between 50–150 ppm for CO₂ and 40–80 ppb for CH₄) was used to calculate flux rates (see *Flux processing procedure*, below). While the static chamber approach employed here can have modest artifacts from pressure gradients within the chamber, they nonetheless generally capture small scale variation in fluxes (Davidson and others 2002).

During each soil flux measurement, we measured instantaneous soil temperature and soil moisture in three locations adjacent to the collar. Soil temperature was measured with a digital thermometer inserted 10 cm below the soil surface, and soil moisture was measured with an H2 HydroSense II (Campbell Scientific, Logan, UT,

USA, $\pm 0.03 \text{ m}^3 \text{ m}^{-3}$ accuracy), which integrates soil moisture within the top 10 cm of soil.

Additional Measurements

In August of 2016, we placed 1046 cm^2 litterfall baskets in each plot, adjacent to the soil flux collar. Baskets were lined with fine mesh to capture small leaves and needles while allowing for drainage. Litter was collected approximately every two weeks from September 1 to November 22, 2016, air dried in paper bags, sorted to species, and weighed. The cumulative total litter flux (g air-dried mass) was calculated as the total amount of litter that fell in each basket during the entire autumn period. To estimate the carbon and nitrogen in the litter in each plot, we used species-level litter chemistry data from the nearby Harvard Forest biometry plots (Munger and Wofsy 2022). Based on those species-level estimates, we calculated the total carbon and nitrogen that fell as leaf litter at each collar.

In August 2018, soil flux collars were removed and the depth of the organic horizon within each collar was recorded. We then collected the entire mass of soil from within the collar to a depth of 10 cm. Soil was returned to the lab, where all woody fine roots (diameter $< 2 \text{ mm}$) were separated from the soil during sieving. Herbaceous roots (of which there were very few) were removed. Roots were then dried at 60 degrees C for 48 h, and weighed to calculate fine root biomass. The remaining soil was sieved to 2 mm and homogenized. A subsample was dried at 60 degrees C for 48 h, and reweighed, to determine gravimetric water content (as the proportion of fresh soil that is water). The subsample was then ground with a mortar and pestle and analyzed for carbon and nitrogen content on a Thermo Flash EA 1112 Series CN Soil Analyzer. We used an additional 5-g subsample of fresh soil mixed with 10 mL of deionized water to measure the soil pH (Orion model 410 pH meter with an Orion Sureflow electrode).

Statistical Methods

Flux Processing Procedure

We calculated soil carbon flux rates by fitting a linear model to the relationship between time (seconds) and either CO_2 concentration (ppm) or CH_4 concentration (ppm) within the total volume of the soil flux collar and LGR system (including the chamber and all associated tubing). We used ambient air temperature and pressure collected at the nearby Fisher Meteorological Station (Boose 2022) every 15 min to calculate the number of

moles from the concentrations within the chamber by matching the start time of each chamber measurement to the nearest meteorological measurement. We removed the first 10 s of each measurement to reduce potential errors associated with securing the chamber top. We then converted the slope of this linear model to $\mu\text{g C per m}^2 \text{ per second}$ using the measured chamber volume (as measured by the chamber height and the soil surface area enclosed by the collar). We removed flux measurements of both gases if the linear model for CO_2 flux had an $R^2 < 0.9$ ($n = 7$ observations of both gases). For ease of interpretation, we took the negative of the CH_4 flux, and refer to this as “ CH_4 uptake” in the remainder of the text.

While soil CO_2 and CH_4 flux rates are dynamic over hours or days, the purpose of this project was to test mechanisms that are static over such short timescales. Therefore, we primarily assess the effects of tree species on total growing season fluxes by integrating the CO_2 efflux and CH_4 uptake rates over time. To do this, we first converted the instantaneous flux values to daily estimates by multiplying them by 86,400 (the number of seconds in a day). We then used the ‘auc’ function in the ‘flux’ package in R (Jurasiński and others 2014) to calculate the area under a curve defined by those daily rates before Julian day 150 and day 315 (end of May through mid-November) for each collar in each year. This allowed us to estimate nearly identical time periods in the two measurement years. Previous work in this forest has shown that instantaneous fluxes, even at the sampling frequency of this study, typically capture the total seasonal fluxes very well (Savage and Davidson 2003). However, to ensure that our results were not sensitive to our assumptions for scaling fluxes to a seasonal total, we ran additional statistical models using the instantaneous flux values (see details below).

Statistical models

First, we assessed the overall effects of tree species identity and diversity on the growing season flux of each gas. To do so, we used linear models with growing season CO_2 or CH_4 flux as the outcome and plot type (either *Q. rubra*, *A. rubrum*, *T. canadensis*, *P. strobus*, or mixed) and year (either 2016 or 2017) as the predictors. We also tested the interaction of plot type and year. To test whether the use of growing season fluxes was obscuring important variation, we ran similar models predicting the instantaneous flux values. When using instantaneous flux values, we square root trans-

formed the CO₂ flux values to remove heteroscedasticity. We also included instantaneous measures of soil temperature and moisture and their interaction, in addition to plot type and year as predictors. Finally, we included the plot ID as a random effect to account for repeated sampling.

