

1 **Spatiotemporal variability and origin of CO₂ and CH₄ tree stem fluxes in an upland forest**

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3 Running head: CO₂ and CH₄ stem fluxes

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19 system

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25

26 Abstract

27 The exchange of multiple greenhouse gases (i.e., CO₂ and CH₄) between tree stems and the at-
28 mosphere represents a knowledge gap in the global carbon cycle. Stem CO₂ and CH₄ fluxes vary
29 across time and space and is unclear which are their individual or shared drivers. Here we meas-
30 ured CO₂ and CH₄ fluxes at different stem heights combining manual (biweekly; n=678) and au-
31 tomated (hourly; n>38,000) measurements in a temperate upland forest. All trees showed CO₂
32 and CH₄ emissions despite 20% of measurements showing net CH₄ uptake. Stem CO₂ fluxes pre-
33 sented clear seasonal trends from manual and automated measurements. Only automated meas-
34 urements captured the high temporal variability of stem CH₄ fluxes revealing clear seasonal
35 trends. Despite that temporal integration, the limited number of automated chambers made stand-
36 level mean CH₄ fluxes sensitive to “hot spots”, resulting in mean fluxes with high uncertainty.
37 Manual measurements provided better integration of spatial variability, but their lack of tem-
38 poral-variability integration hindered the detection of temporal trends and stand-level mean
39 fluxes. These results highlight potential bias of previous studies of stem CH₄ fluxes solely based
40 on manual or automated measurements. Stem height, temperature and soil moisture only ex-
41 plained 7 and 11% of the stem CH₄ flux variability compared to 42 and 81% for CO₂ (manual
42 and automated measurements, respectively). This large unexplained variability, in combination
43 with high CH₄ concentrations in the trees’ heartwood suggests that stem CH₄ fluxes might be
44 more influenced by gas transport and diffusivity through the wood than by drivers of respiratory
45 CO₂ flux, which has crucial implications for developing process-based ecosystem models. We
46 postulate that CH₄ is likely originated within tree stems because of: lack of a consistent vertical
47 pattern in CH₄ fluxes; evidence of CH₄ production in wood incubations; and low CH₄ concentra-
48 tion in the soil profile but high concentrations within the trees’ heartwood.

49 **Introduction**

50 Methane (CH₄) is the second most important greenhouse gas in the atmosphere. With a radiative
51 forcing capacity 25 times that of CO₂ (Forster et al., 2007), it contributes 23% to global warming
52 (Etminan, Myhre, Highwood, & Shine, 2016). Multiple studies have reported that trees can emit
53 CO₂ and CH₄ through stem surfaces. Most efforts have been pursued on stem CO₂ fluxes with
54 examples dating back to more than 60 years ago (Mar, Møller, & Nielsen, 1954). During the last
55 few years efforts have highlighted the relevance of CH₄ fluxes from tree stems in tropical
56 (Pangala et al., 2017; Welch, Gauci, & Sayer, 2018), temperate (Pitz & Megonigal, 2017;
57 Warner, Villarreal, McWilliams, Inamdar, & Vargas, 2017) and boreal forests (Machacova et al.,
58 2016; Vainio, 2019), including in both angiosperms and gymnosperms (Covey & Megonigal,
59 2019). Stem CH₄ fluxes are a widespread phenomenon, potentially relevant at regional scales
60 (Pangala et al., 2017; Wang et al., 2017), but upscaling tree-level flux measurements to the stand
61 level is difficult, precluding the inclusion of stem CH₄ emissions in the global methane budget
62 (Carmichael, Bernhardt, Bräuer, & Smith, 2014; Saunois et al., 2020). This is because our under-
63 standing of magnitudes, patterns and underlying mechanisms of stem CH₄ fluxes (and other
64 greenhouse gases) is still very limited (Vargas & Barba, 2019). Consequently, there is a need to
65 quantify magnitudes and patterns and to incorporate biophysical principles of stem CH₄ fluxes to
66 improve our understanding of the global carbon cycle (Barba, Bradford, et al., 2019). We high-
67 light three interrelated challenges for research regarding stem CH₄ fluxes.

68 First, quantifying the spatial and temporal variability of stem CH₄ fluxes. Even within a
69 single tree, the patterns and magnitudes of fluxes may vary at different stem heights. Most stud-
70 ies have measured stem fluxes at a single stem height (Flanagan et al., 2021; Machacova et al.,
71 2016; Wang et al., 2017; Warner et al., 2017; Welch et al., 2018), but there is mounting evidence

72 that stem CH₄ emissions decrease with stem height (Barba, Poyatos, & Vargas, 2019; Jeffrey,
73 Maher, Tait, & Johnston, 2020; Pitz & Megonigal, 2017; Sjögersten et al., 2020; Wang et al.,
74 2016). Stem CH₄ fluxes also demonstrate large temporal variability at both diurnal and seasonal
75 scales (Barba, Poyatos, et al., 2019) but, the low temporal resolution of measurements found in
76 most studies (i.e., measurements every 2-4 weeks; exceptions: (Barba, Poyatos, et al., 2019;
77 Plain, Ndiaye, Bonnaud, Ranger, & Epron, 2019)) limits the emergence of temporal patterns and
78 the identification of mechanisms underlying such spatial and temporal variability.

79 Second, identifying drivers controlling stem CH₄ fluxes at different spatial and temporal
80 scales. Some studies have suggested that stem CH₄ emissions could be partially explained by
81 abiotic conditions outside the tree stems. For instance, some studies have reported increasing
82 stem CH₄ emissions with increasing air or soil temperature (Barba, Poyatos, et al., 2019; Pitz,
83 Megonigal, Chang, & Szlavecz, 2018; Wang et al., 2016), increasing soil moisture (Barba,
84 Poyatos, et al., 2019; Welch et al., 2018) or decreasing water table depth (Pitz et al., 2018). Other
85 studies have reported that stem CH₄ emissions are also correlated with physiological or biotic
86 factors, such as tree species identity (Sjögersten et al., 2020; Wang et al., 2016; Warner et al.,
87 2017), wood density (Wang et al., 2017), wood structural features that allow gas transport from
88 the soil to the atmosphere (Sjögersten et al., 2020), tree diameter (Pitz et al., 2018) and sap flow
89 dynamics (Barba, Poyatos, et al., 2019; Pitz & Megonigal, 2017). However, it is likely that an
90 interaction of multiple factors influences CH₄ fluxes and patterns across ecosystems, hampering
91 our understanding of the main biophysical drivers of these fluxes.

92 Third, characterizing the origin/source of CH₄ that is ultimately emitted by tree stems.
93 There is evidence that CH₄ could be produced within soils under anoxic conditions, transported
94 through the roots into the stem, and diffused from the tree stems to the atmosphere (Covey &

95 Megonigal, 2019). In that case, the stems would be acting as “straws” by providing physical
96 pathways that connect deep soils with the atmosphere, by-passing the uppermost soil layer domi-
97 nated by methanotrophs (Megonigal & Guenther, 2008). Alternatively, tree stems could emit
98 CH₄ internally produced within the tree’s heartwood (Covey & Megonigal, 2019) by methano-
99 genic archaea (Yip, Veach, Yang, Cregger, & Schadt, 2018). It is hypothesized that this internal
100 production might be responsible for very high CH₄ concentrations found within trees for multiple
101 species and ecosystems (Covey, Wood, Warren, Lee, & Bradford, 2012; Zeikus & Ward, 1974).
102 While CH₄ derived from soils seems to prevail in wetlands and floodplain forests (where CH₄ is
103 produced within anoxic soils), internally-produced CH₄ seems to be the most likely origin in up-
104 land forests, where soils are usually net sinks of CH₄ (Dunfield, 2007; Warner et al., 2017). Sev-
105 eral studies have speculated about the main origin of emitted CH₄ or a potential combination be-
106 tween internal- and soil-produced CH₄ in upland forests, but to date, no clear empirical results
107 have solved this dilemma (Barba, Bradford, et al., 2019; Covey & Megonigal, 2019).

108 If soil and stem internal origin simultaneously occur for CH₄, stem CO₂ and CH₄ fluxes
109 might share some common drivers, as emitted CO₂ also originates from both within the wood
110 (respiration) and from the soil (i.e. transported from belowground through the xylem) (Teskey,
111 McGuire, Bloemen, Aubrey, & Steppe, 2017). While the biogeochemical processes and path-
112 ways of the two gases will probably differ, their response to physical constraints (e.g., gas diffu-
113 sivity through the wood or soil) affected by environmental variables (e.g., temperature or mois-
114 ture) could result in similar temporal and vertical patterns in fluxes from both gases. For exam-
115 ple, Pitz et al. (2018) found that the seasonal dynamics of stem CO₂ and CH₄ fluxes were more
116 similar in wetlands compared to upland forests, suggesting that wetter soils might enhance xy-

117 lem-transported CO₂ and CH₄ emissions. High-frequency measurements would bring the oppor-
118 tunity to test whether temporal patterns of CH₄ and CO₂ fluxes are correlated. If demonstrated, it
119 may enable scientists to estimate CH₄ fluxes from measurements or modelled CO₂ fluxes, which
120 are more feasible to measure than stem CH₄ fluxes (Vargas & Barba, 2019).

