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Spatiotemporal variability and origin of CO_2 and CH_4 tree stem fluxes in an upland forest

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Abstract

The exchange of multiple greenhouse gases (i.e., CO_2 and CH_4) between tree stems and the atmosphere represents a knowledge gap in the global carbon cycle. Stem CO_2 and CH_4 fluxes vary across time and space and are unclear, which are their individual or shared drivers. Here we measured CO_2 and CH_4 fluxes at different stem heights combining manual (biweekly; $n = 678$) and automated (hourly; $n > 38,000$) measurements in a temperate upland forest. All trees showed CO_2 and CH_4 emissions despite 20% of measurements showing net CH_4 uptake. Stem CO_2 fluxes presented clear seasonal trends from manual and automated measurements. Only automated measurements captured the high temporal variability of stem CH_4 fluxes revealing clear seasonal trends. Despite that temporal integration, the limited number of automated chambers made stand-level mean CH_4 fluxes sensitive to "hot spots," resulting in mean fluxes with high uncertainty. Manual measurements provided better integration of spatial variability, but their lack of temporal variability integration hindered the detection of temporal trends and stand-level mean fluxes. These results highlight the potential bias of previous studies of stem CH_4 fluxes solely based on manual or automated measurements. Stem height, temperature, and soil moisture only explained 7% and 11% of the stem CH_4 flux variability compared to 42% and 81% for CO_2 (manual and automated measurements, respectively). This large unexplained variability, in combination with high CH_4 concentrations in the trees' heartwood, suggests that stem CH_4 fluxes might be more influenced by gas transport and diffusivity through the wood than by drivers of respiratory CO_2 flux, which has crucial implications for developing process-based ecosystem models. We postulate that CH_4 is likely originated within tree stems because of lack of a consistent vertical pattern in CH_4 fluxes, evidence of CH_4 production in wood incubations, and low CH_4 concentration in the soil profile but high concentrations within the trees' heartwood.

KEYWORDS
CH_4, CO_2, greenhouse gas fluxes, temperate ecosystem, tree stem fluxes, upland forest

1 | INTRODUCTION

Methane (CH_4) is the second most important greenhouse gas in the atmosphere. With a radiative forcing capacity 25 times that of CO_2 (Forster et al., 2007), it contributes 23% to global warming (Etminan et al., 2016). Multiple studies have reported that trees can emit CO_2 and CH_4 through stem surfaces. Most efforts have been pursued on stem CO_2 fluxes with examples dating back to more than 60 years ago (Mar et al., 1954). During the last few years, efforts have highlighted the relevance of CH_4 fluxes from tree stems in tropical (Pangala et al., 2017; Welch et al., 2018), temperate (Pitz & Megonigal, 2017; Warner et al., 2017), and boreal forests (Machacova et al., 2016; Vainio, 2019), including in both angiosperms and gymnosperms (Covey & Megonigal, 2019). Stem CH_4 fluxes are a widespread phenomenon, potentially relevant at regional scales (Pangala et al., 2017; Wang et al., 2017), but upscaling tree-level flux measurements to the stand level is difficult, precluding the inclusion of stem CH_4 emissions in the global methane budget (Carmichael et al., 2014; Saunois et al., 2020). This is because our understanding of magnitudes, patterns, and underlying mechanisms of stem CH_4 fluxes (and other greenhouse gases) is still very limited (Vargas & Barba, 2019). Consequently, there is a need to quantify magnitudes and patterns and to incorporate biophysical principles of stem CH_4 fluxes to improve our understanding of the global carbon cycle (Barba, Bradford, et al., 2019). We highlight three interrelated challenges for research regarding stem CH_4 fluxes.

First, quantifying the spatial and temporal variability of stem CH_4 fluxes. Even within a single tree, the patterns and magnitudes of fluxes may vary at different stem heights. Most studies have measured stem fluxes at a single stem height (Flanagan et al., 2021; Machacova et al., 2016; Wang et al., 2017; Warner et al., 2017; Welch et al., 2018), but there is mounting evidence that stem CH_4 emissions decrease with stem height (Barba et al., 2019; Jeffrey et al., 2020; Pitz & Megonigal, 2017; Sjögersten et al., 2020; Wang et al., 2016). Stem CH_4 fluxes also demonstrate large temporal variability at both diurnal and seasonal scales (Barba, Poyatos, et al., 2019) but, the low temporal resolution of measurements found in most studies (i.e., measurements every 2–4 weeks; exceptions: Barba, Poyatos, et al., 2019; Plain et al., 2019) limits the emergence of temporal patterns and the identification of mechanisms underlying such spatial and temporal variability.

Second, identifying drivers controlling stem CH_4 fluxes at different spatial and temporal scales. Some studies have suggested that stem CH_4 emissions could be partially explained by abiotic conditions outside the tree stems. For instance, some studies have reported increasing stem CH_4 emissions with increasing air or soil temperature (Barba, Poyatos, et al., 2019; Pitz et al., 2018; Wang et al., 2016), increasing soil moisture (Barba, Poyatos, et al., 2019; Welch et al., 2018), or decreasing water table depth (Pitz et al., 2018). Other studies have reported that stem CH_4 emissions are also correlated with physiological or biotic factors, such as tree species identity (Sjögersten et al., 2020; Wang et al., 2016; Warner et al., 2017), wood density (Wang et al., 2017), wood structural features that allow gas transport from the soil to the atmosphere (Sjögersten et al., 2020), tree diameter (Pitz et al., 2018), and sap flow dynamics (Barba, Poyatos, et al., 2019; Pitz & Megonigal, 2017). However, it is likely that an interaction of multiple factors influences CH_4 fluxes and patterns across ecosystems, hampering our understanding of the main biophysical drivers of these fluxes.

Third, characterizing the origin/source of CH_4 that is ultimately emitted by tree stems. There is evidence that CH_4 could be produced within soils under anoxic conditions, transported through the roots into the stem, and diffused from the tree stems to the atmosphere (Covey & Megonigal, 2019). In that case, the stems would be acting as "straws" by providing physical pathways that connect deep soils with the atmosphere, by-passing the uppermost soil layer dominated by methanotrophs (Megonigal & Guenther, 2008). Alternatively, tree stems could emit CH_4 internally produced within the tree's heartwood (Covey & Megonigal, 2019) by methanogenic archaea (Yip et al., 2018). It is hypothesized that this internal production might be responsible for very high CH_4 concentrations found within trees for multiple species and ecosystems (Covey et al., 2012; Zeikus & Ward, 1974). While CH_4 derived from soils seems to prevail in wetlands and floodplain forests (where CH_4 is produced within anoxic soils), internally produced CH_4 seems to be the most likely origin in upland forests, where soils are usually net sinks of CH_4 (Dunfield, 2007; Warner et al., 2017). Several studies have speculated about the main origin of emitted CH_4 or a potential combination between internal- and soil-produced CH_4 in upland forests, but to date, no clear empirical results have solved this dilemma (Barba, Bradford, et al., 2019; Covey & Megonigal, 2019).

If soil and stem internal origin simultaneously occur for CH_4, stem CO_2, and CH_4 fluxes might share some common drivers, as emitted CO_2 also originates from both within the wood (respiration) and from the soil (i.e., transported from belowground through the xylem; Teskey et al., 2017). While the biogeochemical processes and pathways of the two gases will probably differ, their response to physical constraints (e.g., gas diffusivity through the wood or soil) affected by environmental variables (e.g., temperature or moisture) could result in similar temporal and vertical patterns in fluxes from both gases. For example, Pitz et al. (2018) found that the seasonal dynamics of stem CO_2 and CH_4 fluxes were more similar in wetlands compared with upland forests, suggesting that wetter soils might enhance xylem-transported CO_2 and CH_4 emissions. High-frequency measurements would bring the opportunity to test whether temporal patterns of CH_4 and CO_2 fluxes are correlated. If demonstrated, it may enable scientists to estimate CH_4 fluxes from measurements or modeled CO_2 fluxes, which are more feasible to measure than stem CH_4 fluxes (Vargas & Barba, 2019).