Next, we tested the relationship between CO₂ and CH₄. We expected that fluxes of the two gases may covary, and that the effect of the tree species could either drive or moderate that relationship. For example, if certain tree species were associated with conditions that facilitate microbial activity, we might expect that fluxes from beneath that species would be greater for both CO₂ and CH₄. However, tree species could also decouple the fluxes of these two gases: for example, if soil moisture tends to be higher beneath one species, that could cause greater CO₂ emissions but lower CH₄ uptake. To assess how the two gases covary with one another and with tree species, we evaluated a linear mixed effects model that predicted CH₄ flux as a function of CO₂ flux (continuous variable), plot type, their interaction, and year, with plot as a random effect to account for repeated measurements. The choice of CH₄ as the response variable was arbitrary. The inference would have been the same whether we used CO₂ as the response variable and CH₄ as the predictor.

To test whether the effects of combinations of tree species were additive or synergistic (for example, deviates either positively or negatively from the assumption of additivity), we constructed null predictions. To do this, we took the average flux of each gas from the single species plots on each date ($n = 5$ for *A. rubrum*, $n = 5$ for *P. strobus*, $n = 7$ for *Q. rubra* and $n = 8$ for *T. canadensis*). Then, for each mixed species plot, we calculated the weighted basal area of the three surrounding trees. We then estimated a “predicted” flux by multiplying the mean flux on that date of each of the constitutive species by the proportional basal area. Thus these predictions represent the expectation that fluxes should respond additively to the combination of species present. We also tested whether a flux prediction procedure using simple means of the species averages, rather than basal area weighted means, produced similar results. To test whether our observed fluxes deviated from that expectation, we ran a paired t-test of the observed and expected flux from each collar on each date. Non-additivity would be indicated by observed fluxes being systematically higher or lower than the predicted fluxes.

Next, to determine whether CO₂ and CH₄ fluxes are driven by similar factors, we evaluated linear

models predicting the growing season flux of CO₂ and CH₄ with all hypothesized drivers as independent variables: soil C:N, organic horizon depth, soil pH, soil gravimetric water content, total litter C, litter C:N, and dry root biomass, mean soil temperature, mean soil moisture, and year. We used variance inflation factors (VIFs) to test models for multicollinearity, using a square-root VIF value of > 2 as an indication that two variables could not be included in the same model: in the case of mean soil moisture and year, the covariation was high given the strong drought in 2016 (Supplemental Figure S2). We therefore removed year from these models. However, we also ran the models without soil moisture but including year to confirm that the other predictors in the models did not differ in magnitude or direction. To test whether the hypothesized drivers were able to fully capture the variation in fluxes explained by plot type, we then re-ran the same models with all independent variables but including Plot type as an additional categorical variable. We compared models with and without Plot type using Akaike information criterion (AIC) values.

In all models, predictor variables were standardized by subtracting the mean and dividing by 2 standard deviations, to allow for the direct comparison of the relative effects of continuous variables with different units (permitting comparison of the effect size of a change in terms of the standard deviation in each predictor variable) (Gelman 2008). We report both standardized and unstandardized coefficients for each model. All statistical models were run in lme4 (Bates and others 2015) and visualized using sjPlot (Lüdtke 2021) in R version 4.0.5.

RESULTS

Total growing season soil carbon fluxes varied among plots beneath different tree species (CO₂: $F_{5,92} = 2.90$, $p = 0.026$; CH₄: $F_{5,92} = 17.55$, $p < 0.001$; Figure 2). On average, we found soil CO₂ emission of 661 g CO₂-C m⁻² growing season⁻¹ and CH₄ uptake of 301 mg CH₄-C m⁻² growing season⁻¹. For soil CO₂ flux, differences in flux rates among species was driven by lower fluxes in the *T. canadensis* plots, which were on average 17% lower than all the other plot types (Figure 2a, Supplemental Table S1). *T. canadensis* plots also had the lowest CH₄ uptake rates, but the magnitude of the difference was greater and there were bigger differences among the other plot types: average uptake in *Q. rubra* plots was 447 mg CH₄-C m⁻² year⁻¹, nearly twice the uptake in *T. canadensis* plots, and

31% higher than *A. rubrum* plots, which had the next greatest uptake (Figure 2b, Supplemental Table S1). Overall, tree species were much better predictors of CH₄ fluxes than CO₂ fluxes (Table 1; R^2 of 0.45 vs 0.12). We found very similar patterns when using instantaneous flux measurements rather than growing season aggregates (Supplemental Table S2).

Soil CO₂ emission showed a strong seasonal pattern, with peak rates occurring in mid-August in both years (Figure 3a). Both years also showed similar patterns and rates of CO₂ fluxes. In contrast, soil CH₄ uptake varied less dramatically with the seasons, and peaked in early (2016) or late (2017) September (Figure 3b). CH₄ uptake was also on average 20% lower in 2017 than in 2016 (Supplemental Table S1).