121 We measured in a temperate upland forest the following variables: (1) CO₂ and CH₄ stem
122 fluxes with manual (biweekly resolution) and automated chambers (hourly resolution) at differ-
123 ent stem heights over a growing season, (2) CO₂ and CH₄ concentrations within stems and in the
124 soil profile and (3) CH₄ production capacity in different wood tissues. We used these data to: a)
125 explore the spatiotemporal variability and environmental drivers of stem fluxes; b) test emergent
126 relationships between CO₂ and CH₄ stem fluxes; and c) provide insights about the potential
127 origin of CH₄ (either soil or heartwood production) emitted through stems. We postulate the fol-
128 lowing hypotheses:

129 H1. Seasonal patterns in stem CO₂ and CH₄ fluxes can be identified by high-frequency
130 measurements, which also will provide more accurate seasonal mean fluxes and trends by inte-
131 grating the high variability of stem fluxes throughout the experimental period. Manual measure-
132 ments, on the other hand, will provide better integration of spatial variability of stem CO₂ and
133 CH₄ fluxes, but will miss potential large pulses (i.e., “hot moments”) which could influence esti-
134 mates of the seasonal mean and trends.

135 H2. Underlying biophysical controls of CO₂ and CH₄ could be explained by stem temper-
136 ature and soil moisture, where stem CO₂ and CH₄ fluxes would increase with higher temperature
137 and soil moisture (H2a). Thus, because of this potential co-dependency in temperature and mois-
138 ture, there may be a positive correlation between CO₂ and CH₄ fluxes (H2b) as previously pro-
139 posed (Flanagan et al., 2021; Vargas & Barba, 2019).

140 H3. Tree diameter and stem height will positively influence the magnitude of CO₂ and
141 CH₄ fluxes, interacting with the environmental controls. This is expected because bigger trees
142 might have higher capacity to transporting gases from a large soil volume, which might have
143 stronger effect closer to the soil (base of the tree) than upper in the stem. Additionally, larger
144 trees have more sapwood and heartwood volume, which might enhance their potential for pro-
145 ducing CO₂ and CH₄.

146 H4. We postulate that stem CH₄ emissions may be attributed from CH₄ produced in soils
147 if: a) the magnitude of stem CH₄ emissions decreases with stem height, and b) there is a positive
148 correlation between soil CH₄ concentrations (at different soil depths) and stem CH₄ emissions. In
149 contrast, stem CH₄ emissions may be attributed from CH₄ produced within the stem if a) the
150 magnitude of stem CH₄ emissions does not decrease with stem height, and b) heartwood CH₄
151 concentrations show a positive correlation with stem CH₄ emissions. This study provides unique
152 information of the temporal variability of CO₂ and CH₄ fluxes from tree stems which is relevant
153 for identifying controls and functional relationships of these important greenhouse gases.

154

155 **Materials and Methods**

156 *Study site*

157 We carried out this study in an upland forested area at the St. Jones Reserve [39°5'20"N,
158 75°26'21"W], a component of the Delaware National Estuarine Research Reserve (DNERR).
159 The site has a temperate climate with a mean annual temperature of 13.3 °C and a mean annual
160 precipitation of 1119 mm. Soils are Othello silt loam with a texture of 40, 48 and 12% of sand,
161 silt and clay, respectively (Petraakis et al 2018). The dominant vegetation species are bitternut
162 hickory (*Carya cordiformis*), eastern red cedar (*Juniperus virginiana* L.), American holly (*Ilex*

163 *opaca* (Ashe)), sweet gum (*Liquidambar styraciflua* L.) and black gum (*Nyssa sylvatica* (Mar-
164 shall)), with an overall tree density of 678 stems ha⁻¹ and mean diameter at breast height (DBH)
165 of 25.7±13.9 cm (mean±sd). We studied bitternut hickory, which is one of the most important
166 species in the study site, accounting for 24.9% of the total basal area. The length of the studied
167 area was around 70 m. Additional information on the study site can be found in Petrakis et al.
168 (2018).

169

170 *Flux measurements*

171 We measured stem CO₂ and CH₄ fluxes throughout a growing season and after leaf senescence
172 (from April to December 2017) in 18 hickory trees. Trees' DBH ranged from 24.7 to 75 cm
173 (43±13 cm, mean±sd). To better understand the temporal variability of CO₂ and CH₄ fluxes, we
174 performed automated measurements (i.e., hourly resolution) on three individual stems at 50 and
175 150 cm stem heights as described by Barba, et al. (2019). Briefly, at each stem height, we in-
176 stalled 317.8 cm² PVC collars where automated chambers (Li-COR 8100-104, Lincoln, Ne-
177 braska) were placed. The chambers were controlled by a multiplexer (Li-COR 8150, Lincoln,
178 Nebraska) which was connected to a closed path IRGA (infrared gas analyzer) (Li-8100A, Lin-
179 coln, Nebraska). Additionally, we connected a cavity ring-down spectrometer (Picarro G2508,
180 Santa Clara, California) in series with the IRGA as described in other studies (Barba, Poyatos, et
181 al., 2019; Capooci, Barba, Seyfferth, & Vargas, 2019; Petrakis, Seyfferth, Kan, Inamdar, &
182 Vargas, 2017). For each flux observation, we measured CO₂ and CH₄ concentrations every sec-
183 ond with the Picarro G2508 for 300 s and calculated fluxes (at 1 h time intervals) from the mole
184 dry fraction of each gas (i.e., corrected for water vapor dilution) using the SoilFluxPro software

185 (v4.0; Li-COR, Lincoln, Nebraska, USA). We estimated the fluxes with both linear and exponen-
186 tial fits and kept the flux with the highest R^2 . We applied a quality assurance/quality control
187 (QA/QC) protocol based on CO_2 fluxes established in previous studies (Barba, Poyatos, et al.,
188 2019; Capooci et al., 2019; Petrakis et al., 2018). Briefly, when the R^2 for the CO_2 flux is very
189 high (generally higher than 0.95), it means that the micrometeorological conditions inside the
190 chamber are suitable for measuring stem fluxes (e.g. chamber properly sealed), and therefore, we
191 are confident of keeping CH_4 measurements even if the R^2 for the CH_4 flux is low (which is usu-
192 ally the case when the fluxes close to 0). We also measured soil volumetric water content (SWC)
193 and soil temperature at 10 cm, and stem temperature at 5 cm at each chamber location (EC-5,
194 Decagon Devices, Pullman, WA).

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

$$202 \quad F = \left(\frac{dC}{dt}\right) \left(\frac{V_c}{A_c}\right) \frac{P}{(R * (T + 273.15))}$$

203 where F is the flux of a particular gas, dC/dt is the change in concentration over time (ppm^{-1}) es-
204 timated with both linear and exponential fits, V_c is the system volume (0.001135 m^3), A_c is the
205 measured area (0.0095 m^2), P is the atmospheric pressure measured at the center of the plot for
206 each particular time, R is the ideal gas law constant ($0.00831447 \text{ kg m}^2 \mu\text{mol}^{-1} \text{ K}^{-1} \text{ s}^{-2}$), T is stem
207 temperature ($^{\circ}\text{C}$), and 273.15 is the conversion factor from Celsius to Kelvin. We applied the

208 same QA/QC protocol as for the automated measurements. Additionally, we used a non-contact
209 infrared thermometer (Nubee NUB8500H) for measuring the stem surface temperature associ-
210 ated with each flux measurement.

211

212 *Ancillary data*

213 We installed PVC pipes (5 cm in diameter) into the soil at 10, 25, 50, 75 and 100 cm and at
214 groundwater depth (around 150 cm) within 1 m of each tree equipped with automated measure-
215 ments, for a total of 3 soil profiles. The top of each pipe was sealed whereas the bottom was open
216 to allow the pipe's internal concentrations of CO₂ and CH₄ to equilibrate with soil atmosphere at
217 each target depth. We measured soil CO₂ and CH₄ concentrations at each depth using a closed-
218 loop approach during three campaigns (October 2017, August 2018 and March 2019) with an Ul-
219 tra-Portable Greenhouse Gas Analyzer (Los Gatos Research, California). In addition, we meas-
220 ured SWC and soil temperature at 10 cm (5TE, METER Group, Pullman, WA), air temperature,
221 atmospheric pressure and relative humidity (VP-4 Sensor (Temp/RH/Barometer), METER
222 Group, Pullman, WA), and wind speed and wind direction (DS-2m METER Group, Pullman,
223 WA) during the entire experiment using digital data loggers (Em50, METER Group, Pullman,
224 WA). We also measured water table level every 15 min at the center of the plot (WL16U-003-10,
225 Global Water, Gold River, CA).

226

227 *Heartwood CH₄ concentrations and tree core incubations*

228 On August 20th 2018, we extracted tree cores with an increment borer at each tree stem height
229 for each of the 18 trees measured throughout the study. After extracting the sample but before
230 removing the increment borer we directly measured CO₂ and CH₄ concentrations within the stem

231 with a CO2Meter (MH-Z92 Dual Gas CO₂/CH₄ meter, Ormond Beach, FL), suitable for measur-
232 ing high concentrations (range 0-100% vol), coupled with an Ultra-Portable Greenhouse Gas An-
233 alyzer (Los Gatos Research, California), suitable for accurate low concentrations (0-500 ppm). In
234 order to compare internal stem concentrations with stem fluxes, we measured stem fluxes 5 days
235 before sampling the tree cores at all stem heights with the same instrumental setup as described
236 for manual measurements.

