# Tree stem bases are sources of $CH_4$ and $N_2O$ in a tropical forest on upland soil during the dry to wet season transition

Welch, Bertie; Gauci, Vincent; Sayer, Emma J.

*DOI:*
10.1111/gcb.14498



*Document Version*
Peer reviewed version

*Citation for published version (Harvard):*
Welch, B, Gauci, V & Sayer, EJ 2019, 'Tree stem bases are sources of $CH_4$ and $N_2O$ in a tropical forest on upland soil during the dry to wet season transition', *Global Change Biology*, vol. 25, no. 1, pp. 361-372. https://doi.org/10.1111/gcb.14498

# Tree stem bases are sources of $CH_4$ and $N_2O$ in a tropical forest on upland soils during the dry to wet season transition

**Authors:** Bertie Welch[1], Vincent Gauci[1] & Emma J. Sayer[2,3]

**Addresses:**

1. School of Environment, Earth & Ecosystem Science, The Open University, Milton Keynes, MK7 6AA, United Kingdom

2. Lancaster Environment Centre, Lancaster University, Lancaster, LA1 4YQ, United Kingdom

3. Smithsonian Tropical Research Institute, Apartado 0843-03092, Balboa, Ancón, Republic of Panama.

**Corresponding author:**

E.J. Sayer

e.sayer@lancaster.ac.uk

**Keywords:** Methane, nitrous oxide, tree stem emissions, upland tropical forest, leaf litter, trace greenhouse gases, soil

**Type:** Primary Research Article

**Abstract**

Tropical forests on upland soils are assumed to be a methane ($CH_4$) sink and a weak source of nitrous oxide ($N_2O$), but studies of wetland forests have demonstrated that tree stems can be a substantial source of $CH_4$, and recent evidence from temperate woodlands suggests that tree stems can also emit $N_2O$. Here, we measured $CH_4$ and $N_2O$ fluxes from the soil and from tree stems in a semi-evergreen tropical forest on upland soil. To examine the influence of seasonality, soil abiotic conditions, and substrate availability (litter inputs) on trace greenhouse gas (GHG) fluxes, we conducted our study during the transition from the dry to the wet season in a long-term litter manipulation experiment in Panama, Central America. Trace GHG fluxes were measured from individual stem bases of two common tree species and from soils beneath the same trees. Soil $CH_4$ fluxes varied from uptake in the dry season to minor emissions in the wet season. Soil $N_2O$ fluxes were negligible during the dry season but increased markedly after the start of the wet season. By contrast, tree stem bases emitted $CH_4$ and $N_2O$ throughout the study. Although we observed no clear effect of litter manipulation on trace GHG fluxes, tree species and litter treatments interacted to influence $CH_4$ fluxes from stems and $N_2O$ fluxes from stems and soil, indicating complex relationships between tree species traits and decomposition processes that can influence trace GHG dynamics. Collectively, our results show that tropical trees can act as conduits for trace GHGs that most likely originate from deeper soil horizons, even when they are growing on upland soils. Coupled with the finding that the soils may be a weaker sink for $CH_4$ than previously thought, our research highlights the need to reappraise trace gas budgets in tropical forests.

## Introduction

Methane ($CH_4$) and nitrous oxide ($N_2O$) are important trace greenhouse gases (GHGs) with radiative effects 25 and 298 times greater than $CO_2$, respectively (Houghton et al., 2001). Interest in trace GHG exchange in tropical forests has grown in recent years, particularly in saturated wetland areas of the tropics such as the Amazon floodplain (Graffman et al., 2008; Pangala et al., 2017) and mangrove swamps (Kreuzwieser et al., 2003; Krithika et al., 2008). Natural wetlands are the single largest individual source of atmospheric methane contributing 177-284 Tg $CH_4$ $yr^{-1}$ (IPCC, 2013), to which tropical wetland emissions from a variety of sources (including waterlogged soils) are a significant contributor, but the contribution of tropical forests on upland soils (i.e. soils that are rarely flooded and only temporarily water-saturated) has not yet been quantified. Globally, emissions of nitrous oxide from soils in natural ecosystems account for 37% of total global surface emissions (IPCC, 2007), estimated at 3.37-6.60 Tg N $yr^{-1}$ (Zhuang et al., 2012) and tropical rainforests are the single biggest natural source of $N_2O$ (Bouwman et al., 1995).