We measured in a temperate upland forest the following variables: (1) CO_2 and CH_4 stem fluxes with manual (biweekly resolution) and automated chambers (hourly resolution) at different stem heights over a growing season; (2) CO_2 and CH_4 concentrations within stems and in the soil profile; and (3) CH_4 production capacity in different wood tissues. We used these data to (a) explore the spatiotemporal variability and environmental drivers of stem fluxes; (b) test emergent relationships between CO_2 and CH_4 stem fluxes; and (c) provide insights into the potential origin of CH_4 (either soil or heartwood production) emitted through stems. We postulate the following hypotheses:

Hypothesis 1 *Seasonal patterns in stem CO_2 and CH_4 fluxes can be identified by high-frequency measurements, which also will provide more accurate seasonal mean fluxes and trends by integrating the high variability of stem fluxes throughout the experimental period. Manual measurements, on the other hand,* *will provide better integration of spatial variability of stem CO_2 and CH_4 fluxes but will miss potential large pulses (i.e., "hot moments"), which could influence estimates of the seasonal mean and trends.*

Hypothesis 2 *Underlying biophysical controls of CO_2 and CH_4 could be explained by stem temperature and soil moisture, where stem CO_2 and CH_4 fluxes would increase with higher temperature and soil moisture (a). Thus, because of this potential codependency in temperature and moisture, there may be a positive correlation between CO_2 and CH_4 fluxes (b) as previously proposed (Flanagan et al., 2021; Vargas & Barba, 2019).*

Hypothesis 3 *Tree diameter and stem height will positively influence the magnitude of CO_2 and CH_4 fluxes, interacting with the environmental controls. This is expected because bigger trees might have higher capacity to transporting gases from a large soil volume, which might have a stronger effect closer to the soil (base of the tree) than upper in the stem. Additionally, larger trees have more sapwood and heartwood volume, which might enhance their potential for producing CO_2 and CH_4.*

Hypothesis 4 *We postulate that stem CH_4 emissions may be attributed from CH_4 produced in soils if (a) the magnitude of stem CH_4 emissions decreases with stem height; and (b) there is a positive correlation between soil CH_4 concentrations (at different soil depths) and stem CH_4 emissions. In contrast, stem CH_4 emissions may be attributed to CH_4 produced within the stem if (a) the magnitude of stem CH_4 emissions does not decrease with stem height; and (b) heartwood CH_4 concentrations show a positive correlation with stem CH_4 emissions. This study provides unique information of the temporal variability of CO_2 and CH_4 fluxes from tree stems, which is relevant for identifying controls and functional relationships of these important greenhouse gases.*

2 | MATERIALS AND METHODS

2.1 | Study site

We carried out this study in an upland forested area at the St. Jones Reserve (39°5′20″N, 75°26′21″W], a component of the Delaware National Estuarine Research Reserve. The site has a temperate climate with a mean annual temperature of 13.3°C and a mean annual precipitation of 1119 mm. Soils are Othello silt loam with a texture of 40%, 48%, and 12% of sand, silt, and clay, respectively (Petrakis et al., 2018). The dominant vegetation species are bitternut hickory (*Carya cordiformis*), eastern red cedar (*Juniperus virginiana* L.), American holly (*Ilex opaca* (Ashe)), sweet gum (*Liquidambar styraciflua* L.), and black gum (*Nyssa sylvatica* (Marshall)), with an overall tree density of 678 stems ha^{-1} and mean diameter at breast height (DBH) of 25.7 ± 13.9 cm (mean ± SD). We studied bitternut hickory, which is one of the most important species in the study site, accounting for 24.9% of the total basal area. The length of the studied area was around 70 m. Additional information on the study site can be found in Petrakis et al. (2018).

2.2 | Flux measurements

We measured stem CO_2 and CH_4 fluxes throughout a growing season and after leaf senescence (from April to December 2017) in 18 hickory trees. Trees' DBH ranged from 24.7 to 75 cm (43 ± 13 cm, mean ± SD). To better understand the temporal variability of CO_2 and CH_4 fluxes, we performed automated measurements (i.e., hourly resolution) on three individual stems at 50 and 150 cm stem heights as described by Barba, Bradford, et al. (2019). Briefly, at each stem height, we installed 317.8 cm^2 PVC collars where automated chambers (Li-COR 8100-104) were placed. The chambers were controlled by a multiplexer (Li-COR 8150) which was connected to a closed path IRGA (infrared gas analyzer; Li-8100A). Additionally, we connected a cavity ring-down spectrometer (Picarro G2508) in series with the IRGA as described in other studies (Barba, Poyatos, et al., 2019; Capooci et al., 2019; Petrakis et al., 2017). For each flux observation, we measured CO_2 and CH_4 concentrations every second with the Picarro G2508 for 300 s and calculated fluxes (at 1 h time intervals) from the mole dry fraction of each gas (i.e., corrected for water vapor dilution) using the SoilFluxPro software (v4.0; Li-COR). We estimated the fluxes with both linear and exponential fits and kept the flux with the highest R^2. We applied a quality assurance/quality control (QA/QC) protocol based on CO_2 fluxes established in previous studies (Barba, Poyatos, et al., 2019; Capooci et al., 2019; Petrakis et al., 2018). Briefly, when the R^2 for the CO_2 flux is very high (generally higher than 0.95), it means that the micrometeorological conditions inside the chamber are suitable for measuring stem fluxes (e.g., chamber properly sealed), and therefore, we are confident of keeping CH_4 measurements even if the R^2 for the CH_4 flux is low (which is usually the case when the fluxes close to 0). We also measured soil volumetric water content (SWC) and soil temperature at 10 cm and stem temperature at 5 cm at each chamber location (EC-5; Decagon Devices).

In addition, to better understand the spatial variability of CO_2 and CH_4 stem fluxes, we manually measured fluxes at an additional 15 trees, at three stem heights (50, 100, and 150 cm), every 2 weeks between April and December 2017. We installed 78.5 cm^2 PVC collars at each height and performed manual measurements (4-min observations) with a cavity ringdown spectroscopy gas analyzer (Ultra-Portable Greenhouse Gas Analyzer; Los Gatos Research) around midday (10:00–15:00). We calculated stem fluxes from manual measurements using the following equation (Pumpanen et al., 2004):

$$F = \left(\frac{dC}{dt}\right)\left(\frac{V_c}{A_c}\right)\frac{P}{(R * (T + 273.15))},$$

where F is the flux of a particular gas, dC/dt is the change in concentration over time (ppm^{-1}) estimated with both linear and exponential fits, V_c is the system volume (0.001135 m^3), A_c is the measured area (0.0095 m^2), P is the atmospheric pressure measured at the center of the plot for each particular time, R is the ideal gas law constant (0.00 831447 kg m^2 μmol^{-1} K^{-1} s^{-2}), T is stem temperature (°C), and 273.15

is the conversion factor from Celsius to Kelvin. We applied the same QA/QC protocol as for the automated measurements. Additionally, we used a noncontact infrared thermometer (Nubee NUB8500H) for measuring the stem surface temperature associated with each flux measurement.

2.3 | Ancillary data

We installed PVC pipes (5 cm in diameter) into the soil at 10, 25, 50, 75, and 100 cm and at groundwater depth (around 150 cm) within 1 m of each tree equipped with automated measurements, for a total of three soil profiles. The top of each pipe was sealed, whereas the bottom was open to allow the pipe's internal concentrations of CO_2 and CH_4 to equilibrate with soil atmosphere at each target depth. We measured soil CO_2 and CH_4 concentrations at each depth using a closed-loop approach during three campaigns (October 2017, August 2018, and March 2019) with an Ultra-Portable Greenhouse Gas Analyzer (Los Gatos Research). In addition, we measured SWC and soil temperature at 10 cm (5TE, METER Group), air temperature, atmospheric pressure and relative humidity (VP-4 Sensor [Temp/RH/Barometer]; METER Group), and wind speed and wind direction (DS-2m; METER Group) during the entire experiment using digital data loggers (Em50; METER Group). We also measured the water table level every 15 min at the center of the plot (WL16U-003-10; Global Water).

2.4 | Heartwood CH_4 concentrations and tree core incubations

On August 20, 2018, we extracted tree cores with an increment borer at each tree stem height for each of the 18 trees measured throughout the study. After extracting the sample but before removing the increment borer, we directly measured CO_2 and CH_4 concentrations within the stem with a CO_2Meter (MH-Z92 Dual Gas CO_2/CH_4 meter), suitable for measuring high concentrations (range 0%–100% vol), coupled with an Ultra-Portable Greenhouse Gas Analyzer (Los Gatos Research), suitable for accurate low concentrations (0–500 ppm). In order to compare internal stem concentrations with stem fluxes, we measured stem fluxes 5 days before sampling the tree cores at all stem heights with the same instrumental setup as described for manual measurements.