237 Right after collecting each core, we split samples into sapwood and heartwood fractions,
238 placed each fragment in an incubation jar (350 ml) and flushed the jars in the field with He for 2
239 min at 2 L min⁻¹. Over the following 3 days, we replaced the air in the jars twice using an anaero-
240 bic chamber (95% N₂ and 5% H₂), in order to guarantee a CH₄-free atmosphere for the incuba-
241 tions. After that, we kept samples at constant temperature and under dark conditions for 6 h, be-
242 fore transferring a 15 mL headspace sample into a pre-evacuated glass vial (Exetainer, Labco,
243 High Wycombe, UK) to be analyzed with a gas chromatograph equipped with a flame ionization
244 detector for CH₄ and a thermal conductivity detector for CO₂ (Shimadzu Model 2014, Bucking-
245 hamshire, UK) (Covey et al., 2012). Since we only measured concentrations in the headspace at
246 one time and the humidity was not controlled during the incubation, we could not calculate abso-
247 lute CO₂ and CH₄ production rates. Instead, we report the production potential of each sample.

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

253

254 *Statistical analyses*

255 For manual flux measurements, we analyzed if stem height (factor with 3 levels: 50 cm, 100 cm
256 and 150 cm) influenced CO₂ and CH₄ fluxes. We used linear mixed-effects models (LMM) as
257 implemented in the ‘nlme’ R package (Pinheiro, Bates, DebRoy, & Sarkar, 2013) with tree iden-
258 tity as a random factor. We applied the Bonferroni test when stem height was statistically signifi-
259 cant (p -value<0.05). For the manual flux measurements, we also performed a second set of
260 LMMs to analyze the effect of stem diameter, stem temperature, SWC and the first-order interac-
261 tion between each of the two latter variables and stem height on CO₂ and CH₄ fluxes. The pur-
262 pose of this additional analysis was to test whether environmental responses of fluxes depend on
263 stem height. Additionally, the same LMMs models including total wood moisture and density,
264 and sapwood moisture and density on top of stem diameter, stem temperature and SWC were
265 tested for a subset of manual measurements (just for those with information about wood proper-
266 ties at 150 cm stem height [667 measurements, 14 trees]) to test the potential effect of wood den-
267 sity and moisture on CH₄ fluxes.

268 For automated flux measurements, we analyzed CO₂ and CH₄ fluxes using LMMs that
269 included the second-order interaction between stem height (in this case, there are two levels: 50
270 and 150 cm), stem temperature and SWC. Given the high correlation between SWC and water
271 table level ($R^2=0.87$), the latter was not included in the models.

272 In order to explore the relationship between CO₂ and CH₄ stem fluxes and its dependency
273 with stem height, we used LMMs for both manual and automated measurements with CH₄ as the
274 dependent variable and the interaction between CO₂ and stem height as predictors.

275 All models for CO₂ and CH₄ fluxes (manual and automated measurements) included ran-
276 dom variation of the intercept and, when applicable, of the temperature coefficient associated

277 with tree identity (random slopes model). Because of lack of convergence, the model for manual
278 CH₄ fluxes was fitted allowing only the intercept to vary with tree identity (random intercept
279 model). In order to achieve normality of the residuals, CO₂ flux was log-transformed and CH₄
280 flux was Box-Cox transformed ('bcnPower' function in 'car' R package (Fox & Weisberg,
281 2019)) with lambda values (-0.055 and -0.087 for manual and automated CH₄ fluxes, respec-
282 tively) obtained using maximum-likelihood, as implemented in the 'powerTransform' function
283 from the R package 'car' (Fox & Weisberg, 2019). For the LMM with automated CH₄ measure-
284 ments, we modelled the different variance per tree using the 'varIdent' function from the 'nlme'
285 R package. In all cases, we considered temporal autocorrelation by introducing a discrete-time
286 first-order autocorrelation error structure and calculated the fraction of variance explained by
287 fixed effects (marginal r-squared, R²_m) and by fixed and random effects (conditional r-squared,
288 R²_c), using the approach from Nakagawa & Schielzeth (2013).

289 In order to understand whether stem internal concentrations for each gas could be related
290 to stem fluxes or to stem height, we tested different LMMs between concentrations measured at
291 the final campaign (August 2018) and stem height or stem fluxes measured (i) during the same
292 week or (ii) the mean stem fluxes throughout the 2017 growing season. We also tested if wood
293 rot presence (visually identified from tree cores) was related to stem CH₄ fluxes. First, predic-
294 tions of tree- and height-specific stem CH₄ fluxes using the model for manual measurements (Ta-
295 ble 1) were obtained by using the tree-level random coefficients of the model and fixing stem
296 temperature at 20 °C, SWC at 0.25 v/v, DBH at 30 cm and DOY at 220. Predictions were also
297 made at the tree-level by additionally fixing stem height at 50 cm. Then, we tested whether these
298 predictions were affected by the presence of wood rot at the stem height or at the tree level using
299 a linear model.

300 Finally, we tested the difference between heartwood and sapwood production of CO₂ and
301 CH₄ using LMMs (random intercept model), with stem height nested within tree.

302

303 **Results**

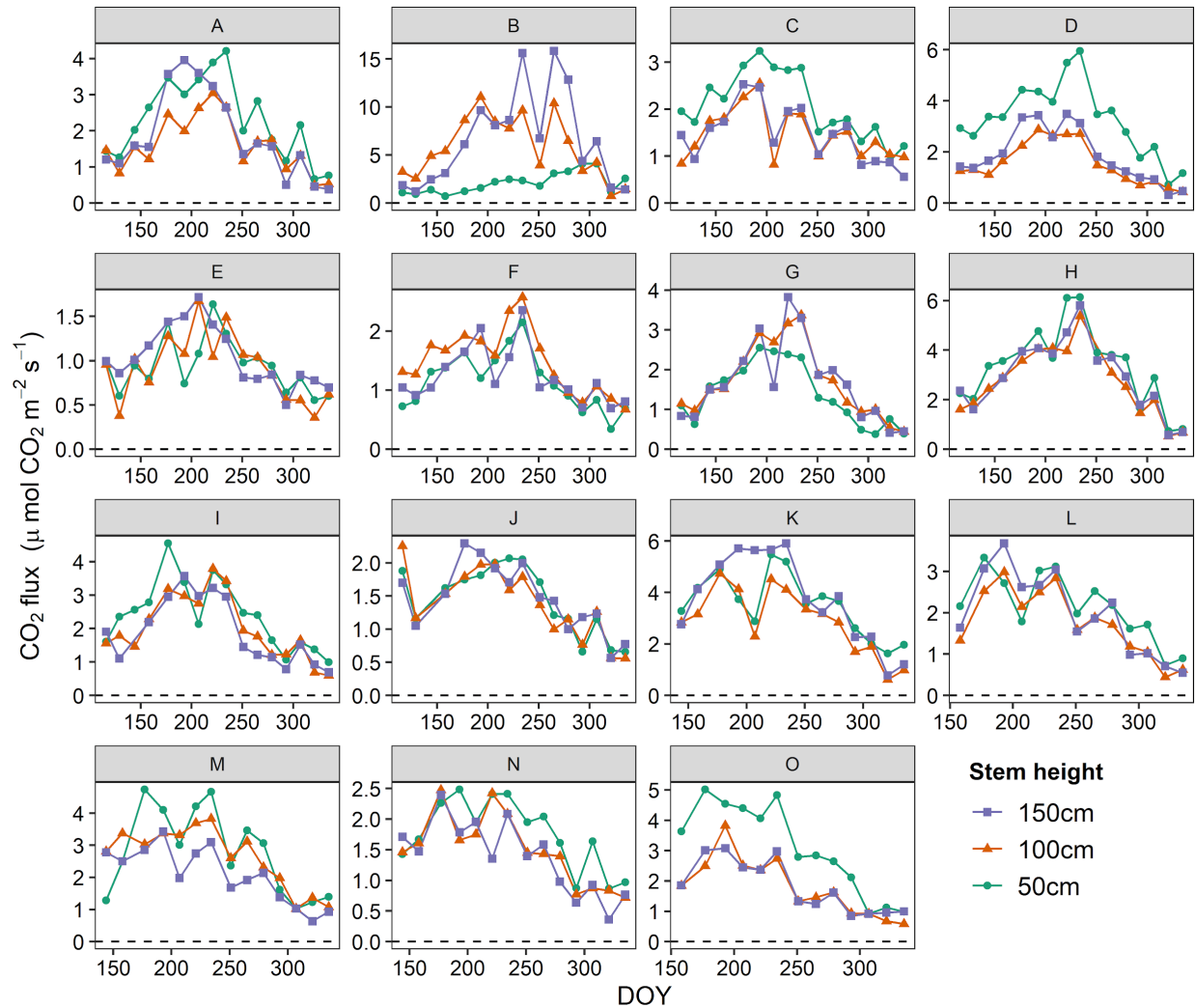
304 *Magnitudes and temporal and spatial variability of stem fluxes*

305 During the study period, we collected over 38,000 automated measurements and 678 manual
306 measurements of CO₂ and CH₄ stem fluxes from 18 hickory trees. Mean CO₂ stem fluxes during
307 the study period were 2.97 ± 0.41 and 2.17 ± 0.49 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (mean \pm 95% confidence interval
308 [CI]) for automated and manual measurements, respectively. On average, trees were net sources
309 of CH₄ (2.54 ± 4.35 and 0.24 ± 0.16 $\text{nmol m}^{-2} \text{s}^{-1}$ derived from automated and manual measure-
310 ments, respectively). Despite generally being sources of CH₄, occasionally trees also showed
311 CH₄ uptake from the atmosphere (17.7 and 22.9% of measurements for automated and manual
312 measurements, respectively).