Tree stems can also emit significant amounts of $CH_4$ in temperate (Gauci et al., 2010) and tropical (Pangala et al., 2013; Pangala et al., 2017) wetland ecosystems, and mesocosm experiments showed that black alder trees, typical of European temperate wetlands, can also act as a pathway for $N_2O$ emissions to the atmosphere (Rusch and Rennenberg, 1998). $CH_4$ and $N_2O$ are produced under anoxic conditions in waterlogged soils by methanogenic consortia of archaea or denitrifying bacteria, respectively, and the gases diffuse into soil water, and then from water into roots as either a solute or a gas. The gases move up the tree stem by either mass flow (transpiration or pressurized ventilation) or diffusion, then diffuse from the xylem through the stem tissue to the atmosphere via lenticels and other structures that aid gas exchange (Carmichael et al., 2014). Findings extrapolated from glasshouse experiments suggest that wetland hardwood trees could account for emissions of around 60 Tg $CH_4$ $yr^{-1}$ (Rice et al.,

2010); tree stem fluxes accounted for 62-87% of total ecosystem $CH_4$ flux in tropical peat forests in Indonesia (Pangala et al. 2013) and contribute half of all emitted methane in the Amazon floodplain (~20 Tg; Pangala et al., 2017).

We are only just beginning to understand the role of tropical tree stems as conduits of soil-produced trace GHGs, and the vast majority of research on this subject to date has been in forested wetlands. Wetland tree species have evolved a variety of specialist tissues to aid oxygen transport to roots in anoxic soils such as aerenchyma, increased stem lenticel density (Pangala et al., 2014) and adventitious roots, which can transport soil-generated $CH_4$ in mangrove tree species (Purvaja et al., 2004). Inter-species variations in wood specific density and stem lenticel numbers could be important controls of stem emissions, as tree stem $CH_4$ flux is negatively related to wood density and positively related to lenticel number (Pangala et al., 2013). Tree stem emissions of trace GHGs can occur on upland soils when such soils become saturated with water, which reduces soil oxygen concentrations and facilitates the activity of anoxic methanogenic archaea and denitrifying bacteria that thrive in such soil conditions. Tree stem emissions of $CH_4$ and $N_2O$ have been observed in temperate trees that lack aerenchyma and other adaptations to wet, anoxic soil conditions (Machacova *et al*., 2013, 2016; Wen et al., 2017). In addition, recent work demonstrates production of $CH_4$ within tree stems (Wang et al., 2016) and high abundance of methanogens in heartwood (Yip et al., 2018). However, despite the global importance of tropical forests in GHG budgets, we know very little about $CH_4$ emissions from tropical tree stems on upland soils and we do not know whether they are also capable of emitting $N_2O$.

Although tropical forests on upland soils cover a larger land-surface area than tropical wetlands (Pan et al., 2013), they are generally not considered to be a major source of $CH_4$ and $N_2O$ emissions. However, the role of tree stems as conduits of trace GHGs in tropical forests on upland soils warrants further attention because many tropical tree species have large buttress

roots and the greatest stem gas emissions are measured within 0.3-m of the soil surface (Rusch and Rennenberg, 1998; Gauci et al., 2010; Pangala et al., 2013). Hence, even minor trace GHG emissions from tropical trees on upland soils could represent a significant source of $CH_4$ and $N_2O$. Indeed, recent work in temperate woodland demonstrated that tree stem emissions diminish the methane sink of upland forests (Pitz & Megonigal, 2017), if the same applies to tropical forests, it would affect global trace GHG budgets.