Right after collecting each core, we split samples into sapwood and heartwood fractions, placed each fragment in an incubation jar (350 ml), and flushed the jars in the field with He for 2 min at 2 L min^{-1}. Over the following 3 days, we replaced the air in the jars twice using an anaerobic chamber (95% N_2 and 5% H_2), in order to guarantee a CH_4-free atmosphere for the incubations. After that, we kept samples at constant temperature and under dark conditions for 6 h, before transferring a 15 ml headspace sample into a preevacuated glass vial (Exetainer; Labco) to be analyzed with a gas chromatograph equipped with a flame ionization detector for CH_4 and a thermal conductivity detector for CO_2 (Shimadzu Model 2014; Covey et al., 2012). Since we only measured concentrations in the headspace at one time and the humidity was not controlled during the incubation, we could not calculate absolute CO_2 and CH_4 production rates. Instead, we report the production potential of each sample.

Additionally, we took an extra tree core per tree at 150 cm stem height on the same day as the other cores (August 20, 2018). For each of these extra cores, we measured the thickness of sapwood, the fresh and dry weight (48 h in an oven at 70°C) of sapwood and heartwood fractions, and wood density of both fractions in order to test the potential effect of wood density and moisture on stem fluxes.

2.5 | Statistical analyses

For manual flux measurements, we analyzed if stem height (factor with three levels: 50, 100, and 150 cm) influenced CO_2 and CH_4 fluxes. We used linear mixed-effects models (LMM) as implemented in the “nlme” R package (Pinheiro et al., 2021) with tree identity as a random factor. We applied the Bonferroni test when stem height was statistically significant ($p < 0.05$). For the manual flux measurements, we also performed a second set of LMMs to analyze the effect of stem diameter, stem temperature, SWC, and the first-order interaction between each of the two latter variables and stem height on CO_2 and CH_4 fluxes. The purpose of this additional analysis was to test whether environmental responses of fluxes depend on stem height. Additionally, the same LMM models including total wood moisture and density, and sapwood moisture and density on top of stem diameter, stem temperature, and SWC were tested for a subset of manual measurements (just for those with information about wood properties at 150 cm stem height [667 measurements, 14 trees]) to test the potential effect of wood density and moisture on CH_4 fluxes.

For automated flux measurements, we analyzed CO_2 and CH_4 fluxes using LMMs that included the second-order interaction between stem height (in this case, there are two levels: 50 and 150 cm), stem temperature, and SWC. Given the high correlation between SWC and water table level ($R^2 = 0.87$), the latter was not included in the models.

In order to explore the relationship between CO_2 and CH_4 stem fluxes and its dependency with stem height, we used LMMs for both manual and automated measurements with CH_4 as the dependent variable and the interaction between CO_2 and stem height as predictors.

All models for CO_2 and CH_4 fluxes (manual and automated measurements) included a random variation of the intercept and, when applicable, of the temperature coefficient associated with tree identity (random slopes model). Because of lack of convergence, the model for manual CH_4 fluxes was fitted allowing only the intercept to vary with tree identity (random intercept model). In order to achieve normality of the residuals, CO_2 flux was log-transformed and CH_4 flux was Box-Cox transformed ("bcnPower" function in "car" R package, Fox & Weisberg, 2019) with lambda values (−0.055 and −0.087 for manual and automated CH_4 fluxes, respectively) obtained using maximum-likelihood, as implemented in the "powerTransform" function from the R package car (Fox & Weisberg, 2019). For the LMM with automated CH_4 measurements, we modeled the different variance per tree using the "varIdent" function from the nlme R package. In all cases, we considered temporal autocorrelation by introducing a discrete-time first-order autocorrelation error structure and calculated the fraction of variance explained by fixed effects (marginal r^2, R^2_m) and by fixed and random effects (conditional r^2, R^2_c), using the approach from Nakagawa and Schielzeth (2013).

In order to understand whether stem internal concentrations for each gas could be related to stem fluxes or to stem height, we tested different LMMs between concentrations measured at the final campaign (August 2018) and stem height or stem fluxes measured (i) during the same week or (ii) the mean stem fluxes throughout the 2017 growing season. We also tested if wood rot presence (visually identified from tree cores) was related to stem CH_4 fluxes. First, predictions of tree- and height-specific stem CH_4 fluxes using the model for manual measurements (Table 1) were obtained by using the tree-level random coefficients of the model and fixing stem temperature at 20°C, SWC at 0.25 *v*/*v*, DBH at 30 cm, and day of the year (DOY) 220. Predictions were also made at the tree level by additionally fixing stem height at 50 cm. Then, we tested whether these predictions were affected by the presence of wood rot at the stem height or at the tree level using a linear model.

Finally, we tested the difference between heartwood and sapwood production of CO_2 and CH_4 using LMMs (random intercept model), with stem height nested within tree.

3 | RESULTS

3.1 | Magnitudes and temporal and spatial variability of stem fluxes

During the study period, we collected over 38,000 automated measurements and 678 manual measurements of CO_2 and CH_4 stem fluxes from 18 hickory trees. Mean CO_2 stem fluxes during the study period were 2.97 ± 0.41 and 2.17 ± 0.49 μmol m^{-2} s^{-1} (mean ± 95% CI) for automated and manual measurements, respectively. On average, trees were net sources of CH_4 (2.54 ± 4.35 and 0.24 ± 0.16 nmol m^{-2} s^{-1} derived from automated and manual measurements, respectively). Despite generally being sources of CH_4, occasionally trees also showed CH_4 uptake from the atmosphere (17.7% and 22.9% of measurements for automated and manual measurements, respectively).

Stem CO_2 emissions measured with manual chambers showed a clear seasonal pattern among all trees over the study period, with emissions increasing throughout spring and summer, peaking at the beginning of August (DOY≃220), and then decreasing towards the end of the experiment (Figure 1).

TABLE 1 Summary of the linear mixed-effects models for manual measurements of CO_2 and CH_4 stem fluxes. Stem height at 50 cm was used as a reference category and included in the intercept. CO_2 and CH_4 fluxes were log and Box-Cox transformed, respectively, to achieve normality in the residuals. R^2_m is the variance explained by the fixed effects (marginal) and $R^2_c$is the variance explained by the entire model, including both fixed and random effects (conditional)

Manual CO_2	Variables	Estimate (SE)	*p*-value
	(Intercept)	−3.17 (0.32)	<0.001
R^2_m: 0.42	Temperature	0.070 (0.005)	<0.001
R^2_c: 0.67	Height 100 cm	−0.075 (0.36)	0.835
	Height 150 cm	0.20 (0.36)	0.571
	SWC	5.10 (0.55)	<0.001
	DBH	0.013 (0.006)	0.040
	Temp*Height 100 cm	0.0086 (0.004)	0.022
	Temp*Height 150 cm	−0.0020 (0.004)	0.594
	SWC*Height 100 cm	−0.49 (0.95)	0.602
	SWC*Height 150 cm	−0.34 (0.95)	0.724
Manual CH_4	(Intercept)	−4.64 (0.98)	<0.001
	Temperature	0.045 (0.007)	<0.001
R^2_m: 0.068	Height 100 cm	0.93 (1.09)	0.394
R^2_c: 0.37	Height 150 cm	0.75 (1.08)	0.489
	SWC	3.35 (1.66)	0.045
	DBH	0.0058 (0.02)	0.745
	Temp*Height 100 cm	−0.016 (0.01)	0.203
	Temp*Height 150 cm	−0.0061 (0.01)	0.630
	SWC*Height 100 cm	−2.87 (2.88)	0.319
	SWC*Height 150 cm	−1.95 (2.88)	0.499

Abbreviations: DBH, diameter at breast height; SWC, soil volumetric water content.

Similar seasonal patterns were found both within and between trees, despite the differences in the magnitudes of CO_2 flux from tree to tree. Manual stem CO_2 fluxes presented a vertical pattern ($p < 0.001$) with higher emissions at 50 cm than at upper heights (i.e., 100 and 150 cm). Automated stem CO_2 fluxes also showed a clear seasonal pattern among trees, with emissions increasing until mid-summer and then decreasing until the end of autumn (Figure 2a,c,e). For trees measured with our automated flux system, CO_2 emissions at 50 cm were higher than at 150 cm (3.89 ± 0.46 and 2.06 ± 0.29 μmol m^{-2} s^{-1} [mean ± SD], respectively).