Soil CO₂ and CH₄ flux magnitudes were positively correlated, but the relative rates of CO₂ efflux and CH₄ uptake were modified by tree species (Figure 4, Supplemental Table S3). For example, soils beneath *Q. rubra* had greater CH₄ uptake rates relative to CO₂ emission, and *P. strobus* and *T. canadensis* had lower CH₄ uptake rates relative to CO₂ emission. Soils beneath all species exhibited

similar slopes, and there was no difference in slope in the two years (Supplemental Table S3).

Soil carbon fluxes beneath mixtures of species were typically in the middle of the range across single-species carbon flux (Figure 2). CO₂ fluxes beneath species mixtures matched expectations if species effects on gas flux rates are additive (Supplemental Figure S3): observed fluxes were indistinguishable from our predictions (mean difference = 1.19 mg C m⁻² h⁻¹, 95% CI = -2.75 to 5.13). Observed soil CH₄ uptake, however, was significantly lower than predictions based on single species plots (mean difference = -0.13 mg C m⁻² h⁻¹, 95% CI = -0.15 to -0.11).

Although soil CO₂ emission and CH₄ uptake were positively correlated, they were also associated with different suites of drivers (Table 1). Total litter carbon input was positively associated with both soil CO₂ efflux and CH₄ uptake (Figure 5). Soil CO₂ efflux was positively associated with mean soil temperatures and negatively associated with the C:N of the soil (Figure 5). CH₄ uptake was negatively associated with soil moisture and organic horizon depth (Figure 5). Overall, models using hypothesized drivers captured less variation in CO₂ ($R^2_{\text{adjusted}} = 26\%$) than CH₄ fluxes

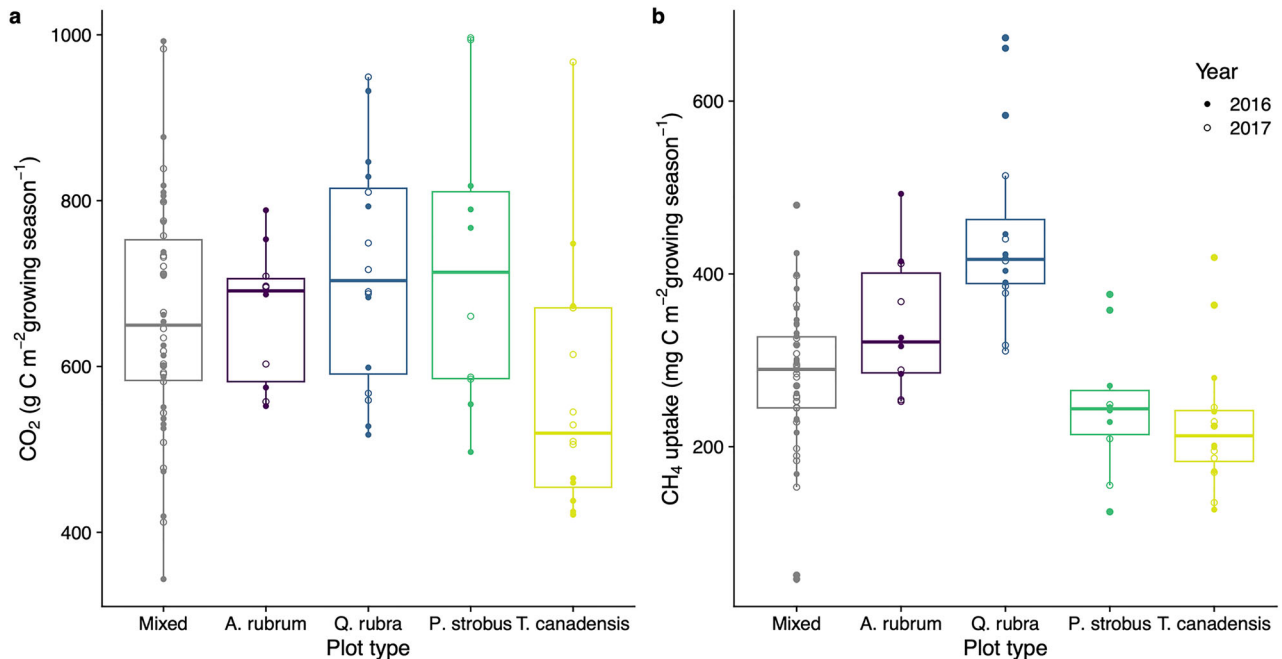


Figure 2. Total growing season soil carbon fluxes, including a) soil CO₂ efflux (g CO₂-C m⁻²) and b) soil CH₄ uptake (mg CH₄-C m⁻²). Points represent individual locations, scaled to the growing season in each year ($N = 98$), with solid circles representing 2016 growing season fluxes and open circles representing 2017 growing season fluxes. Growing season was calculated as Julian day 150–315 (May 29 through November 10). Boxes represent interquartile range (IQR); whiskers extend to 1.5 times the IQR. Thick lines represent the median. Lowercase letters indicate significant differences among plot types according to Tukey's HSD test ($p < 0.05$).