313 Stem CO₂ emissions measured with manual chambers showed a clear seasonal pattern
314 among all trees over the study period, with emissions increasing throughout Spring and Summer,
315 peaking at the beginning of August (DOY \approx 220), and then decreasing towards the end of the ex-
316 periment (Fig. 1).

317

318 Figure 1. Manual measurements of CO₂ emissions from 15 individual trees (different letters in
319 the panels headers indicate different trees) at different stem heights. Emissions were measured
320 every two weeks from April to December 2017. Note that the scale of y axis is adjusted for each
321 tree to improve clarity.



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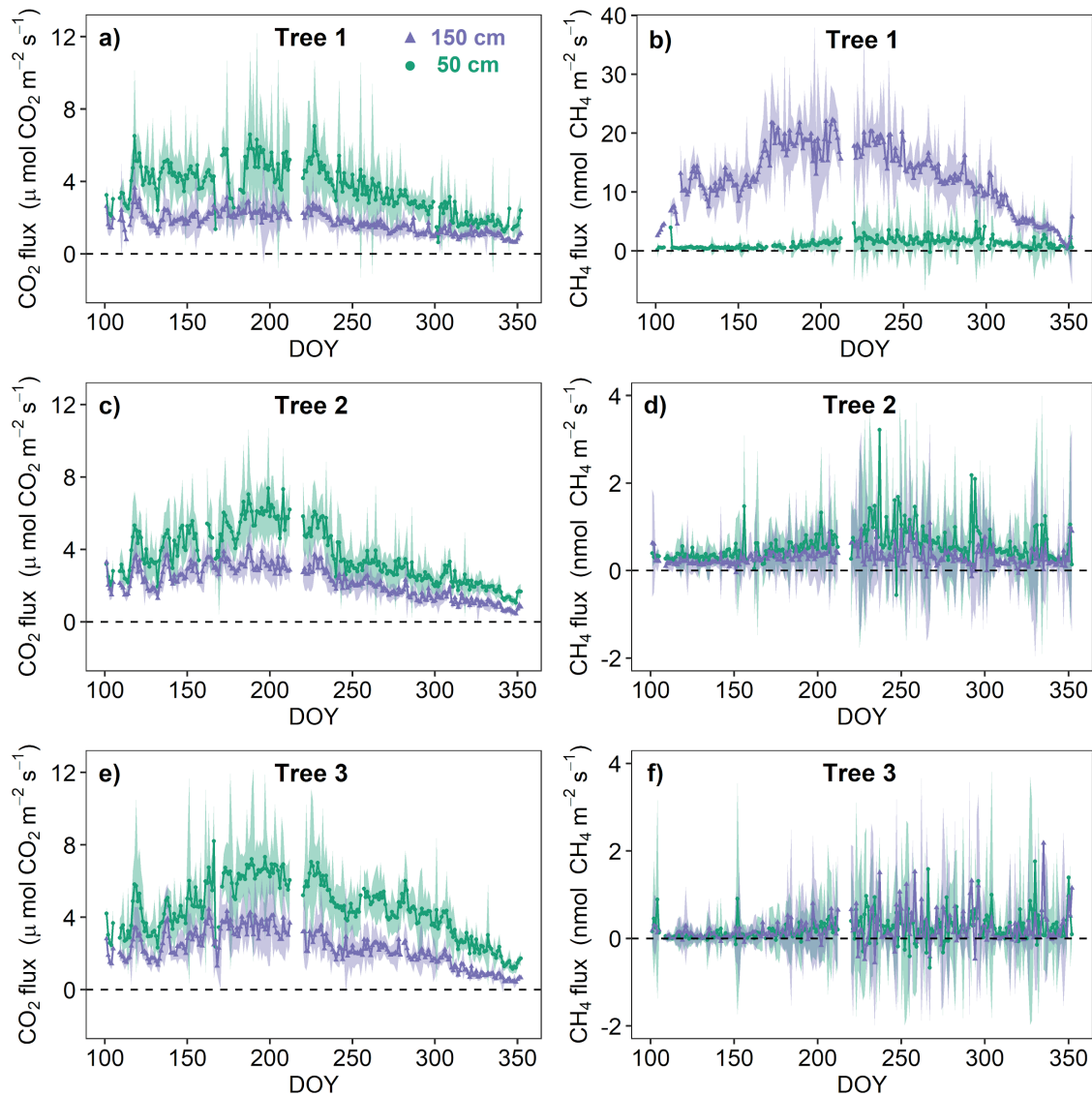
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Similar seasonal patterns were found both within and between trees, despite the differences in the magnitudes of CO₂ flux from tree to tree. Manual stem CO₂ fluxes presented a vertical pattern (p-value < 0.001) with higher emissions at 50 cm than at upper heights (i.e., 100 and 150 cm). Automated stem CO₂ fluxes also showed a clear seasonal pattern among trees, with emissions increasing until mid-Summer and then decreasing until the end of Fall (Fig. 2 a, c and e). For trees measured with our automated flux system, CO₂ emissions at 50 cm were higher than at 150 cm (3.89 ± 0.46 and $2.06 \pm 0.29 \mu\text{mol m}^{-2} \text{s}^{-1}$ (mean \pm sd), respectively).

332 Figure 2. Automated measurements of CO₂ and CH₄ stem fluxes during one growing season
333 (April - December 2017) at three different trees and two different stem heights (red for 50 cm
334 and blue for 150 cm). Dots represent daily averages of hourly measurements and shaded areas
335 represent standard deviations. Plotted standard deviations in panels d) and f) are constricted be-
336 tween -2 and 4 nmol m⁻² s⁻¹. The y axis for panel b) has a different scale because of extremely
337 high fluxes compared with the other 2 trees.
338