Manual measurements of stem CH_4 fluxes did not show a clear seasonal pattern (Figure 3). Stem CH_4 emissions decreased with stem height ($p < 0.001$), where magnitudes at 50 cm were the highest ($p < 0.001$) and magnitudes at 100 cm were marginally higher than at 150 cm ($p = 0.068$). In contrast, automated measurements of stem CH_4 fluxes varied greatly within days, but when hourly measurements

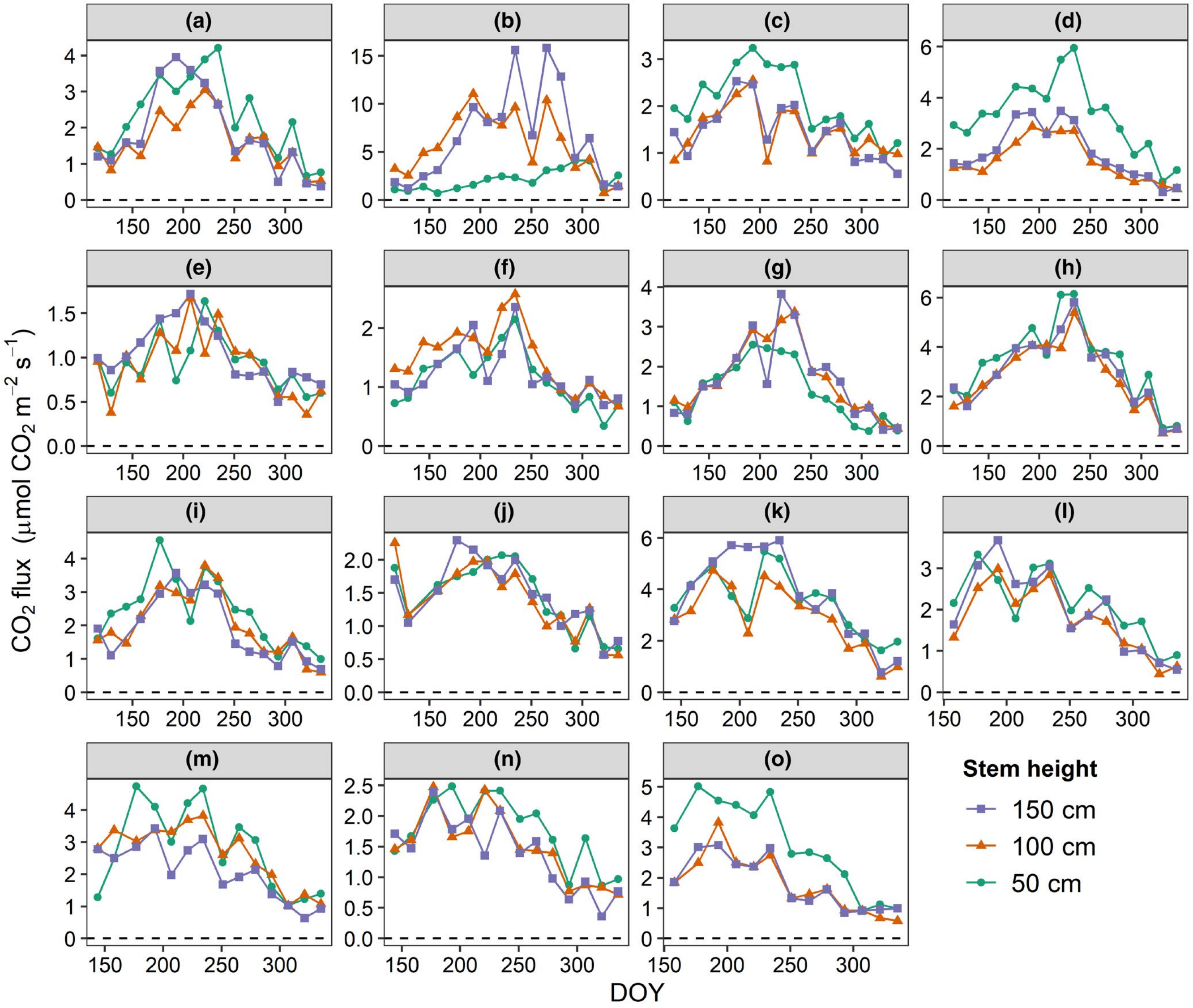

FIGURE 1 Manual measurements of CO_2 emissions from 15 individual trees (different letters in the panels' headers indicate different trees) at different stem heights. Emissions were measured every 2 weeks from April to December 2017. Note that the scale of *y*-axis is adjusted for each tree to improve clarity [Colour figure can be viewed at wileyonlinelibrary.com]

were integrated into daily means, these showed clear seasonal trends, with emissions peaking around the end of the summer and decreasing toward the end of autumn (Figure 2b,d,f). Five out of six chambers presented similar magnitudes of stem CH_4 fluxes (0.51 ± 0.45 nmol m^{-2} s^{-1}; mean ± SD), but one chamber showed mean fluxes that were 20 times higher (12.37 ± 5.33 nmol m^{-2} s^{-1}; mean ± SD).

3.2 | Drivers of stem CO_2 and CH_4 fluxes

The LMM of manual stem CO_2 emissions as a function of environmental drivers was able to explain 67% of the variance, although only 42% was explained by fixed effects (i.e., stem height, DBH, SWC, and temperature; Table 1). This model showed a positive effect of temperature and a marginal interaction of temperature with stem height (Type III ANOVA, $\chi^2 = 3.42$, $df = 1$, $p = 0.064$). Soil moisture also had a strong positive effect on stem CO_2 emissions but this effect did not vary with stem height (Table 1). Stem CO_2 emissions also increased with increasing DBH (Table 1). For the CH_4 manual measurements, stem temperature and soil moisture showed a positive effect, independent of stem height (Table 1). The model explained 37% of stem CH_4 fluxes variability, but only 7% was explained by fixed effects (Table 1). When we tested the effect of wood density and moisture (total or sapwood component) in a subset of data (where wood properties information was available), no effect of those variables was detected on stem manual CH_4 fluxes (Table S2).

Automated stem CO_2 emissions positively responded to the interaction between stem temperature and SWC (Table 2). In addition, the interaction between stem height and SWC was also significant, while that of stem height and stem temperature was only marginally significant (Table 2). This complex model outcome resulted in higher CO_2 emissions with high SWC at high temperatures, particularly at 150 cm (Figure S1). Overall, fixed effects in the model explained 81% of the

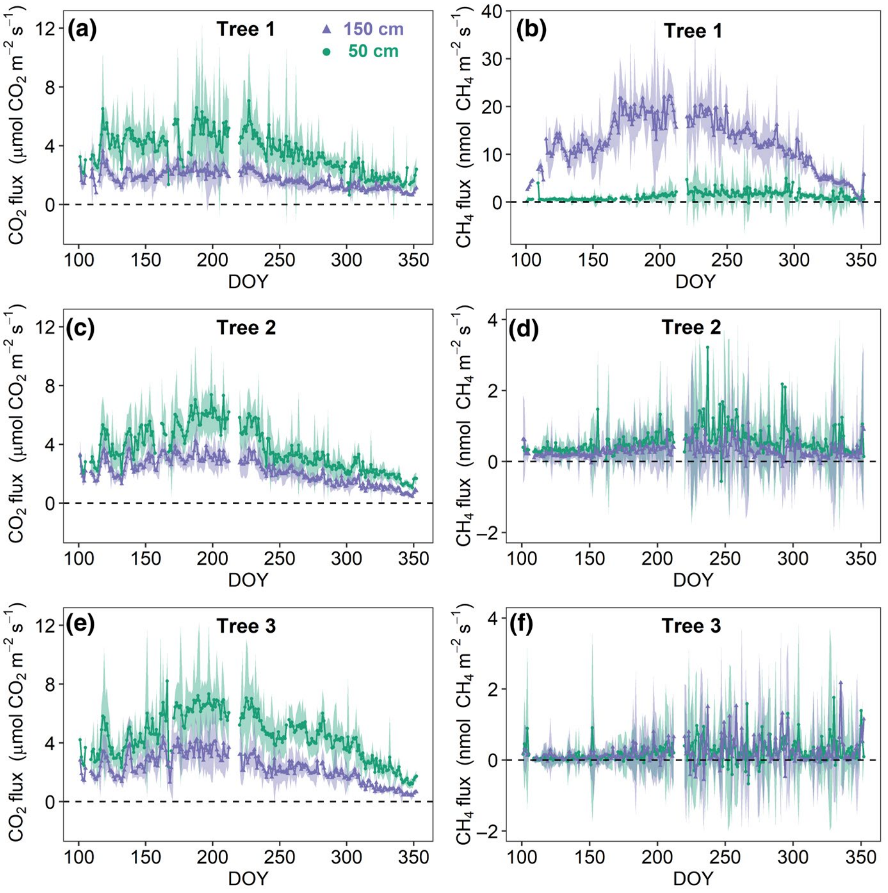

FIGURE 2 Automated measurements of CO_2 and CH_4 stem fluxes during one growing season (April–December 2017) at three different trees and two different stem heights (red for 50 cm and blue for 150 cm). Dots represent daily averages of hourly measurements, and shaded areas represent SDs. Plotted standard deviations in panels (d) and (f) are constricted between −2 and 4 nmol m^{-2} s^{-1}. The *y*-axis for panel (b) has a different scale because of extremely high fluxes compared with the other two trees [Colour figure can be viewed at wileyonlinelibrary.com]

variability of stem CO_2 emissions (Table 2). In contrast, automated CH_4 fluxes were partially explained by SWC interacting with stem height (Table 2), with fluxes at 50 cm increasing as SWC increased but those at 150 cm decreasing at high SWC (Figure S2). The interaction between stem temperature and SWC was only marginally significant (Table 2), with CH_4 fluxes responding more to a temperature at higher levels of SWC. The model explained 89% of the variability in stem CH_4 fluxes, but only 11% was explained by fixed effects.