Table 1. Linear Models Growing Season CO₂ Emission and CH₄ Uptake as a Function of Tree Related Variables, Soil Characteristics, and Microclimate

	CO ₂ production (g CO ₂ -C m ⁻² growing season ⁻¹)			CH ₄ uptake (mg CH ₄ -C m ⁻² growing season ⁻¹)		
	Estimate (SE)	Standardized Beta (SE)	<i>p</i>	Estimate (SE)	Standardized Beta (SE)	<i>p</i>
Intercept	- 649.8 (846.8)	0.0 (0.09)	0.445	- 173.41 (521.1)	0.0 (0.07)	0.740
Soil C:N	- 13.3 (6.23)	- 0.25 (0.12)	0.036	- 2.72 (3.84)	- 0.07 (0.10)	0.481
Organic horizon depth (cm)	7.73 (8.53)	0.10 (0.11)	0.368	- 28.48 (5.25)	- 0.50 (0.09)	< 0.001
Gravimetric water content (g/g)	37.3 (36.3)	0.11 (0.10)	0.307	- 22.1 (22.3)	- 0.08 (0.08)	0.325
pH	- 28.3 (99.1)	- 0.04 (0.12)	0.776	<i>119.9 (61.0)</i>	<i>0.20 (0.10)</i>	<i>0.052</i>
Root biomass (g)	20.5 (12.6)	0.17 (0.10)	0.107	1.59 (7.75)	0.02 (0.08)	0.838
Total litter mass (g C)	1.29 (0.35)	0.34 (0.09)	< 0.001	0.87 (0.22)	0.31 (0.08)	< 0.001
Litter C:N	0.49 (1.99)	0.03 (0.13)	0.805	- 0.58 (1.22)	- 0.05 (0.11)	0.634
Mean soil temperature (C)	<i>90.6 (46.8)</i>	<i>0.20 (0.10)</i>	<i>0.056</i>	<i>26.9 (28.8)</i>	<i>0.08 (0.08)</i>	<i>0.353</i>
Mean soil moisture (m ³ /m ³)	324.2 (223.2)	0.14 (0.09)	0.150	- 385.5 (137.4)	- 0.21 (0.08)	0.006
R ² /R ² adjusted	0.33/0.26			0.55/0.51		

Each model contains 98 observations. Shown here are both the unstandardized and standardized coefficients. Rows in bold are significant at $p < 0.05$, rows in italics are significant at $p < 0.1$. See also Table S4.

($R^2_{\text{adjusted}} = 50\%$; Table 1). Including the species composition of the plot dramatically improved the fit of the CH₄ model ($R^2_{\text{adjusted}} = 60\%$, delta AIC = - 17.7), but the CO₂ model less so ($R^2_{\text{adjusted}} = 31\%$, delta AIC = - 3.17; Supplemental Table S4).

DISCUSSION

The growing season estimates of CO₂ fluxes were broadly consistent with previous estimates of annual soil respiration at this site, with our estimates predictably somewhat lower than the total annual fluxes as they do not include the snow-covered season. For example, our estimate of mean deciduous (*Q. rubra* and *A. rubrum*) growing season flux was 689 g C m⁻² year⁻¹ (range 518–949 g C m⁻² year⁻¹), 8% lower than the annual estimate of 748 g C m⁻² year⁻¹ from deciduous stands (Giasson and others 2013). Similarly, our estimate of 661 g C m⁻² year⁻¹ for mixed plots and 557 g C m⁻² year⁻¹ for hemlock plots were 5% and 18% lower than annual estimates for mixed and hemlock stands, respectively (Giasson and others 2013). Instantaneous measurements of CO₂ (Savage and

Davidson 2003; Savage and others 2013) and CH₄ (Castro and others 1994) were well within the range of previously reported values at this site.

Our results were consistent with the hypothesis that tree species identity is related to soil CO₂ and CH₄ fluxes. However, effects were much more apparent for CH₄ than for CO₂. CO₂ fluxes were mostly similar among the species, with slightly lower fluxes in *T. canadensis* plots. This may be partially due to the presence of hemlock woolly adelgid (HWA) in this stand, which can temporarily depress soil respiration (Nuckolls and others 2009; Finzi and others 2014). We also found that once we accounted for other hypothesized drivers of CO₂ fluxes, tree species explained little additional variation. For example, soils beneath *T. canadensis* not only had lower CO₂ fluxes but also tended to have a higher C:N, which was related to lower CO₂ fluxes overall. This is consistent with other work showing that tree species distributions may be associated with soil factors that are responsible for driving differences in soil carbon fluxes, rather than tree traits themselves (Lang and others 2020).