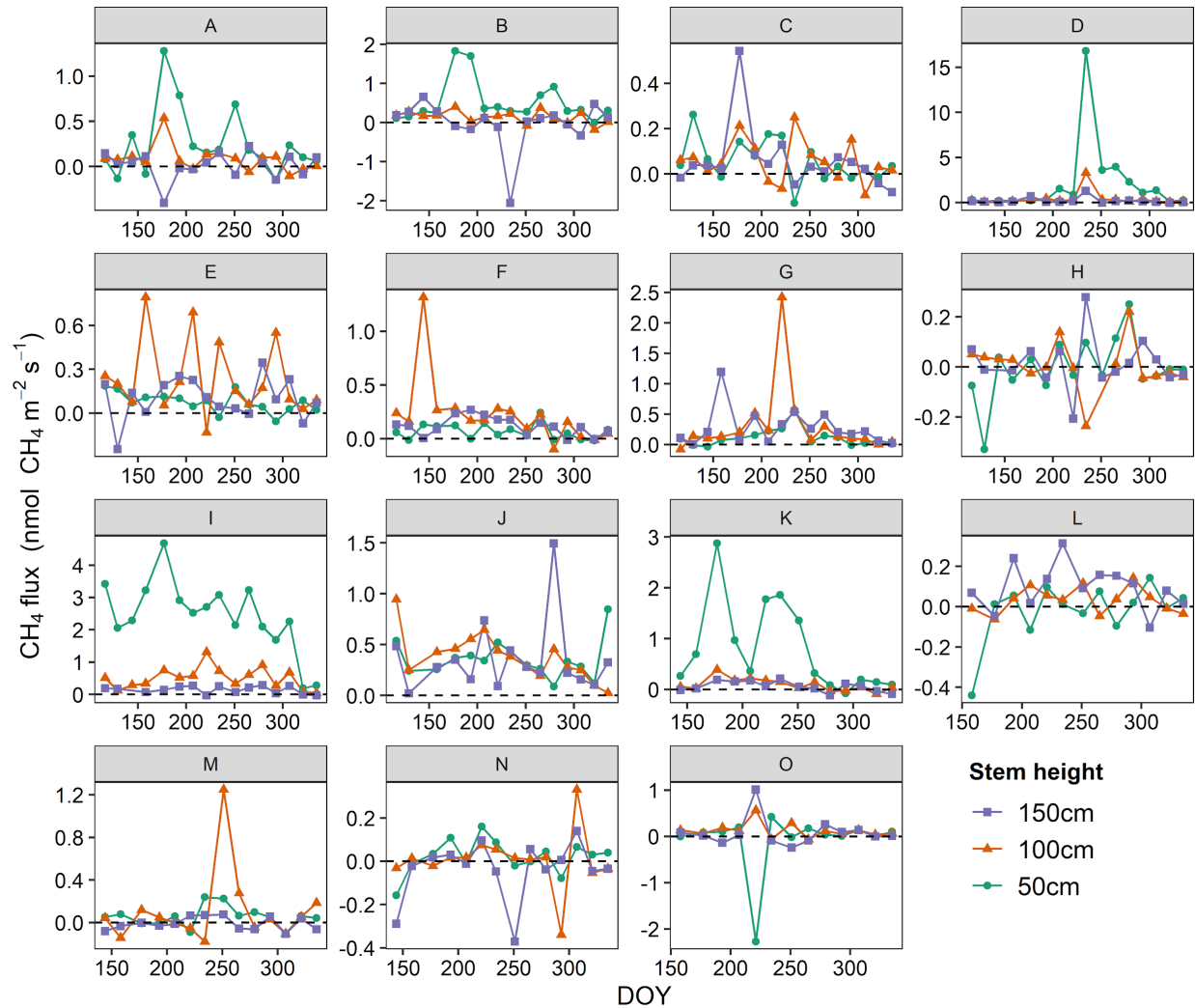


340

341 Manual measurements of stem CH₄ fluxes did not show a clear seasonal pattern (Fig. 3).
342 Stem CH₄ emissions decreased with stem height (p-value<0.001), where magnitudes at 50 cm
343 were the highest (p-value<0.001) and magnitudes at 100 cm were marginally higher than at 150
344 cm (p-value=0.068). In contrast, automated measurements of stem CH₄ fluxes varied greatly
345 within days, but when hourly measurements were integrated into daily means, these showed
346 clear seasonal trends, with emissions peaking around the end of the Summer and decreasing to-
347 wards the end of Fall (Fig. 2 b, d and f). Five out of six chambers presented similar magnitudes
348 of stem CH₄ fluxes ($0.51 \pm 0.45 \text{ nmol m}^{-2} \text{ s}^{-1}$; mean \pm sd), but one chamber showed mean fluxes
349 that were 20 times higher ($12.37 \pm 5.33 \text{ nmol m}^{-2} \text{ s}^{-1}$; mean \pm sd).

350

351 Figure 3. Manual measurements of CH₄ fluxes of 15 trees (different letters in the panels headers
352 indicate different trees) at three different stem heights. Fluxes were measured every two weeks
353 from April to December 2017. Note that the scale of y axis is adjusted for each tree to improve
354 clarity.



356

357 *Drivers of stem CO₂ and CH₄ fluxes*

358 The LMM of manual stem CO₂ emissions as a function of environmental drivers was able to ex-
 359 plain 67% of the variance, although only 42% was explained by fixed effects (i.e. stem height,
 360 DBH, SWC and temperature) (Table 1). This model showed a positive effect of temperature and
 361 a marginal interaction of temperature with stem height (Type III ANOVA, $\chi^2= 3.42$, $df=1$, p -
 362 value=0.064). Soil moisture also had a strong positive effect on stem CO₂ emissions but this ef-
 363 fect did not vary with stem height (Table 1). Stem CO₂ emissions also increased with increasing
 364 DBH (Table 1). For the CH₄ manual measurements, stem temperature and soil moisture showed

365 a positive effect, independent of stem height (Table 1). The model explained 37% of stem CH₄
 366 fluxes variability, but only 7% was explained by fixed effects (Table 1). When we tested the ef-
 367 fect of wood density and moisture (total or sapwood component) in a subset of data (where wood
 368 properties information was available), no effect of those variables was detected on stem manual
 369 CH₄ fluxes (S Table 2).

370

371 Table 1. Summary of the linear mixed-effects models for manual measurements of CO₂ and CH₄
 372 stem fluxes. Stem height at 50 cm was used as a reference category and included in the intercept.
 373 CO₂ and CH₄ fluxes were log and Box-Cox transformed, respectively to achieve normality in the
 374 residuals. R²_m is the variance explained by the fixed effects (marginal) and R²_c is the variance
 375 explained by the entire model, including both fixed and random effects (conditional).

376

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 100cm	-0.075 (0.36)	0.835
	Height 150cm	0.20 (0.36)	0.571
	SWC	5.10 (0.55)	<0.001
	DBH	0.013 (0.006)	0.040
	Temp*Height 100cm	0.0086 (0.004)	0.022
	Temp*Height 150cm	-0.0020 (0.004)	0.594
	SWC*Height 100cm	-0.49 (0.95)	0.602

	SWC*Height 150cm	-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 100cm	0.93 (1.09)	0.394
R ² _c : 0.37	Height 150cm	0.75 (1.08)	0.489
	SWC	3.35 (1.66)	0.045
	DBH	0.0058 (0.02)	0.745
	Temp*Height 100cm	-0.016 (0.01)	0.203
	Temp*Height 150cm	-0.0061 (0.01)	0.630
	SWC*Height 100cm	-2.87 (2.88)	0.319
	SWC*Height 150cm	-1.95 (2.88)	0.499

377

378 Automated stem CO₂ emissions positively responded to the interaction between stem
379 temperature and SWC (Table 2). In addition, the interaction between stem height and SWC was
380 also significant, while that of stem height and stem temperature was only marginally significant
381 (Table 2). This complex model outcome resulted in higher CO₂ emissions with high SWC at high
382 temperatures, particularly at 150 cm (S Figure 1). Overall, fixed effects in the model explained
383 81% of the variability of stem CO₂ emissions (Table 2). In contrast, automated CH₄ fluxes were
384 partially explained by SWC interacting with stem height (Table 2), with fluxes at 50 cm increas-

385 ing as SWC increased but those at 150 cm decreasing at high SWC (S Fig. 2). The interaction be-
386 tween stem temperature and SWC was only marginally significant (Table 2), with CH₄ fluxes re-
387 sponding more to temperature at higher levels of SWC. The model explained 89% of the varia-
388 bility in stem CH₄ fluxes, but only 11% was explained by fixed effects.

389

390 Table 2. Summary of the LMMs for automated measurements of CO₂ and CH₄ stem fluxes. Stem
391 height at 50cm is used as a reference category and included in the intercept. CO₂ and CH₄ fluxes
392 were log and box-cox transformed, respectively to achieve normality in the residuals. R²_m is the
393 variance explained by the fixed effects (marginal) and R²_c is the variance explained by the entire
394 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 150cm	-1.42 (0.27)	<0.001
	SWC	-3.87 (0.90)	<0.001
	Temp*Height 150cm	0.025 (0.014)	0.070
	Temp*SWC	0.19 (0.047)	<0.001
	SWC* Height 150cm	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 150cm	4.29 (0.70)	<0.001
R ² _c : 0.89	SWC	2.72 (2.00)	0.174
	Temp*Height 150cm	-0.016 (0.038)	0.700
	Temp*SWC	0.19 (0.10)	0.068
	SWC*Height 150cm	-13.90 (2.48)	<0.001
	Temp*SWC*Height	0.073 (0.13)	0.588

395

396 We explored to what extent stem CO₂ fluxes could explain the temporal patterns in CH₄
397 fluxes. Stem CH₄ fluxes were positively related to stem CO₂ fluxes but the slope of this relation-
398 ship tended to diminish with stem height, for both manual and automated measurements (S Table
399 1). However, the variability in stem CH₄ fluxes explained by the fixed effects (i.e. stem CO₂
400 fluxes and stem height) was lower than 10% in both cases (S Fig. 3).