We explored to what extent stem CO_2 fluxes could explain the temporal patterns in CH_4 fluxes. Stem CH_4 fluxes were positively related to stem CO_2 fluxes but the slope of this relationship tended to diminish with stem height, for both manual and automated measurements (Table S1). However, the variability in stem CH_4 fluxes explained by the fixed effects (i.e., stem CO_2 fluxes and stem height) was lower than 10% in both cases (Figure S3).

3.3 | Insights about the origin of stem CO_2 and CH_4 fluxes

Stem CO_2 concentrations measured at the end of August 2018 showed high values in the heartwood of the stems (median = 15,000 ppm; Figure 4a), with no significant differences with stem height (p = 0.86). The heartwood also showed high CH_4 concentrations (median = 1000 ppm), with no significant differences with stem height either (p = 0.32). Neither CO_2 nor CH_4 concentrations presented significant correlations with stem fluxes measured 5 days before nor with the mean fluxes over the whole experiment ($p > 0.05$ in all cases).

CO_2 concentrations in the soil profile showed similar patterns during the three measurement campaigns, with lower values at the beginning of spring compared with the summer and the autumn (Figure 4c). In general, concentrations increased with soil depth down to 25 cm (March) or 50 cm (August and October), and from there, decreased with depth, achieving the minimum concentration at the groundwater level (~150 cm depth). CH_4 concentrations in the soil profile showed similar trends across seasons, dropping below the atmospheric concentration (1.71 ± 0.03 ppm; mean ± SD) along the first 25 cm, and from then, increasing until 50 cm depth, achieving concentrations slightly above atmospheric. At deeper layers, CH_4 concentrations did not show a clear pattern, with no major changes compared to concentrations at 50 cm.

While Bitternut Hickory trees usually present hard, strong, and durable wood which makes the species relatively resistant to heart rot (Berry & Beaton, 1972), we found that 8 tree cores out of 51 (from four different trees) presented visual evidence of wood rot. That said, no significant relationship between rot presence and modeled CH_4 fluxes was found at the tree level (rot presence anywhere in the tree; p = 0.28) or at the core level (p = 0.46).

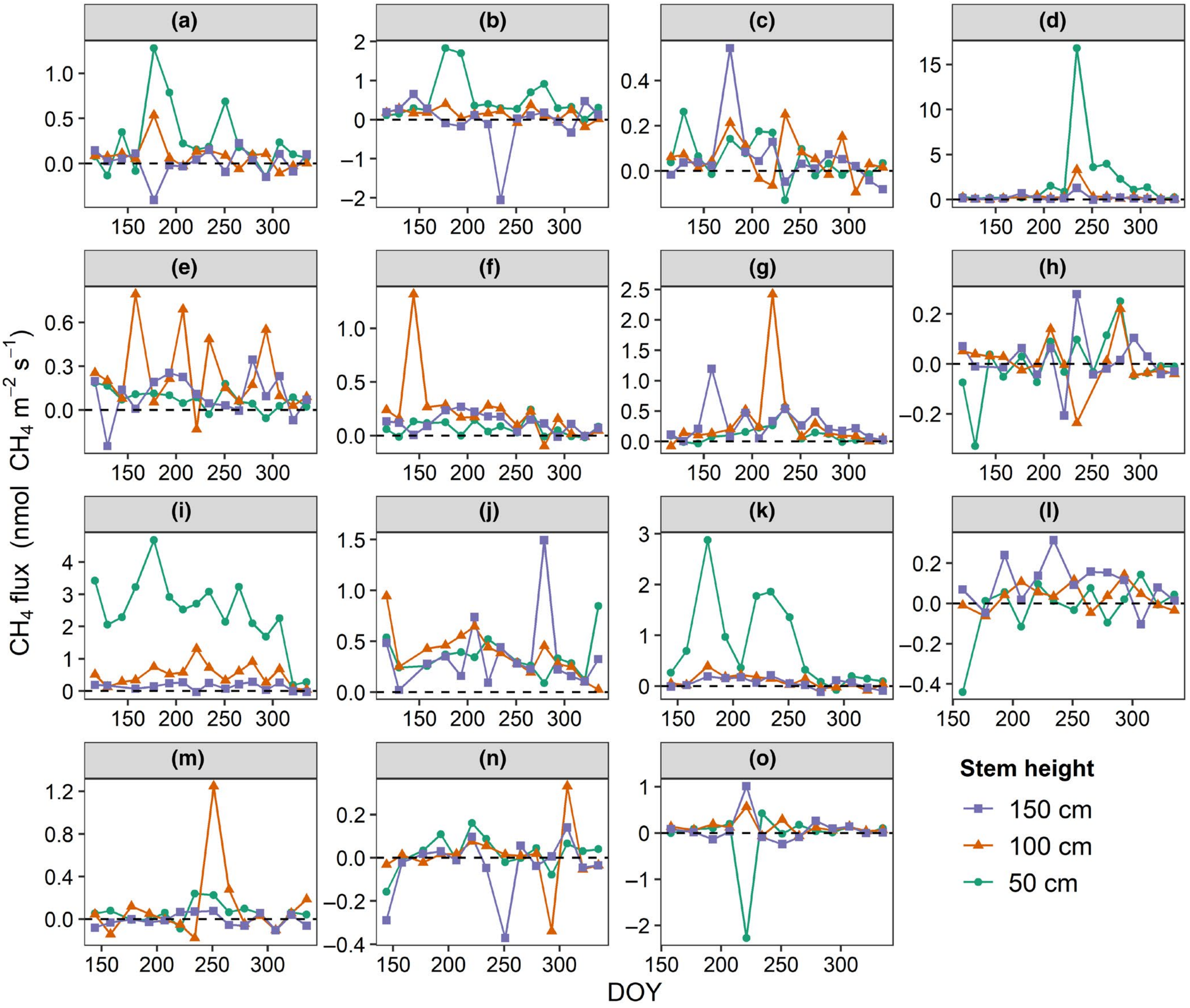

FIGURE 3 Manual measurements of CH_4 fluxes of 15 trees (different letters in the panels' headers indicate different trees) at three different stem heights. Fluxes were measured every 2 weeks from April to December 2017. Note that the scale of *y*-axis is adjusted for each tree to improve clarity [Colour figure can be viewed at wileyonlinelibrary.com]

Tree core incubations revealed that all tree cores produced CO_2 (Figure 5a), with higher production from the sapwood than from the heartwood ($p < 0.001$). All tree cores were able to produce CH_4 as well (Figure 5b), with higher production in the heartwood than in the sapwood ($p = 0.013$).

4 | DISCUSSION

Our results demonstrate that all tree stems (18 individuals) emit CH_4 throughout the experimental period at all stem heights. Moreover, around 20% of the measurements (both automated and manual) showed a net CH_4 uptake by tree stems, indicating that net stem-atmosphere CH_4 fluxes might be a balance between CH_4 production and consumption that may be co-occurring in this interface. This cryptic balance between production and consumption complicates estimates of net stem CH_4 fluxes and the identification of dominant drivers. Several studies measuring stem CH_4 emissions have reported some uptake measurements as well (e.g., Pitz & Megonigal, 2017; Warner et al., 2017; Welch et al., 2018), but little has been discussed about the potential of tree stems acting not only as sources but also as sinks of CH_4 (but see Machacova et al., 2020), which might have implications for the development of future process-based ecosystem models.

4.1 | Magnitudes and temporal patterns of stem CO_2 and CH_4 fluxes using manual and automated measurements

Automated and manual measurements presented comparable mean stem CO_2 emissions (2.97 ± 0.41 and 2.17 ± 0.49 μmol m^{-2} s^{-1}, automated and manual, respectively), but this was not the case for CH_4.