Regardless of whether soils are waterlogged or well-drained, the production of trace GHGs in soils depends on the availability of suitable substrates (Li et al., 2000). Litter quantity can influence the rates of trace GHG emissions from forest soils as it provides substrate (acetate and hydrogen) for methanogens and the nitrate used in denitrification (Teh et al., 2008). The potential link between litter inputs and trace GHG emissions from soil was explored by a study of soil $N_2O$ emissions from a wet forest in Costa Rica, in which doubling leaf litter inputs increased rates of $N_2O$ emissions by 43% relative to controls, with a corresponding decline of 42% in litter removal plots (Wieder et al., 2011). However, litter manipulation treatments in subtropical forest in Southern China had no significant effect on soil $CH_4$ uptake or $N_2O$ production, implying that abiotic conditions in the mineral soil may be more important than litter quantity (Tang et al., 2006). The effects of litter manipulation on trace GHG emissions from tree stems is presently unknown, but as changes in mineral soil chemistry from litter were the primary driver of changes in $CH_4$ and $N_2O$ fluxes (Fender et al., 2013), it is conceivable that litter manipulation could also affect tree stem emissions.

Hence, although trees can be a major conduit for $CH_4$ in tropical floodplains and peat forests, we know very little about tree stem fluxes of $CH_4$ in tropical forests on upland soils, and there are no field data on tree stem $N_2O$ emissions in the tropics. We aimed to address these gaps in our knowledge of tropical GHG emissions by measuring $CH_4$ and $N_2O$ fluxes from the soil and tree stems in a seasonal moist tropical forest on upland soils. We focussed our attention on

quantifying seasonal changes in $CH_4$ and $N_2O$ fluxes at the base of tree stems, and we assessed the specific role of litter in providing substrate for trace GHG production. Accordingly, we conducted our study in an existing long-term litter manipulation experiment during the transition from the dry season to the wet season to test the following hypotheses:

1) Tree stems in tropical forests on upland soils will act as a conduit for $CH_4$ and $N_2O$ produced in the soil; patterns in trace GHG emissions from tree stems will therefore mimic those from the soil, increasing in the wet season and decreasing in the dry season.
2) Litter manipulation treatments alter substrate availability to microorganisms and will therefore influence $CH_4$ and $N_2O$ fluxes from soils and tree stems; hence, trace GHG emissions will be greater in litter addition treatments and lower in litter removal plots relative to controls.

## Materials and Methods

### *Field area and sampling*

The study was carried out within the Gigante Litter Manipulation Project, approximately 5 km south of Barro Colorado Island (BCI) in Panama, Central America (Sayer & Tanner, 2010). The 15 plots were set up between 2000 and 2002; each plot measures 45-m × 45-m and the edges of the plots were trenched to a depth of 0.5-m, lined with plastic and then backfilled. Starting in January 2003, the litter is raked up and removed from five plots every month (L-) and added to five plots where it is spread as evenly as possible (L+); five plots were left as controls (CT; see Sayer et al. 2006 and Sayer & Tanner 2010 for a full description). The mean annual temperature at the weather station on nearby Barro Colorado Island (within 2 km of the study site) is 26$^{o}$C, mean annual rainfall is 2,600 mm and there is a strong dry season from mid-December to mid-April (Leigh, 1999). During the study period, maximum and minimum air temperatures were 32.4$^{o}$C and 24.3$^{o}$C respectively, soil temperature ranged from 24.9 – 29.2$^{o}$C

144 and soil water content (SWC) was between 14% and 40%. The soil in the plots is characterised
145 as a moderately acidic Oxisol (Cavelier, 1992).

146 Two tree species were selected for this study: the fast-growing canopy tree *Simarouba amara*
147 (Aubl.) and the shade-tolerant subcanopy tree *Heisteria concinna* (Standl.), which occur
148 frequently throughout the study forest and are among the most abundant tree species in the
149 experimental plots (12% of all trees with dbh >10 cm; Sayer and Tanner, 2010). Both species
150 have relatively smooth bark and straight stems, which facilitated sampling and the species have
151 distinct specific wood densities (0.38 g $cm^{-3}$ for *Simarouba* and 0.64 g $cm^{-3}$ for *Heisteria*;
152 Condit et al., 2013), which is likely to influence trace GHG fluxes from stems (Pangala et al.,
153 2013). Trees were mapped and marked using handheld GPS. One individual per species was
154 chosen per plot but only 13 of the 15 experimental plots contained live mature individuals of
155 *Simarouba*; hence the present study included trees in four plots per treatment, making 12
156 *Heisteria* and 12 *Simarouba* trees in total.