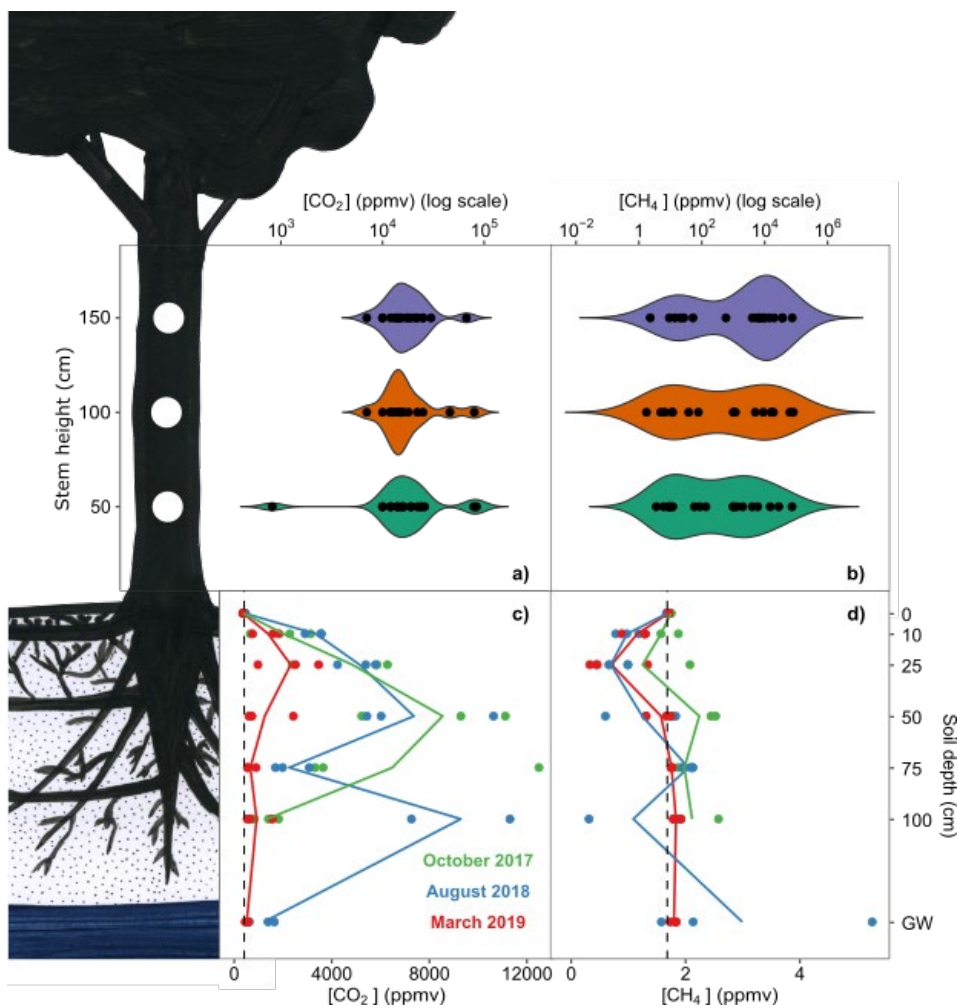
401

402 *Insights about the origin of stem CO₂ and CH₄ fluxes*

403 Stem CO₂ concentrations measured at the end of August 2018 showed high values in the heart-
404 wood of the stems (median=15,000 ppm; Fig. 4a), with no significant differences with stem
405 height (p-value=0.86). The heartwood also showed high CH₄ concentrations (median=1,000
406 ppm), with no significant differences with stem height either (p-value=0.32). Neither CO₂ nor
407 CH₄ concentrations presented significant correlations with stem fluxes measured five days before
408 nor with the mean fluxes over the whole experiment (p-value higher than 0.05 in all cases).

409

410 Figure 4. CO₂ and CH₄ concentrations in the stem heartwood and in the soil profile. Heartwood
411 concentrations (panels a and b) were measured in Aug 2018. Violin plots represent log CO₂ and
412 CH₄ concentrations at different stem measuring heights (black dots), with colored areas depicting
413 a kernel density plot showing the distribution of concentrations. Soil concentrations (panels c and
414 d) were measured at three soil profiles including groundwater (GW; around 150cm deep) in the
415 vicinity of the trees with automated measurements. For panels c and d, different colors represent
416 different sampling dates, dots represent single measurements, solid lines represent average fluxes
417 of the three profiles for each depth, and dashed lines represent atmospheric concentrations of
418 each gas.



420

421 CO₂ concentrations in the soil profile showed similar patterns during the three measure-

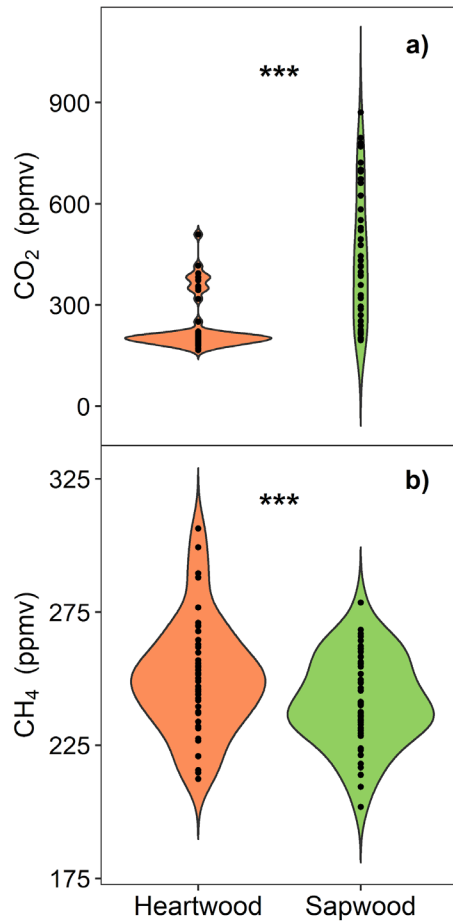
422 ment campaigns, with lower values at the beginning of Spring compared to the Summer and the

423 Fall (Fig. 4c). In general, concentrations increased with soil depth down to 25 cm (March) or 50
424 cm (August and October), and from there, decreased with depth, achieving the minimum concen-
425 tration at the groundwater level (~150 cm depth). CH₄ concentrations in the soil profile showed
426 similar trends across seasons, dropping below the atmospheric concentration (1.71 ± 0.03 ppm;
427 mean \pm sd) along the first 25 cm, and from then, increasing until 50 cm depth, achieving concen-
428 trations slightly above atmospheric. At deeper layers, CH₄ concentrations did not show a clear
429 pattern, with no major changes compared to concentrations at 50 cm.

430 While Bitternut Hickory trees usually present hard, strong and durable wood which
431 makes the species relatively resistant to heart rot (Berry & Beaton, 1972), we found that 8 tree
432 cores out of 51 (from four different trees) presented visual evidence of wood rot. That said, no
433 significant relationship between rot presence and modelled CH₄ fluxes was found at the tree level
434 (rot presence anywhere in the tree; p-value=0.28) or at the core level (p-value=0.46).

435

436 Figure 5. CO₂ and CH₄ tree core incubations. CO₂ and CH₄ concentrations in the incubation jars
437 (panels a and b, respectively) after anaerobic incubations of heartwood and sapwood tissues of
438 the different stem heights from the 18 studied trees (sampled in Aug 2018). Violin plots repre-
439 sent CO₂ and CH₄ concentrations (black dots), with colored areas depicting a kernel density plot
440 showing the distribution of concentrations. Asterisks indicate differences between heartwood and
441 sapwood concentrations.



443

444 Tree core incubations revealed that all tree cores produced CO₂ (Fig. 5a), with higher
445 production from the sapwood than from the heartwood (p-value<0.001). All tree cores were able
446 to produce CH₄ as well (Fig. 5b), with higher production in the heartwood than in the sapwood
447 (p=0.013).

448

449 Discussion

450 Our results demonstrate that all tree stems (18 individuals) emit CH₄ throughout the experi-
451 mental period at all stem heights. Moreover, around 20% of the measurements (both automated
452 and manual) showed a net CH₄ uptake by tree stems, indicating that net stem-atmosphere CH₄

453 fluxes might be a balance between CH₄ production and consumption that may be co-occurring in
454 this interface. This cryptic balance between production and consumption complicates estimates
455 of net stem CH₄ fluxes, and the identification of dominant drivers. Several studies measuring
456 stem CH₄ emissions have reported some uptake measurements as well (e.g., Pitz & Megonigal,
457 2017; Warner et al., 2017; Welch et al., 2018), but little has been discussed about the potential of
458 tree stems acting not only as sources but also as sinks of CH₄ (but see Machacova et al., 2020),
459 which might have implications for development of future process-based ecosystem models.

460

461 *Magnitudes and temporal patterns of stem CO₂ and CH₄ fluxes using manual and automated*
462 *measurements*

463 Automated and manual measurements presented comparable mean stem CO₂ emissions
464 (2.97 ± 0.41 and 2.17 ± 0.49 $\mu\text{mol m}^{-2} \text{s}^{-1}$, automated and manual, respectively), but this was not the
465 case for CH₄. Mean stem CH₄ flux from automated chambers was 10 times larger than that ob-
466 tained from manual measurements (2.54 ± 4.35 and 0.24 ± 0.16 $\text{nmol m}^{-2} \text{s}^{-1}$ for automated and
467 manual measurements, respectively). This discrepancy was influenced by one automated cham-
468 ber that presented mean fluxes 20 times higher than all the other automated chambers (Fig. 2b),
469 also resulting in a wider confidence interval (i.e., uncertainty) for the automated measurements
470 compared to manual measurements. When that particular chamber was removed, the mean CH₄
471 flux from automated measurements was about 50% higher than that from manual measurements
472 (0.51 ± 0.45 $\text{nmol m}^{-2} \text{s}^{-1}$ and 0.24 ± 0.16 $\text{nmol m}^{-2} \text{s}^{-1}$ for automated and manual measurements,
473 respectively). These results indicate that annual estimates based on automated measurements
474 might be sensitive to “hot spots”, both between trees and/or within trees, due to the limited num-
475 ber of automated chambers associated with logistical challenges and costs of automated systems.