TABLE 2 Summary of the LMMs for automated measurements of CO_2 and CH_4 stem fluxes. Stem height at 50 cm is used as a reference category and included in the intercept. CO_2 and CH_4 fluxes were log and Box-Cox transformed, respectively, to achieve normality in the residuals. R^2_m is the variance explained by the fixed effects (marginal) and R^2_c is the variance explained by the entire model, including both fixed and random effects (conditional)

Automated CO_2	Variables	Estimate (SE)	*p*-value
	(Intercept)	1.53 (0.27)	<0.001
R^2_m: 0.81	Temperature	−0.0044 (0.014)	0.750
R^2_c: 0.84	Height 150 cm	−1.42 (0.27)	<0.001
	SWC	−3.87 (0.90)	<0.001
	Temp*Height 150 cm	0.025 (0.014)	0.070
	Temp*SWC	0.19 (0.047)	<0.001
	SWC* Height 150 cm	2.24 (0.93)	0.016
	Temp*SWC*Height	−0.052 (0.034)	0.285
Automated CH_4	(Intercept)	−2.00 (0.82)	0.015
	Temperature	−0.035 (0.037)	0.336
R^2_m: 0.11	Height 150 cm	4.29 (0.70)	<0.001
R^2_c: 0.89	SWC	2.72 (2.00)	0.174
	Temp*Height 150 cm	−0.016 (0.038)	0.700
	Temp*SWC	0.19 (0.10)	0.068
	SWC*Height 150 cm	−13.90 (2.48)	<0.001
	Temp*SWC*Height	0.073 (0.13)	0.588

Abbreviations: DBH, diameter at breast height; SWC, soil volumetric water content.

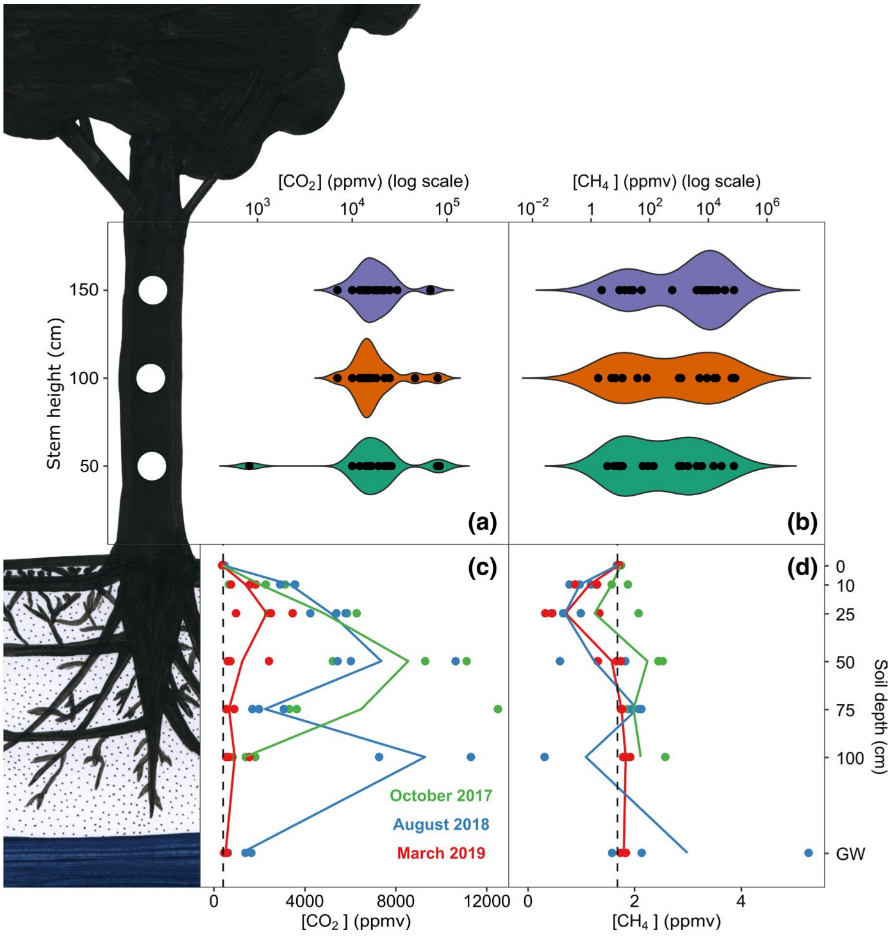

FIGURE 4 CO_2 and CH_4 concentrations in the stem heartwood and in the soil profile. Heartwood concentrations (panels a and b) were measured in August 2018. Violin plots represent log CO_2 and CH_4 concentrations at different stem measuring heights (black dots), with colored areas depicting a kernel density plot showing the distribution of concentrations. Soil concentrations (panels c and d) were measured at three soil profiles including groundwater (GW; around 150 cm deep) in the vicinity of the trees with automated measurements. For panels (c) and (d), different colors represent different sampling dates, dots represent single measurements, solid lines represent average fluxes of the three profiles for each depth, and dashed lines represent atmospheric concentrations of each gas [Colour figure can be viewed at wileyonlinelibrary.com]

Mean stem CH_4 flux from automated chambers was 10 times larger than that obtained from manual measurements (2.54 ± 4.35 and 0.24 ± 0.16 nmol m^{-2} s^{-1} for automated and manual measurements, respectively). This discrepancy was influenced by one automated chamber that presented mean fluxes 20 times higher than all the other automated chambers (Figure 2b), also resulting in a wider

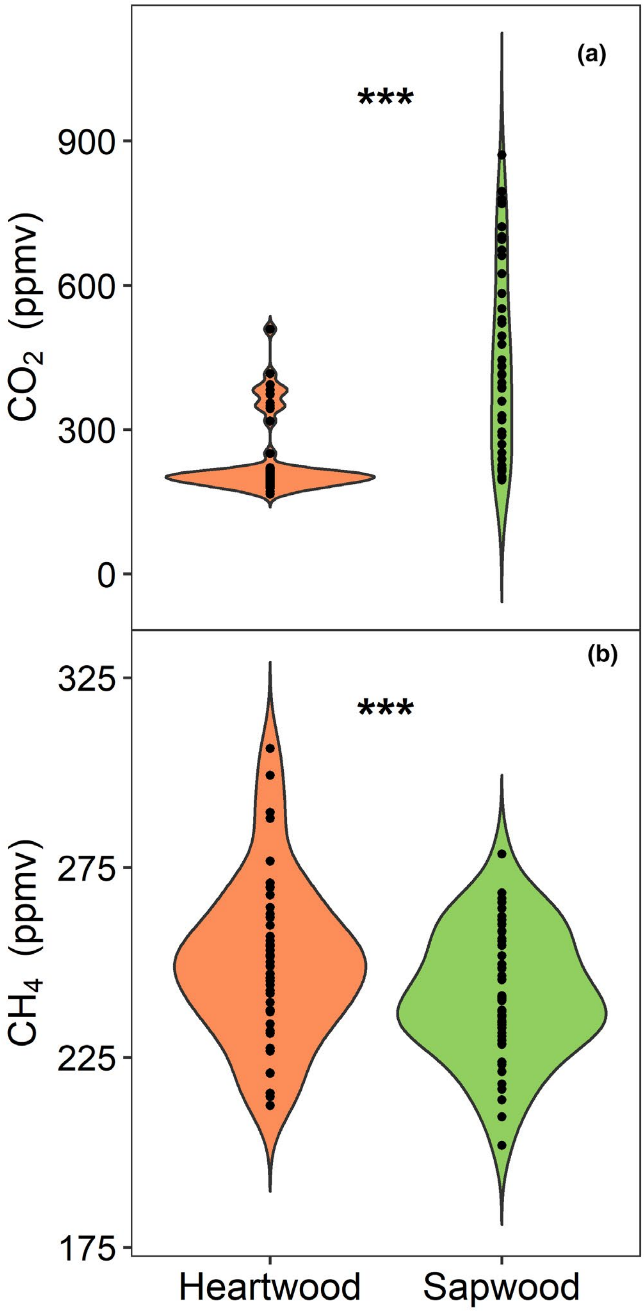

FIGURE 5 CO_2 and CH_4 tree core incubations. CO_2 and CH_4 concentrations in the incubation jars (panels a and b, respectively) after anaerobic incubations of heartwood and sapwood tissues of the different stem heights from the 18 studied trees (sampled in August 2018). Violin plots represent CO_2 and CH_4 concentrations (black dots), with colored areas depicting a kernel density plot showing the distribution of concentrations. Asterisks indicate differences between heartwood and sapwood concentrations [Colour figure can be viewed at wileyonlinelibrary.com]

CI (i.e., uncertainty) for the automated measurements compared with manual measurements. When that particular chamber was removed, the mean CH_4 flux from automated measurements was about 50% higher than that from manual measurements (0.51 ± 0.45 nmol m^{-2} s^{-1} and 0.24 ± 0.16 nmol m^{-2} s^{-1} for automated and manual measurements, respectively). These results indicate that annual estimates based on automated measurements might be sensitive to hot spots, both between trees and/or within trees, due to the limited number of automated chambers associated with logistical challenges and costs of automated systems.