157 Greenhouse gas fluxes from the soil were measured using permanently installed soil collars
158 located 2-3 m to the north and south of each tree. The collars were made from 120-mm long
159 sections of polyvinyl chloride pipe (internal diameter: 200 mm), which were embedded into the
160 soil to 30-mm depth. All collars were installed at least two weeks prior to sampling in March
161 2014 and an appropriate amount of litter was placed into the collars in the CT and L+ plots to
162 achieve consistency with the surrounding forest floor. To determine $CH_4$ and $N_2O$ emissions
163 from the soil, a PVC lid with an inner seal of gas-tight neoprene foam was placed on top of the
164 collar; a 15-ml air sample was taken by syringe via a septum in the lid immediately after closure
165 and then again after 3, 6 and 10 minutes. Each sample was injected into pre-evacuated 12-ml
166 borosilicate vials (Exetainer™, LabCo Ltd, High Wycombe, UK). The suction when removing
167 the lid after sampling demonstrated the integrity of the seal on the soil chamber. Soil

168 temperature at 0-6 cm depth was recorded adjacent to the collars using a Thermapen (ETI Ltd,
169 Worthing, UK).

170 Tree stem gas fluxes were measured using a flexible chamber made from a 450-mm × 300-
171 mm sheet of polycarbonate, lined with neoprene foam (19 mm wide, 25 mm thick; Siegenthaler
172 et al. 2016). The chambers were secured to the tree stems at 0.3-m above the forest floor using
173 cam buckle straps. Gas samples were taken by syringe from a septum in the middle of the
174 chamber at 0, 5, 10 and 15 minutes, and injected into pre-evacuated 12-ml vials as described
175 above.

176 Air samples from the tree stem and soil chambers were collected every two weeks between
177 30 March and 20 July 2014. Air pressure and temperature outside the stem chamber were
178 recorded at the start of sampling using a Thermometer-Hygrometer-Barometer probe
179 (Commeter C4141; Comet Systems, Czech Republic). Soil temperature at 0-10-cm depth was
180 measured adjacent to the collars using a soil temperature probe and volumetric soil water
181 content at 0-6 cm depth was measured using a Thetaprobe (Delta-T Devices, Cambridge, UK)
182 calibrated to local soil conditions following the manufacturer's instructions. Collection of soil
183 temperature and soil water content data during gas sampling was limited to 28 May - 14 June
184 2014 and 2 - 6 July 2014 due to equipment malfunction. We therefore used monthly values
185 measured in the plots as part of a separate study (Sayer et al. unpublished data) and weekly
186 rainfall data measured at the meteorological tower on Barro Colorado Island (courtesy of the
187 Physical Monitoring Program of the Smithsonian Tropical Research Institute).

188 The $CH_4$ concentrations of the air samples were analysed within a week of sampling using
189 off-axis Integrated Cavity Output Spectroscopy (FMA-200 Fast Methane Analyser; Los Gatos
190 Research, Mountain View, CA, USA). The $N_2O$ concentrations of the air samples were
191 analysed using gas chromatography (Ai 94 Gas Chromatograph, Cambridge Instruments,
192 Ellutia, Ely, UK). All methods for the measurement of GHGs, including the testing of the

chamber method and sensitivity of measurements are discussed fully in Siegenthaler et al. (2016).

*Data analyses*

The data were inspected visually before further analysis; we considered extreme outliers that lay outside of the $5^{th}$ - $95^{th}$ interquartile range as measurement errors and removed them from the dataset. For soil fluxes, we omitted two data-points for $CH_4$ and one value for $N_2O$ (out of 188 and 108, respectively) and for tree stem fluxes, we omitted 20 data-points for $CH_4$ and three data-points for $N_2O$ (out of 201 and 115, respectively).