In contrast to CO₂, soil CH₄ fluxes varied by nearly 100% beneath the different tree species. Indeed, even after accounting for our hypothesized

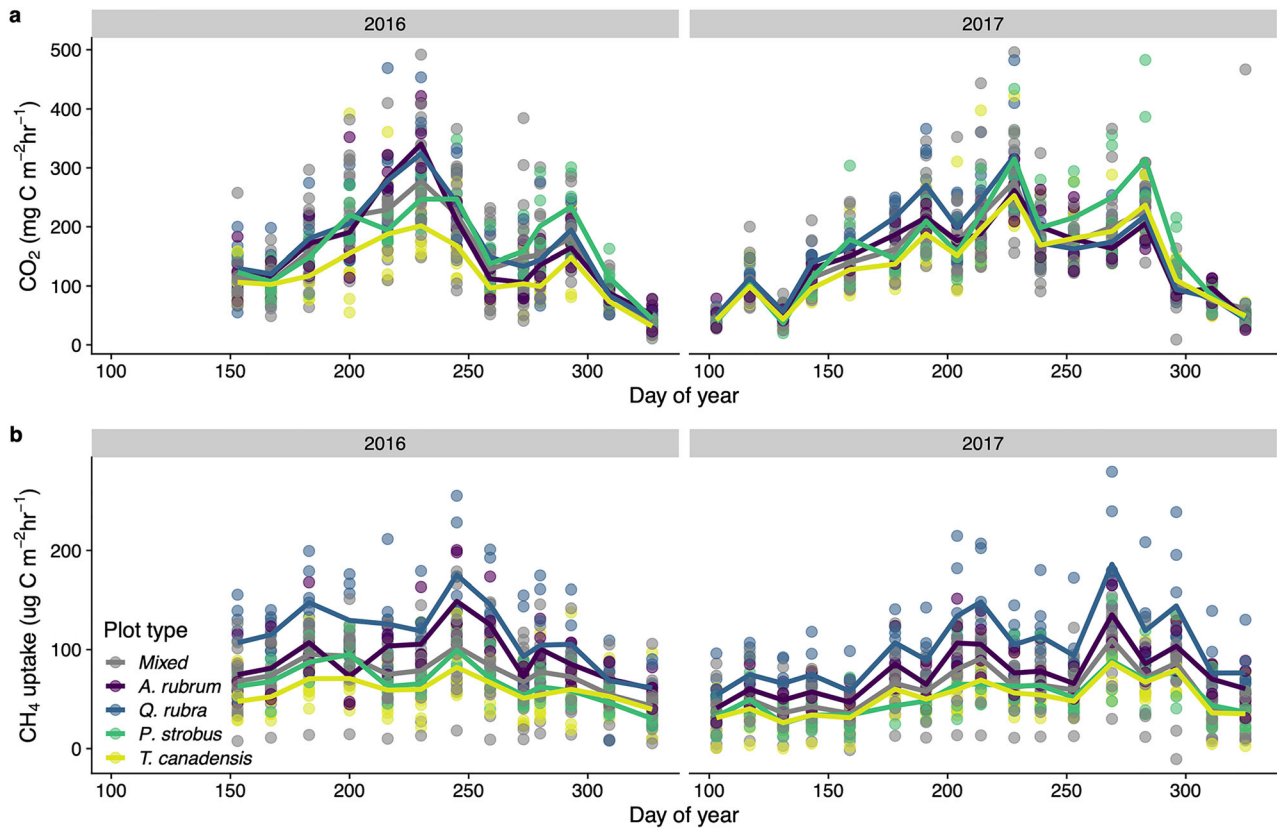


Figure 3. Seasonal variation in soil CO₂ efflux (a) and CH₄ uptake (b). Points represent individual instantaneous measurements ($n = 49$ points per date), lines connect the mean of each plot type on each date.

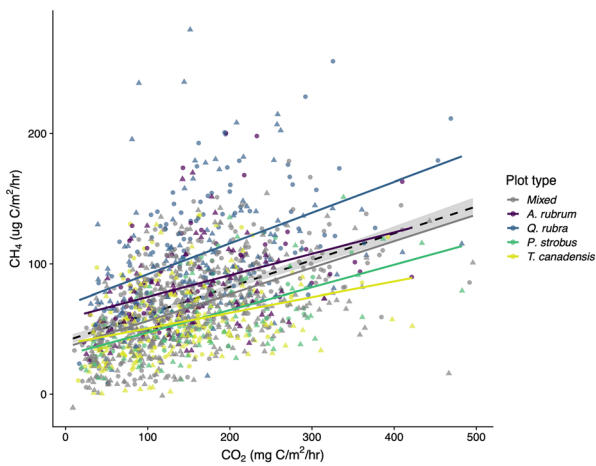


Figure 4. Points represent individual collars on a single date. Circles represent measurements from 2016, triangles from 2017. Points are colored by plot type. Solid colored lines represent simple linear regressions of soil CO₂ and CH₄ fluxes for each plot type. The dashed black line shows the overall correlation, with the gray shading showing the 95% confidence interval. $N = 1463$. Slopes do not differ by plot type (Supplemental Table S3).

drivers of CH₄ fluxes, tree species identity provided high additional information content for predicting CH₄ uptake (delta AIC = -17.7). This could possibly be due to unmeasured soil properties that covary with both tree species and methane uptake rates; for example soil texture or mineralogy, which can influence methane oxidation rate due to their effects on gas diffusivity (Nazaries and others 2013). However, they are not likely to be influenced by tree species at small spatial scales. Instead, we hypothesize that species-specific effects on soil microbial activity may explain the differences in CH₄ uptake beneath different tree species, which could be driven by community interactions between roots, methanotrophs, and other soil- and root-associated microbiota. However, this observational study cannot isolate the cause of this pattern. Future work that links tree species distributions with CH₄ uptake should include additional biogeochemical measurements, such as micronutrient concentrations and soil nitrogen dynamics, as well as focus on identifying specific mechanisms using controlled experiments.