476 The strength of manual measurements is to better integrate spatial variability of stem
477 fluxes (including “hot spots”) than automated approaches due their suitability for measuring a
478 larger number of trees, which might result in more spatially representative estimates. That said,
479 because of the high temporal variability of stem CH₄ fluxes shown by automated measurements
480 (see next paragraph), our results indicate that manual measurements are clearly missing temporal
481 variability and “hot moments” of CH₄ fluxes (as demonstrated by the lower CV), which might
482 have a strong impact on the calculation of annual fluxes. The mean growing season stem CH₄
483 fluxes estimated with manual measurements ($0.24 \pm 0.16 \text{ nmol m}^{-2} \text{ s}^{-1}$) is within the range of val-
484 ues reported for other upland forests (Pitz et al., 2018 and references therein) but our results chal-
485 lenge the studies that only rely on manual measurements to estimate annual fluxes.

486 Stem CO₂ emissions presented a clear seasonal pattern with both manual and automated
487 measurements, again, showing coherence between both approaches. Seasonal patterns of stem
488 CO₂ emissions are consistent with tree physiological activity peaking during the growing season
489 (Teskey, Saveyn, Steppe, & McGuire, 2008). Stem CH₄ fluxes also presented a clear seasonal
490 pattern (supporting H1), but this was only evident with the automated measurements where the
491 integration of the high variability of hourly measurements (including “hot moments”) into daily
492 means allowed the seasonal pattern to emerge. We argue that the lack of observed seasonal pat-
493 terns in other studies using manual measurements in upland forests (with a typical measurement
494 frequency of 2-3 weeks) (Pitz & Megonigal, 2017; Pitz et al., 2018; Warner et al., 2017; Welch
495 et al., 2018) may be because sub-daily variability was not accounted for. Studies performing
496 high-frequency measurements could integrate the high variability within days (Barba, Bradford,
497 et al., 2019) contributing to an emergent seasonal pattern (Barba, Poyatos, et al., 2019; Plain et
498 al., 2019).

499 Overall, these results underline the challenges that manual measurements may have for
500 estimating temporal patterns, and the challenges that both manual and automated measurements
501 may have for estimating mean annual CH₄ fluxes from tree stems. Worldwide studies are needed
502 to test the temporal variability of stem CH₄ fluxes combining manual and automated measure-
503 ments and to evaluate the consistency between both approaches to accurately estimate temporal
504 patterns and annual fluxes. These efforts are crucial if we aim to include tree stem CH₄ fluxes
505 into local or global carbon budgets.

506

507 *Drivers of stem CO₂ and CH₄ fluxes*

508 Temperature and SWC played an important role controlling CO₂ and CH₄ stem fluxes as pro-
509 posed in H2a. For CO₂ fluxes, temperature, SWC, stem height and some of their interactions ex-
510 plained a large proportion of stem CO₂ emissions variability (R^2_m of 0.81 and 0.42, automated
511 and manual measurements, respectively). These environmental variables as well as plant activity
512 have previously been identified as drivers of stem CO₂ emissions (Ceschia, Damesin, Lebaube,
513 Pontauiller, & Dufrene, 2002; Gansert & Burgdorf, 2005). Stem CO₂ emissions also increased
514 with tree DBH, consistent with more sapwood tissue contributing to stem CO₂ efflux via stem
515 respiration in larger trees (Teskey et al., 2017). Our results support the fact that identifying func-
516 tional relationships for stem CO₂ fluxes is consistent when derived from manual or automated
517 measurements.

518 The role of stem temperature and SWC for stem CH₄ fluxes presented some similarities
519 between approaches (Table 1 and 2). Both variables showed a positive independent effect for
520 CH₄ manual measurements but an interacting effect for automated measurements (positive mar-
521 ginal significance). (Table 1 and 2). Our results thus partly agree with other studies (derived

522 from manual measurements) reporting larger CH₄ fluxes with increasing soil temperature (Pitz et
523 al., 2018; Wang et al., 2016). Stem CH₄ fluxes increased consistently with SWC (Table 1, Table
524 2), although in the case of automated measurements, this was supported only for the 50 cm
525 height (Fig. S2). These results clearly differ from other studies (derived from manual measure-
526 ments) showing no effect of SWC on stem CH₄ fluxes (Pitz & Megonigal, 2017; Warner et al.,
527 2017; Welch et al., 2018). Thus, our results compound the challenge of finding consensus for in-
528 corporating stem CH₄ fluxes and associated functional relationships into process-based models.

529 We found a positive relationship between stem CO₂ and CH₄ supporting H2b, which was
530 also reported in a riparian cottonwood forest ecosystem (slope of correlation changed seasonally)
531 (Flanagan et al., 2021) and in a previous study using daily means of CO₂ and CH₄ fluxes (Vargas
532 & Barba, 2019). This correlation could be attributed to gas diffusivity heterogeneity through the
533 wood, which might similarly affect both gases (Barba, Bradford, et al., 2019). However, stem
534 CO₂ fluxes only explain a small fraction of stem CH₄ flux variability in this study ($R^2_m < 0.1$ for
535 both manual and automated measurements), hindering predictions of stem CH₄ fluxes based on
536 stem CO₂ fluxes as previously suggested (Vargas & Barba, 2019). We recognize that the under-
537 lying mechanisms controlling stem CH₄ fluxes should be properly elucidated to move beyond
538 empirical relationships and build process-based models to predict stem CH₄ fluxes.

539 The effect of tree attributes such as DBH and stem height in modulating environmental
540 controls of stem fluxes supported our hypothesis H3, but these effects were not consistent across
541 CO₂ and CH₄ fluxes. Tree DBH was associated with larger stem CO₂ fluxes but we observed no
542 DBH effect on stem CH₄ fluxes (DBH only tested for manual measurements due to the limited
543 number of trees for automated measurements). There is no consensus on the effect of DBH on
544 CH₄ fluxes in upland forests, with positive relationships found in some studies (Pitz et al., 2018;

545 Wang et al., 2017), but not in others (Warner et al., 2017). However, we expected bigger trees to
546 present higher fluxes because of their potentially higher capacity of transporting CH₄ from a
547 larger soil volume, and/or because their higher volume of heartwood for internally producing
548 methane (see next section for CH₄ origin discussion). The fact that we only studied mature trees
549 with a relatively narrow range of diameters might hinder our capacity to detect a DBH effect that
550 may be evident with a larger gradient of stem diameters. The effect of stem height was also more
551 evident for CO₂ than for CH₄ (manual measurements). The observed decline in CH₄ fluxes with
552 stem height was only detected when stem height was the sole factor in the model, disappearing
553 when other drivers were included (Table 1). For automated measurements, the influence of stem
554 height on stem CH₄ fluxes was only detected in interaction with SWC, and the model outcome
555 did not support a strong decline in stem CH₄ fluxes with stem height (Fig. S2). Additionally,
556 concurrent stem CH₄ uptake, as seen in other studies (Jeffrey et al., 2021; Machacova et al.,
557 2016, 2020; Pitz & Megonigal, 2017; Welch et al., 2018), may mask potential patterns between
558 CH₄ fluxes and stem height (Barba, Bradford, et al., 2019).

559 Our results on stem height effect contrast with other studies reporting CH₄ fluxes (mainly
560 from manual measurements) declining with stem height (Jeffrey et al., 2020; Pitz & Megonigal,
561 2017; Sjögersten et al., 2020; Wang et al., 2016), suggesting that the relationship between fluxes
562 and stem height might be species- or site-specific. However, most of the stem flux measurements
563 are usually limited to the first 2-3 meters above the soil due to logistical constraints, indicating
564 that further investigations measuring CH₄ fluxes upper in the canopy (e.g. branches and twigs)
565 may be crucial in order to properly upscale chamber measurements to a whole-tree level, and to
566 properly model the effect of changes (if any) in radial CH₄ diffusion and production throughout
567 tree stems (Barba, Bradford, et al., 2019).

568 The proportion of CH₄ variability explained by SWC, temperature, stem height and asso-
569 ciated interactions was small (6.8 and 11% for manual and automated measurements, respec-
570 tively), contributing to the increasing evidence on the complications involved for modeling CH₄
571 fluxes from multiple land surfaces (Ringeval et al., 2014; Vázquez-Lule & Vargas, 2021). The
572 consistency between manual and automated measurements in the lack of explanatory power of
573 the measured drivers might suggest that we probably did not measure the appropriate variables.
574 Other variables, such as soil CH₄ fluxes or plant phenology, may explain more variability of
575 stem fluxes (Barba, Poyatos, et al., 2019). However, most of the CH₄ flux variability in the cur-
576 rent study was explained by tree identity, allocated in the random part of the models. We did not
577 expect such large effect of tree identity (i.e., differences between trees) because all measured
578 trees were mature and from the same species. They also grew in flat and apparently homogene-
579 ous terrain (Petraakis et al., 2018) and were relatively close to each other (maximum distance be-
580 tween trees around 70 m). This might suggest that tree traits in the vicinity of the flux measure-
581 ment, or processes that affect methane production, consumption and transport (e.g., methano-
582 genic and methanotrophic microbial communities), might be more relevant determining stem
583 CH₄ fluxes at the ecosystem scale than environmental conditions. We found a significant effect
584 of wood density and moisture on CO₂ stem fluxes, but not on CH₄ fluxes, which might imply
585 that CH₄ fluxes are less related to wood features than CO₂, and that the source of CH₄ is much
586 more localized than the source of CO₂ (respiration tissue is everywhere and CO₂ could be trans-
587 ported from soils). However, other wood properties such as tree wounds, water distribution
588 within stems, and lenticels density, might still affect gas diffusivity in the wood, and thus, con-
589 trol CH₄ fluxes. The relatively small magnitudes of stem fluxes measured in this study contrast

590 with the high internal CH₄ concentrations; this suggests that wood properties controlling gas dif-
591 fusivity from the heartwood to the atmosphere might be key factors explaining stem CH₄ fluxes.
592 If that is the case, it could imply that the classical temperature and moisture kinetic relations
593 commonly applied for modelling fluxes of greenhouse gases might not be suitable for upscaling
594 and predicting stem CH₄ fluxes. Overall, these results emphasize the need to incorporate tree-
595 specific traits and processes to better understand spatial and temporal variability of stem CH₄
596 fluxes.