All data analyses were conducted in R 3.3.2 (R Core Team, 2016) using the lme4 package for mixed effects models (Bates et al., 2015). Gas fluxes were calculated for each chamber following Baird et al. (2010), whereby the least squares linear regression slope of the four sample concentrations is plotted against sampling time and the slope to give the gas flux in µg $m^{-2}$ $h^{-1}$. Gas flux measurements were only used for further statistical analysis if the $R^2$ of the regression was >0.7; this cut-off point was chosen following Alm et al. (2007; cited in Cooper et al., 2014), who noted that low fluxes (especially those near to zero) tend to have low $R^2$ values. Concentration changes in $N_2O$ in dry season samples were too small to estimate non-zero fluxes (i.e. $R^2$ <0.7); we therefore only present wet season data for soil and tree stem $N_2O$ fluxes.

Soil water content, soil temperature, and air temperature were strongly correlated; however, as is typical of the tropics, temperature only varied within a narrow range. Given that rainfall and soil moisture exhibited far larger variation (and have a fundamental control on soil $CH_4$ and $N_2O$ production), we investigated the relationships between soil water content and trace GHG fluxes from soils and tree stems using monthly means. Relationships were inspected visually and emerging patterns were then tested using linear models. We then assessed the

influence of tree species, litter treatment, and their interaction on soil and stem trace GHG fluxes using linear mixed effects models (*lmer* function) with plot and time as random effects. The significance of each term was determined by comparing nested models using likelihood ratio tests. Models were simplified by sequentially dropping terms until a minimum adequate model was reached, using AICs and p-values to check for model improvement (Pinheiro and Bates, 2000). The final model fit was inspected using diagnostic plots. Effects of seasonal variation were tested by comparing minimum adequate models with and without time as a random effect. Statistics for mixed effects models are given for the comparison between the best-fit model and the corresponding null model. All results are reported as significant at $p < 0.05$ but due to the low number of replicate plots ($n = 4$), marginally significant trends are also reported at $p < 0.1$.

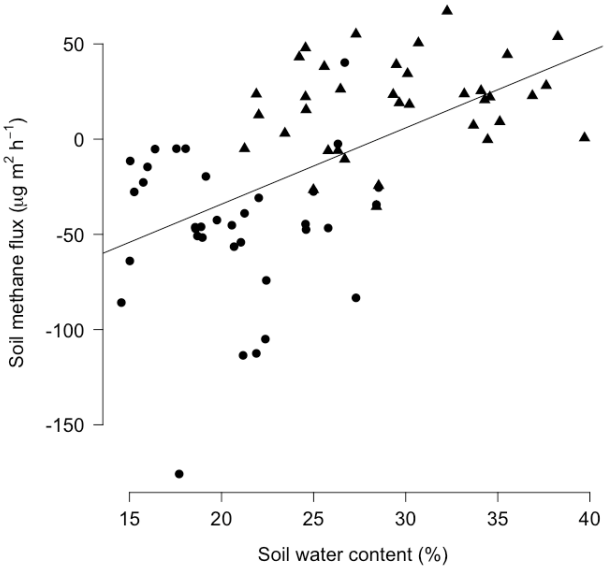


**Figure 1.** The relationship between methane fluxes from the soil and soil water content at 0-6 cm depth in a lowland tropical forest on upland soil in Panama, Central America, during the transition from the dry season (circles) to the wet season (triangles) from March to July 2014.

## Results

### *Seasonal variation in $CH_4$ fluxes*

231 Soil water content was strongly related to total rainfall ($R^2$ = 0.6, $p$ < 0.01) and $CH_4$ fluxes
232 increased with soil water content ($R^2$ = 0.3, $p$ < 0.01; Fig. 1). Soil $CH_4$ fluxes therefore varied
233 significantly between the dry and wet season ($p$ < 0.001, $\chi^2$ = 36.4), whereby soils acted as a
234 $CH_4$ sink during the dry season and switched to being a source within two to three weeks of the
235 start of the wet season (Fig. 2a).

236 There was no clear seasonal pattern for tree stem $CH_4$ fluxes; although stem $CH_4$ emissions
237 tended to be larger during the wet season, they were not significantly so (Fig. 2a) and there
238 were no significant relationships between tree stem fluxes and soil water content.