Lower methanotrophy in soils underlying coniferous species are consistent with previous research,

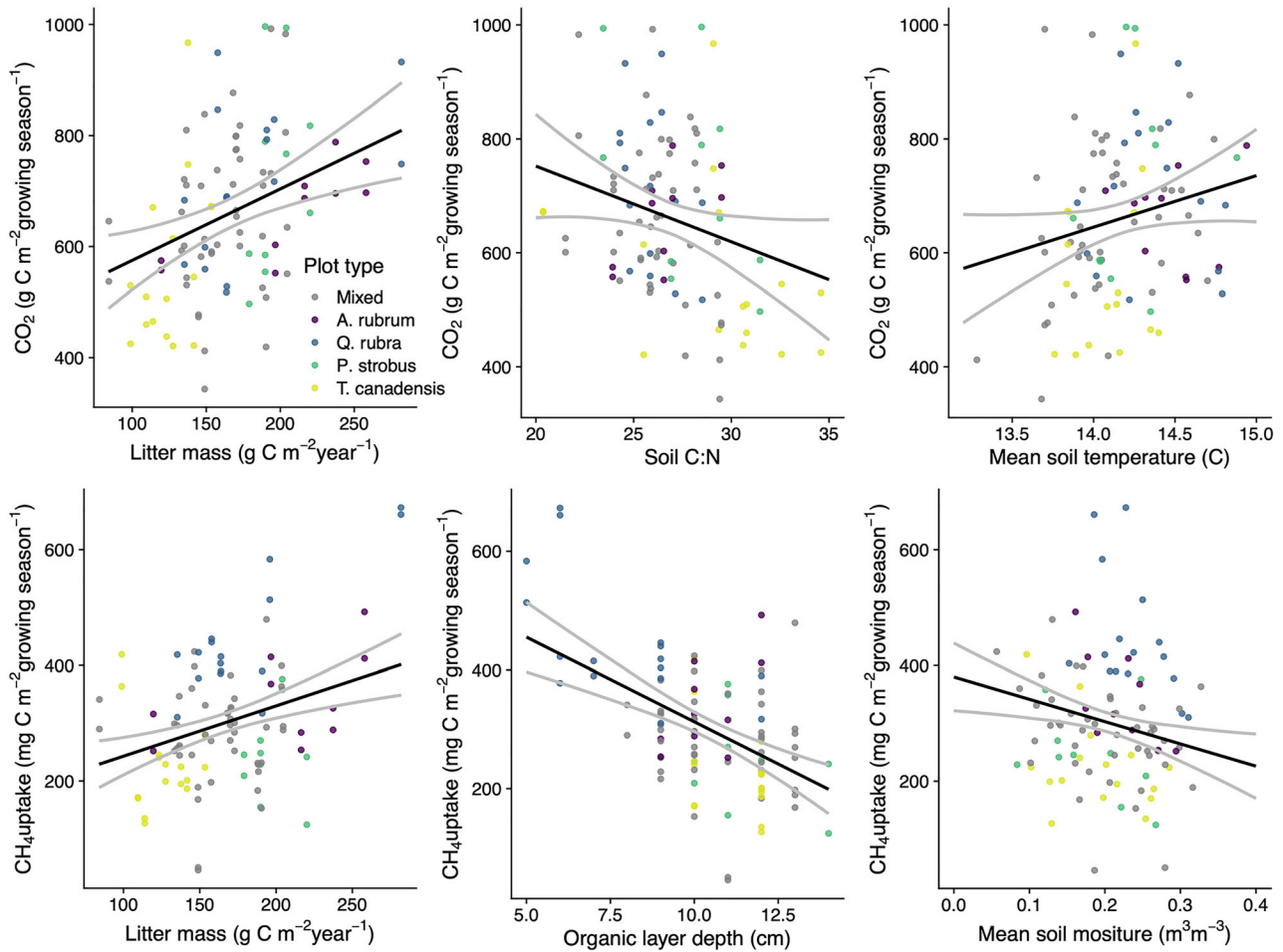


Figure 5. Relationship between hypothesized drivers and growing season soil CO₂ production and CH₄ uptake. Points represent raw observations, while lines depict the modeled, marginal effect each driver on CO₂ production (upper panel) and CH₄ uptake (lower panel) using the unstandardized coefficients from the statistical models (see Table 1 for model coefficients and model fit). The black regression line is plotted while holding the other variables in the model constant at their mean values to isolate the effects of each driver while the points represent the full variability in soil CO₂ and CH₄ flux, which is controlled by multiple factors. Gray lines represent 95% confidence intervals. In each case, the colored points represent the different plot types.