The strength of manual measurements is to better integrate spatial variability of stem fluxes (including hot spots) than automated approaches due to their suitability for measuring a larger number of trees, which might result in more spatially representative estimates. That said, because of the high temporal variability of stem CH_4 fluxes shown by automated measurements (see next paragraph), our results indicate that manual measurements are clearly missing temporal variability and hot moments of CH_4 fluxes (as demonstrated by the lower CV), which might have a strong impact on the calculation of annual fluxes. The mean growing season stem CH_4 fluxes estimated with manual measurements (0.24 ± 0.16 nmol m^{-2} s^{-1}) is within the range of values reported for other upland forests (Pitz et al., 2018 and references therein) but our results challenge the studies that only rely on manual measurements to estimate annual fluxes.

Stem CO_2 emissions presented a clear seasonal pattern with both manual and automated measurements, again, showing coherence between both approaches. Seasonal patterns of stem CO_2 emissions are consistent with tree physiological activity peaking during the growing season (Teskey et al., 2008). Stem CH_4 fluxes also presented a clear seasonal pattern (supporting H1), but this was only evident with the automated measurements where the integration of the high variability of hourly measurements (including hot moments) into daily means allowed the seasonal pattern to emerge. We argue that the lack of observed seasonal patterns in other studies using manual measurements in upland forests (with a typical measurement frequency of 2–3 weeks; Pitz & Megonigal, 2017; Pitz et al., 2018; Warner et al., 2017; Welch et al., 2018) may be because subdaily variability was not accounted for. Studies performing high-frequency measurements could integrate the high variability within days (Barba, Bradford, et al., 2019) contributing to an emergent seasonal pattern (Barba, Poyatos, et al., 2019; Plain et al., 2019).

Overall, these results underline the challenges that manual measurements may have for estimating temporal patterns and the challenges that both manual and automated measurements may have for estimating mean annual CH_4 fluxes from tree stems. Worldwide studies are needed to test the temporal variability of stem CH_4 fluxes combining manual and automated measurements and to evaluate the consistency between both approaches to accurately estimate temporal patterns and annual fluxes. These efforts are crucial if we aim to include tree stem CH_4 fluxes into local or global carbon budgets.

4.2 | Drivers of stem CO_2 and CH_4 fluxes

Temperature and SWC played an important role in controlling CO_2 and CH_4 stem fluxes as proposed in (a) in Hypothesis 2. For CO_2 fluxes, temperature, SWC, stem height, and some of their interactions explained a large proportion of stem CO_2 emissions variability

(R^2_m of 0.81 and 0.42, automated and manual measurements, respectively). These environmental variables as well as plant activity have previously been identified as drivers of stem CO_2 emissions (Ceschia et al., 2002; Gansert & Burgdorf, 2005). Stem CO_2 emissions also increased with tree DBH, consistent with more sapwood tissue contributing to stem CO_2 efflux via stem respiration in larger trees (Teskey et al., 2017). Our results support the fact that identifying functional relationships for stem CO_2 fluxes is consistent when derived from manual or automated measurements.

The role of stem temperature and SWC for stem CH_4 fluxes presented some similarities between approaches (Tables 1 and 2). Both variables showed a positive independent effect for CH_4 manual measurements but an interacting effect for automated measurements (positive marginal significance; Tables 1 and 2). Our results thus partly agree with other studies (derived from manual measurements) reporting larger CH_4 fluxes with increasing soil temperature (Pitz et al., 2018; Wang et al., 2016). Stem CH_4 fluxes increased consistently with SWC (Tables 1 and 2), although in the case of automated measurements, this was supported only for the 50 cm height (Figure S2). These results clearly differ from other studies (derived from manual measurements) showing no effect of SWC on stem CH_4 fluxes (Pitz & Megonigal, 2017; Warner et al., 2017; Welch et al., 2018). Thus, our results compound the challenge of finding consensus for incorporating stem CH_4 fluxes and associated functional relationships into process-based models.

We found a positive relationship between stem CO_2 and CH_4 supporting (b) in Hypothesis 2, which was also reported in a riparian cottonwood forest ecosystem (slope of correlation changed seasonally; Flanagan et al., 2021) and in a previous study using daily means of CO_2 and CH_4 fluxes (Vargas & Barba, 2019). This correlation could be attributed to gas diffusivity heterogeneity through the wood, which might similarly affect both gases (Barba, Bradford, et al., 2019). However, stem CO_2 fluxes only explain a small fraction of stem CH_4 flux variability in this study ($R^2_m < 0.1$ for both manual and automated measurements), hindering predictions of stem CH_4 fluxes based on stem CO_2 fluxes as previously suggested (Vargas & Barba, 2019). We recognize that the underlying mechanisms controlling stem CH_4 fluxes should be properly elucidated to move beyond empirical relationships and build process-based models to predict stem CH_4 fluxes.

The effect of tree attributes such as DBH and stem height in modulating environmental controls of stem fluxes supported our hypothesis H3, but these effects were not consistent across CO_2 and CH_4 fluxes. Tree DBH was associated with larger stem CO_2 fluxes but we observed no DBH effect on stem CH_4 fluxes (DBH only tested for manual measurements due to the limited number of trees for automated measurements). There is no consensus on the effect of DBH on CH_4 fluxes in upland forests, with positive relationships found in some studies (Pitz et al., 2018; Wang et al., 2017) but not in others (Warner et al., 2017). However, we expected bigger trees to present higher fluxes because of their potentially higher capacity of transporting CH_4 from a larger soil volume and/or because of their higher volume of heartwood for internally producing methane (see next section for CH_4 origin discussion). The fact that we only studied mature trees with a relatively narrow range of diameters might hinder our capacity to detect a DBH effect that may be evident with a larger gradient of stem diameters. The effect of stem height was also more evident for CO_2 than for CH_4 (manual measurements). The observed decline in CH_4 fluxes with stem height was only detected when stem height was the sole factor in the model, disappearing when other drivers were included (Table 1). For automated measurements, the influence of stem height on stem CH_4 fluxes was only detected in interaction with SWC, and the model outcome did not support a strong decline in stem CH_4 fluxes with stem height (Figure S2). Additionally, concurrent stem CH_4 uptake, as seen in other studies (Jeffrey et al., 2021; Machacova et al., 2016, 2020; Pitz & Megonigal, 2017; Welch et al., 2018), may mask potential patterns between CH_4 fluxes and stem height (Barba, Bradford, et al., 2019).

Our results on stem height effect contrast with other studies reporting CH_4 fluxes (mainly from manual measurements) declining with stem height (Jeffrey et al., 2020; Pitz & Megonigal, 2017; Sjögersten et al., 2020; Wang et al., 2016), suggesting that the relationship between fluxes and stem height might be species- or site-specific. However, most of the stem flux measurements are usually limited to the first 2–3 m above the soil due to logistical constraints, indicating that further investigations measuring CH_4 fluxes upper in the canopy (e.g., branches and twigs) may be crucial to properly upscale chamber measurements to a whole-tree level and to properly model the effect of changes (if any) in radial CH_4 diffusion and production throughout tree stems (Barba, Bradford, et al., 2019).

The proportion of CH_4 variability explained by SWC, temperature, stem height, and associated interactions was small (6.8% and 11% for manual and automated measurements, respectively), contributing to the increasing evidence on the complications involved for modeling CH_4 fluxes from multiple land surfaces (Ringeval et al., 2014; Vázquez-Lule & Vargas, 2021). The consistency between manual and automated measurements in the lack of explanatory power of the measured drivers might suggest that we probably did not measure the appropriate variables. Other variables, such as soil CH_4 fluxes or plant phenology, may explain more variability of stem fluxes (Barba, Poyatos, et al., 2019). However, most of the CH_4 flux variability in the current study was explained by tree identity, allocated in the random part of the models. We did not expect such a large effect of tree identity (i.e., differences between trees) because all measured trees were mature and from the same species. They also grew in flat and apparently homogeneous terrain (Petrakis et al., 2018) and were relatively close to each other (maximum distance between trees around 70 m). This might suggest that tree traits in the vicinity of the flux measurement, or processes that affect methane production, consumption, and transport (e.g., methanogenic and methanotrophic microbial communities), might be more relevant in determining stem CH_4 fluxes at the ecosystem scale than environmental conditions. We found a significant effect of wood density and moisture on CO_2 stem fluxes, but not on CH_4 fluxes, which might imply that CH_4 fluxes are less related to wood features than CO_2, and that the source of CH_4 is much more localized than the source of CO_2 (respiration tissue is everywhere and CO_2 could be transported from soils). However, other wood properties, such as tree wounds, water distribution within stems, and lenticels density, might still affect gas diffusivity in the wood, and thus, control CH_4 fluxes. The relatively small magnitudes of stem fluxes measured in this study contrast with the high internal CH_4 concentrations; this suggests that wood properties controlling gas diffusivity from the heartwood to the atmosphere might be key factors explaining stem CH_4 fluxes. If that is the case, it could imply that the classical temperature and moisture kinetic relations commonly applied for modeling fluxes of greenhouse gases might not be suitable for upscaling and predicting stem CH_4 fluxes. Overall, these results emphasize the need to incorporate tree-specific traits and processes to better understand spatial and temporal variability of stem CH_4 fluxes.