597

598 *Evidence for the xylem origin of stem CH₄ fluxes*

599 Our results provide evidence that emitted CH₄ is likely produced inside tree stems. First, most
600 trees did not show a consistent decrease in fluxes with stem height, which is usually attributed to
601 the possibility that CH₄ is produced in soils (Jeffrey et al., 2020; Pitz & Megonigal, 2017;
602 Sjögersten et al., 2020; Wang et al., 2016). In this case it is expected that CH₄ produced in the
603 soil is transported by the roots and emitted by stem degasification and consequently CH₄ emis-
604 sions decrease with stem height (Barba, Bradford, et al., 2019).

605 Second, soils in the study site were net sinks of methane (Barba, Poyatos, et al., 2019;
606 Petrakis et al., 2018), and there was a strong decline in CH₄ soil concentrations throughout the
607 soil profile (Fig. 4d). In theory, even in locations where soils are net sinks of CH₄, soils still
608 could be the origin of CH₄ stem fluxes, if larger concentrations of CH₄ are produced (or stored)
609 deeper in the soil profile and transported by roots into the tree, bypassing the soil's uppermost
610 methanotrophic layer (Megonigal & Guenther, 2008). However, this deep soil transport does not
611 seem feasible in our case since the highest soil CH₄ concentration measured in the whole soil
612 profile (up to the ground water) was less than 5 ppm and there was no evidence of CH₄ produced

613 within the soil profile. Additionally, a study performed in the same region measuring *Carya* sp
614 fine roots density showed that most of the fine roots were allocated in the uppermost 15 cm
615 (Davis, Haines, Coleman, & Hendrick, 2004), a soil zone where we found lower-than-atmos-
616 phere CH₄ concentrations (Fig. 4d). Overall, thermodynamic principles and our soil CH₄ meas-
617 urements suggest that it is unlikely that CH₄ was produced in the soil and transported to the tree
618 stems. Further studies measuring soil redox and O₂ concentrations along with characterization of
619 fine roots biomass and distribution in the soil profile could provide more evidences for discard-
620 ing soil as the origin of stem emitted CH₄.

621 Third, CH₄ internal concentrations in most trees were very high (up to 82000 ppm).
622 These internal concentrations did not correlate with stem fluxes measured in the same week or
623 with the study-period integrated flux measurements (likely due to differences in radial wood dif-
624 fusivity). These results are supported by previous observations (from manual measurements)
625 where heartwood concentrations did not correlate with stem fluxes unless longer periods of stem
626 concentrations were integrated in the analysis (Wang et al., 2017). A lack of correlation between
627 stem internal concentrations and fluxes challenge the application of models based on diffusion
628 gradients to estimate stem CH₄ fluxes (Covey et al., 2012; Wang et al., 2021). Furthermore, if we
629 did not find evidence of CH₄ produced or stored in the soil profile, then where do the high inter-
630 nal CH₄ concentrations come from? Further studies measuring internal CH₄ would be useful for
631 identifying the origin of CH₄, not only measuring absolute concentrations but also isotopic com-
632 position of emitted CH₄, which might depend on the composition of the source (CO₂ reduction or
633 acetate fermentation), the fractionation during the transport, or the oxidation by the metha-
634 notrophs (Barba, Bradford, et al., 2019; Jeffrey et al., 2021). Fourth, our incubation experiment

635 demonstrated that stems under anaerobic conditions can produce CH₄. This CH₄ might be pro-
636 duced by methanogenic archaea communities inhabiting heartwood of the trees, as found in East-
637 ern cottonwood trees in temperate forests (Flanagan et al., 2021; Yip et al., 2018). In our case,
638 we could not quantify the absolute CH₄ production rates due to the lack of temporal sampling
639 (see M&M section), but we demonstrated that production capacity in the heartwood was higher
640 than in the sapwood (as found in Wang et al. (2016)). Lower O₂ concentrations in the heartwood
641 than in sapwood (Mugnai & Mancuso, 2010) may result in a more suitable environment for the
642 methanogenic archaea community, enhancing their performance and abundance. All our samples
643 for both sapwood and heartwood were able to produce CH₄, which is unprecedented since previ-
644 ous studies only showed that a small proportion of trees produced CH₄ under lab incubations
645 (Covey et al., 2012; Pangala et al., 2017; Wang et al., 2016). Wood CH₄ production capacity
646 should be complemented with analysis on the inner-stem microbial community (amplicon-based
647 and metagenomic approaches), which would provide additional evidence for the hypothesis of
648 heartwood CH₄ production. A study analyzing methanogenic archaea within stems found its
649 presence in just 34% of the heartwood and 13% of the sapwood samples (Yip et al., 2018). This
650 could suggest that CH₄ production capacity might be species-specific, and by extension, that the
651 origin of CH₄ emitted by stems might also be species-specific. This might imply an additional
652 challenge for process-based models, which may have to consider tree species and the possibility
653 of CH₄ being produced within the tree or transported from soils depending on the environment.

654 We did not find evidence that internal wood rot would favor higher stem CH₄ fluxes as
655 also found for six upland tree species in Northeastern US (Covey et al., 2012), undermining the
656 influence of wood rot for stem CH₄ production. Some studies have speculated that internal wood
657 rot could be a potential source of stem CH₄ production (Covey & Megonigal, 2019), either by

658 enhancing methanogenesis or by eliminating physical barriers (i.e., increasing diffusion) for gas
659 transport. In our case, up to 85% of the measured stem height locations showed positive fluxes
660 despite not showing evidence of rot (visual observation from tree cores). Even the stem with very
661 high emissions measured with automated chambers (Fig. 2b) did not present symptoms of inter-
662 nal wood decay when it was felled by a storm 2.5 years after the experiment. The fact that stem
663 CH₄ emissions might not be related to wood decay could suggest that persistent stem CH₄ emis-
664 sions are a common phenomenon in healthy upland forests.

665

666 **Conclusions**

667 Manual and automated measurements allowed us to identify seasonal patterns of stem CO₂ emis-
668 sions and to show coherence between mean seasonal fluxes and their response to stem tempera-
669 ture and soil moisture. However, we found important discrepancies for stem CH₄ fluxes when
670 using automated and manual measurements and discussed potential bias of previous studies of
671 stem CH₄ fluxes solely based on manual or automated measurements. Stem CH₄ fluxes were
672 highly variable over short time periods, changing from net emissions to net uptake within days.
673 Only automated measurements captured such high variability, allowing seasonal patterns to
674 emerge among-trees and within-trees when data was integrated daily. However, seasonal mean
675 fluxes derived from automated measurements were too sensitive to “hot spots”, due to the lim-
676 ited number of spatial replicates. Temperature and moisture had a positive effect on stem CH₄
677 fluxes for both manual and automated measurements, but the effect of moisture for automated
678 measurements was only positive at the base of the trees. Overall, the abiotic and biotic drivers
679 examined explained a low variability in stem CH₄ fluxes, compared to CO₂.

680 The inconsistent vertical pattern of stem CH₄ emissions, together with low soil CH₄ con-
681 centrations throughout the soil profile, high CH₄ concentrations in the heartwood of the trees,
682 and CH₄ production within the xylem during laboratory incubations, provide several lines of evi-
683 dence that suggest most of the CH₄ emitted through the tree stems may have been produced in-
684 ternally. This study contributes to the general understanding of environmental controls of stem
685 emissions, but also reveals that there is a large proportion of variability that still remains unex-
686 plained, suggesting that other variables not accounted in this study, such as those controlling gas
687 diffusivity and transport in through the wood, might play a major role on controlling stem fluxes.
688 We recognize that our results might be species- or site-specific and, there is a need for more
689 studies that measure stem emissions spanning multiple tree species and ecosystems, as well as
690 incorporate tree-level wood properties. A critical volume of experimental studies and an under-
691 standing of internal controls of stem fluxes are key steps for quantifying the integrated role of
692 trees on the global CH₄ cycle.

693

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701

702 **Authorship**

703 J.B. and R.V. conceived and designed the experiment; J.B. and M. C. performed the experiment;
704 J.B. and R.P. analyzed the data; J.B. wrote the first draft and all authors edited the manuscript.

705

706 **Data availability statement**

707 Data used in this study can be download from Figshare (10.6084/m9.figshare.14885697). The R
708 code used for estimating stem GHG fluxes from the gas analyzer concentrations can be down-
709 loaded from https://github.com/josepbarba/GHG_flux_calculations.

710

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