which has found lower fluxes in coniferous soils in field and laboratory conditions (Ishizuka and others 2000; Menyailo and Hungate 2003; Reay and others 2005) and in stands converted from hardwood to coniferous (Borken and others 2003). This pattern has previously been ascribed to decreased methanotroph abundance or activity in near-surface organic soils (Adamsen and King 1993; Ishizuka and others 2000; Reay and others 2005; Degelmann and others 2009; Walkiewicz and others 2021). However, the reasons for these lower surface fluxes remain elusive. Higher acidity is often suggested as a likely mechanism, and our results suggest that pH could be playing some role here as higher pH tended toward higher CH₄ up-

take (Table 1), and coniferous plots had generally lower pH (Supplemental Figure S4). Secondary compounds in plant litter may also help structure soil microbial communities; monoterpenes found in coniferous litter have been suggested to play a particularly significant role in inhibiting soil methanotrophy (Amaral and Knowles 1998; Amaral and others 1998; Maurer and others 2008). Direct root exudation of carbon, oxygen, or other compounds (Tokida and others 2011; Waldo and others 2019) and the role of root-associated methanotrophs (King 1994) could also play a role in upland forest soil CH₄ fluxes. Finally, the presence of hemlock woolly adelgid in this stand could have cascading effects on soil methane uptake, as

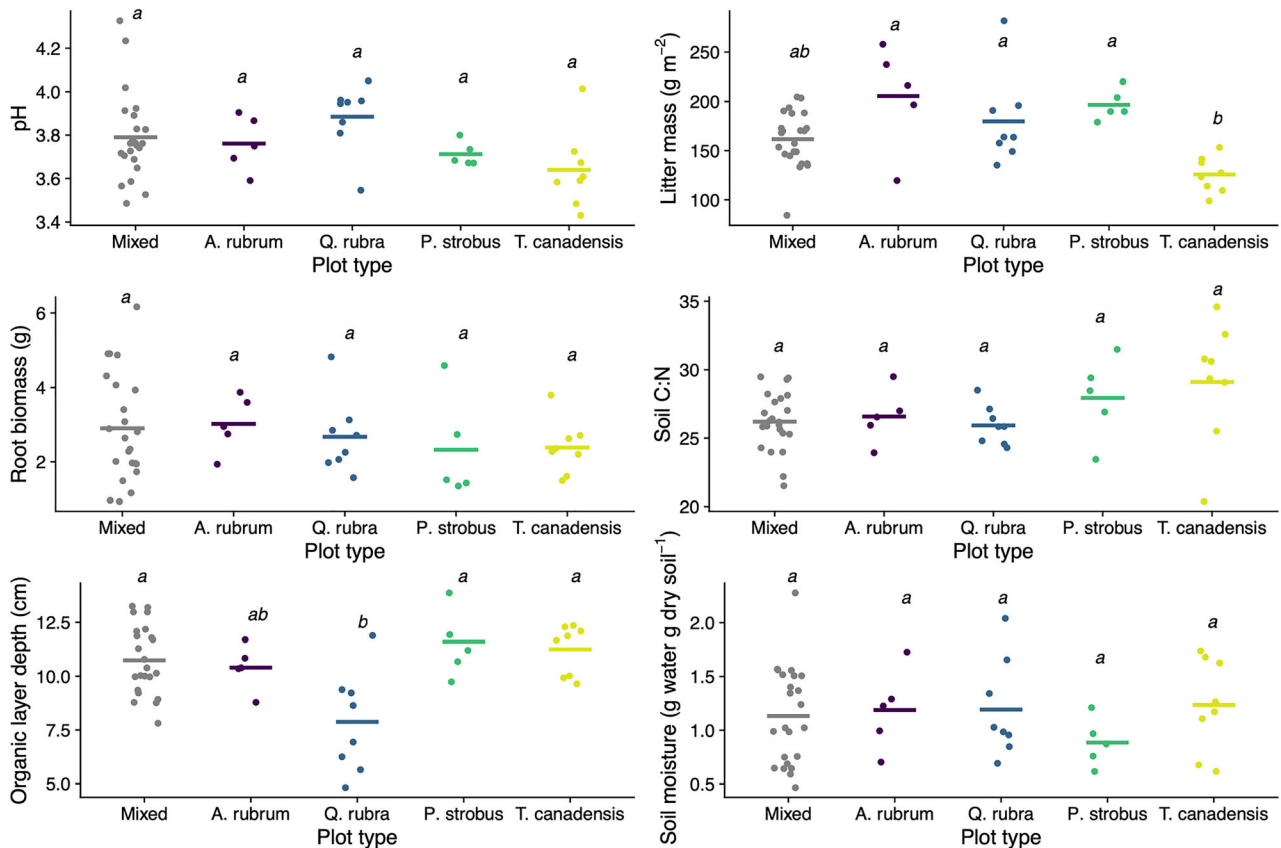


Figure 6. Environmental factors (including soil pH, autumn litterfall mass, root biomass, soil C:N, organic layer depth, and soil moisture) vary by plot type. Points represent individual plots, and bars represent the mean for each plot type. Lowercase letters indicate significant differences among plot types according to Tukey's HSD test ($p < 0.05$).

on soil respiration, by altering the soil microbial community due to the reducing labile carbon supply from roots to soils. More research into the pathways by which tree species and community interactions influence soil CH₄ uptake is sorely needed.