4.3 | Evidence for the xylem origin of stem CH_4 fluxes

Our results provide evidence that emitted CH_4 is likely produced inside tree stems. First, most trees did not show a consistent decrease in fluxes with stem height, which is usually attributed to the possibility that CH_4 is produced in soils (Jeffrey et al., 2020; Pitz & Megonigal, 2017; Sjögersten et al., 2020; Wang et al., 2016). In this case, it is expected that CH_4 produced in the soil is transported by the roots and emitted by stem degasification and consequently CH_4 emissions decrease with stem height (Barba, Bradford, et al., 2019).

Second, soils in the study site were net sinks of methane (Barba, Poyatos, et al., 2019; Petrakis et al., 2018), and there was a strong decline in CH_4 soil concentrations throughout the soil profile (Figure 4d). In theory, even in locations where soils are net sinks of CH_4, soils still could be the origin of CH_4 stem fluxes, if larger concentrations of CH_4 are produced (or stored) deeper in the soil profile and transported by roots into the tree, bypassing the soil's uppermost methanotrophic layer (Megonigal & Guenther, 2008). However, this deep soil transport does not seem feasible in our case as the highest soil CH_4 concentration measured in the whole soil profile (up to the groundwater) was <5 ppm and there was no evidence of CH_4 produced within the soil profile. Additionally, a study performed in the same region measuring *Carya* sp. fine roots density showed that most of the fine roots were allocated is the uppermost 15 cm (Davis et al., 2004), a soil zone where we found lower than atmosphere CH_4 concentrations (Figure 4d). Overall, thermodynamic principles and our soil CH_4 measurements suggest that it is unlikely that CH_4 was produced in the soil and transported to the tree stems. Further studies measuring soil redox and O_2 concentrations along with the characterization of fine roots biomass and distribution in the soil profile could provide more evidence for discarding soil as the origin of stem emitted CH_4.

Third, CH_4 internal concentrations in most trees were very high (up to 82,000 ppm). These internal concentrations did not correlate with stem fluxes measured in the same week or with the study period integrated flux measurements (likely due to differences in radial wood diffusivity). These results are supported by previous observations (from manual measurements), where heartwood concentrations did not correlate with stem fluxes unless longer periods of stem concentrations were integrated with the analysis (Wang et al., 2017). A lack of correlation between stem internal concentrations and fluxes challenges the application of models based on diffusion gradients to estimate stem CH_4 fluxes (Covey et al., 2012; Wang et al., 2021). Furthermore, if we did not find evidence of CH_4 produced or stored in the soil profile, then where do the high internal CH_4 concentrations come from? Further studies measuring internal CH_4 would be useful for identifying the origin of CH_4, not only measuring absolute concentrations but also isotopic composition of emitted CH_4, which might depend on the composition of the source (CO_2 reduction or acetate fermentation), the fractionation during the transport, or the oxidation by the methanotrophs (Barba, Bradford, et al., 2019; Jeffrey et al., 2021). Fourth, our incubation experiment demonstrated that stems under anaerobic conditions can produce CH_4. This CH_4 might be produced by methanogenic archaea communities inhabiting heartwood of the trees, as found in Eastern cottonwood trees in temperate forests (Flanagan et al., 2021; Yip et al., 2018). In our case, we could not quantify the absolute CH_4 production rates due to the lack of temporal sampling (see Section 2), but we demonstrated that production capacity in the heartwood was higher than in the sapwood (as found in Wang et al., 2016). Lower O_2 concentrations in the heartwood than in sapwood (Mugnai & Mancuso, 2010) may result in a more suitable environment for the methanogenic archaea community, enhancing their performance and abundance. All our samples for both sapwood and heartwood were able to produce CH_4, which is unprecedented since previous studies only showed that a small proportion of trees produced CH_4 under lab incubations (Covey et al., 2012; Pangala et al., 2017; Wang et al., 2016). Wood CH_4 production capacity should be complemented with analysis on the inner-stem microbial community (amplicon-based and metagenomic approaches), which would provide additional evidence for the hypothesis of heartwood CH_4 production. A study analyzing methanogenic archaea within stems found its presence in just 34% of the heartwood and 13% of the sapwood samples (Yip et al., 2018). This could suggest that CH_4 production capacity might be species-specific, and by extension, that the origin of CH_4 emitted by stems might also be species-specific. This might imply an additional challenge for process-based models, which may have to consider tree species and the possibility of CH_4 being produced within the tree or transported from soils depending on the environment.

We did not find evidence that internal wood rot would favor higher stem CH_4 fluxes as also found for six upland tree species in Northeastern United States (Covey et al., 2012), undermining the influence of wood rot for stem CH_4 production. Some studies have speculated that internal wood rot could be a potential source of stem CH_4 production (Covey & Megonigal, 2019), either by enhancing methanogenesis or by eliminating physical barriers (i.e., increasing diffusion) for gas transport. In our case, up to 85% of the measured stem height locations showed positive fluxes despite not showing evidence of rot (visual observation from tree cores). Even the stem with very high emissions measured with automated chambers (Figure 2b) did not present symptoms of internal wood decay when it was felled by a storm 2.5 years after the experiment. The fact that stem CH_4 emissions might not be related to wood decay could suggest that persistent stem CH_4 emissions are a common phenomenon in healthy upland forests.

5 | CONCLUSIONS

Manual and automated measurements allowed us to identify seasonal patterns of stem CO_2 emissions and to show coherence between mean seasonal fluxes and their response to stem temperature and soil moisture. However, we found important discrepancies for stem CH_4 fluxes when using automated and manual measurements and discussed potential bias of previous studies of stem CH_4 fluxes solely based on manual or automated measurements. Stem CH_4 fluxes were highly variable over short time periods, changing from net emissions to net uptake within days. Only automated measurements captured such high variability, allowing seasonal patterns to emerge among-trees and within-trees when data were integrated daily. However, seasonal mean fluxes derived from automated measurements were too sensitive to hot spots, due to the limited number of spatial replicates. Temperature and moisture had a positive effect on stem CH_4 fluxes for both manual and automated measurements, but the effect of moisture for automated measurements was only positive at the base of the trees. Overall, the abiotic and biotic drivers explained a low variability in stem CH_4 fluxes compared with CO_2.

The inconsistent vertical pattern of stem CH_4 emissions, together with low soil CH_4 concentrations throughout the soil profile, high CH_4 concentrations in the heartwood of the trees, and CH_4 production within the xylem during laboratory incubations, provide several lines of evidence that suggest most of the CH_4 emitted through the tree stems may have been produced internally. This study not only contributes to the general understanding of environmental controls of stem emissions but also reveals that there is a large proportion of variability that still remains unexplained, suggesting that other variables not accounted for in this study, such as those controlling gas diffusivity and transport in through the wood, might play a major role on controlling stem fluxes. We recognize that our results might be species- or site-specific and, there is a need for more studies that measure stem emissions spanning multiple tree species and ecosystems, as well as incorporate tree-level wood properties. A critical volume of experimental studies and an understanding of internal controls of stem fluxes are key steps for quantifying the integrated role of trees on the global CH_4 cycle.

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AUTHOR CONTRIBUTION

Josep Barba and Rodrigo Vargas conceived and designed the experiment; Josep Barba and Margaret Capooci performed the experiment; Josep Barba and Rafael Poyatos analyzed the data; Josep Barba wrote the first draft and all authors edited the manuscript.

DATA AVAILABILITY STATEMENT

Data used in this study can be download from Figshare (https://doi.org/10.6084/m9.figshare.14885697.v1). The R code used for estimating stem GHG fluxes from the gas analyzer concentrations can be downloaded from (https://github.com/josepbarba/GHG_flux_calculations). The R code for replicating the results can be downloaded from (https://github.com/vargaslab/Barba_etal_GCB_2021).

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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