We found that the magnitude of CO₂ and CH₄ fluxes were highly positively correlated, though rates were modified by tree species (Figure 4). We interpret this as evidence that the activity of these microbial populations may be coupled, though the microbial community structure or relative composition may vary between associated tree species. As the activity of these distinct microbial communities increases, through facilitating factors both measured and unmeasured in this study, biogeochemical process rates and consequent gas exchange increase as well (Figure 5).

Although the magnitude of CO₂ and CH₄ fluxes were positively correlated on the whole, they were associated with some of the same and some different drivers. CO₂ emission and CH₄ uptake were both greater with greater litter carbon inputs: this

suggests that both CO₂ and CH₄ may be partially predicted by aboveground patterns. This has previously been shown for soil respiration (Janssens and others 2001). However the positive relationship between litter inputs and CH₄ fluxes is more surprising, as some previous work has found that a large litter horizon can act as a barrier to atmospheric methane, resulting in lower uptake rates (Ball and others 1997; Brumme and Borken 1999). Therefore, we suspect that the greater CH₄ uptake in locations with greater litterfall are responding not to the depth of the litter layer (which was not measured here) but to greater overall productivity: this is consistent with more recent experimental work showing that increasing litter inputs can stimulate CH₄ uptake (Wu and others 2019).

We also found that CH₄ uptake rates were lower in places with a deeper organic horizon, and thus while the litter layer may not have posed a barrier to diffusive methane transport, the organic horizon may have. This is consistent with previous observations that methanotrophs tend to be most prevalent at the base of the organic layer or top of

the mineral horizon (Amaral and Knowles 1998); thus, a larger organic layer may have created a longer diffusion gradient and thus lower uptake. The effect of the organic horizon depth was stronger than the effect of soil moisture, which is considered a primary driver of CH₄ uptake rates (Castro and others 1994; Bowden and others 1998). We also observed that a shallower organic horizon was most common in *Q. rubra* plots (Supplemental Figure S3), helping to explain why those plots exhibited the greatest CH₄ uptake. The association between *Q. rubra* and shallower organic horizons could be due to unmeasured landscape drivers, or either to effects of trees on the organic horizon or because their habitat preference is related to organic horizon depth. In either case, our results indicate that tree species identity is related to methane uptake in soils, even after direct measurements of soil properties are accounted for; however, future work on both the association between tree species distributions and organic layer depth as well as the importance of organic layer depth to CH₄ fluxes is needed.

The effect of tree species mixtures also varied between soil CO₂ and CH₄ fluxes. We found that typically, soils beneath tree species mixtures produced the amount of CO₂ that would be expected based on a weighted average of the component tree species, suggesting that mechanisms of tree species effects are working additively on soil CO₂ fluxes. This is consistent with the factors we identified as important for driving soil CO₂ fluxes, including mean soil temperature and soil C:N. Unlike some of our other hypothesized factors which may be subject to diversity effects (for example, overyielding), these drivers are unlikely to change synergistically with species mixtures; rather, they are likely to take on intermediate values when species mixtures are present. In contrast, CH₄ fluxes in mixed plots were lower in magnitude than predicted by single species. This may be in part due to the strong effect of the depth of the organic horizon on CH₄ fluxes: plots beneath red oak trees tend to have a shallower organic horizon, and greater CH₄ fluxes, than all other plot types (Figure 6). In mixed plots, organic horizon depths were similar to those of the individual species plots (Supplemental Figure S3). This suggests that while tree species adequately predicted CH₄ fluxes, uncovering the mechanisms driving this pattern will be crucial for accurately scaling carbon flux estimates to the landscape level.

CONCLUSIONS

By comparing simultaneous CO₂ and CH₄ fluxes, we show that despite the theoretically more direct role of trees in soil CO₂ flux (that is, as root respiration), CH₄ uptake rates are much more closely tied to tree species identity within a mixed forest stand. Additionally, environmental conditions associated with tree species identity (for example, litter C:N, microclimate, and so on) were unable to fully explain the effects of tree species on CH₄ uptake, suggesting that the mechanisms by which tree species influence net CH₄ flux from soils are yet unknown. However, these results also demonstrate that tree species composition may be a powerful predictor of soil carbon fluxes, and in particular rates of methane uptake, at the landscape level in temperate forests.

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DATA AVAILABILITY

All data associated with this manuscript as well as the statistical code can be found at Jevon, Fiona (2023), “CO₂ and CH₄ fluxes at Harvard Forest”, Mendeley Data, V2, <https://doi.org/10.17632/z6wybrtpyk.2>.

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