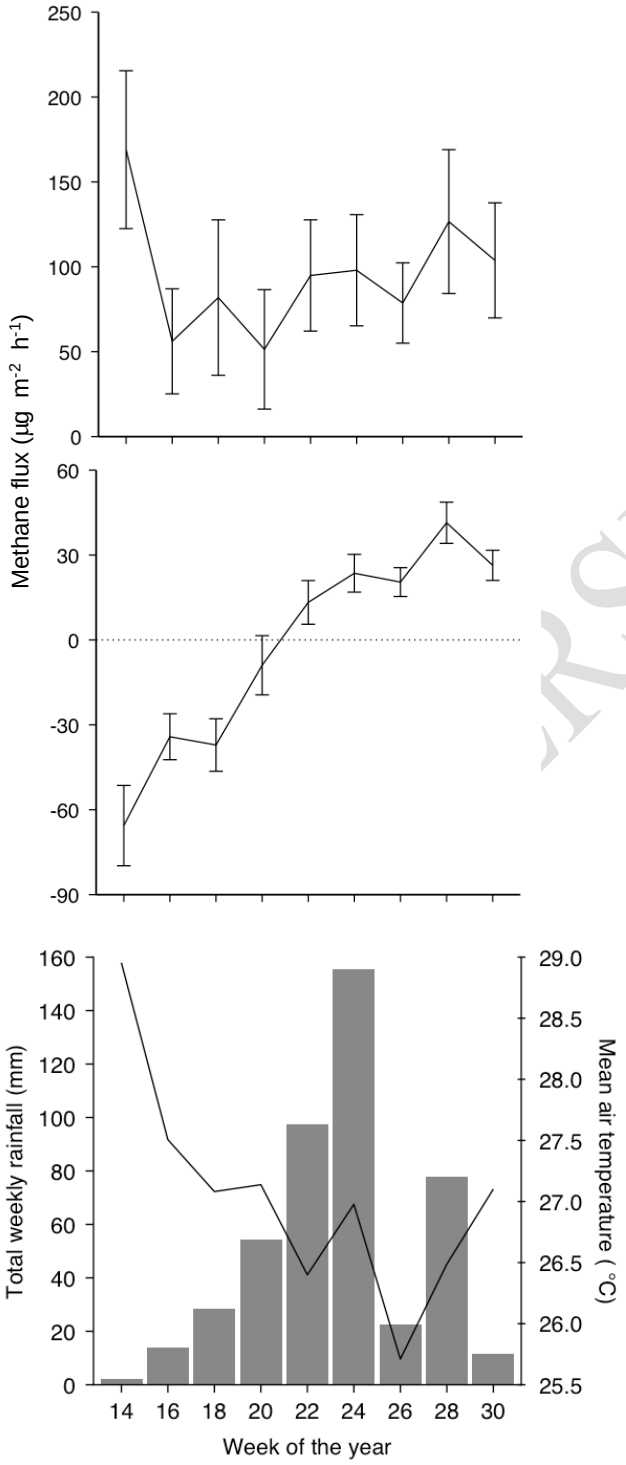


**Figure 2** Methane ($CH_4$) fluxes from tree stems at 0.3-m height (top panel) and soil (centre panel) in a lowland tropical forest on upland soil in Panama, Central America; data shown are pooled across experimental plots with three litter manipulation treatments, means and standard errors are therefore given for $n$ = 12; measurements were made weekly during the transition from the dry season to the wet season (bottom panel, weeks 14-19 and 20-30, respectively), with corresponding changes in total rainfall (bars) and temperature (line).

*Species and treatment effects on soil $CH_4$ fluxes*

There were no effects of species, treatment or their interaction on soil $CH_4$ fluxes. Soil $CH_4$ fluxes remained predominantly negative until week 24 under individuals of *Heisteria* and until week 21 under individuals of *Simarouba*, indicating dry season uptake of $CH_4$ before a transition to emissions within two to four weeks after the first heavy rainfall of the year (Figs. 2b and 3). The median flux beneath *Heisteria* was 8.3 µg $m^{-2}$ $hr^{-1}$, which was slightly higher than the median $CH_4$ flux of 6.3 µg $m^{-2}$ $hr^{-1}$ from chambers under *Simarouba*.

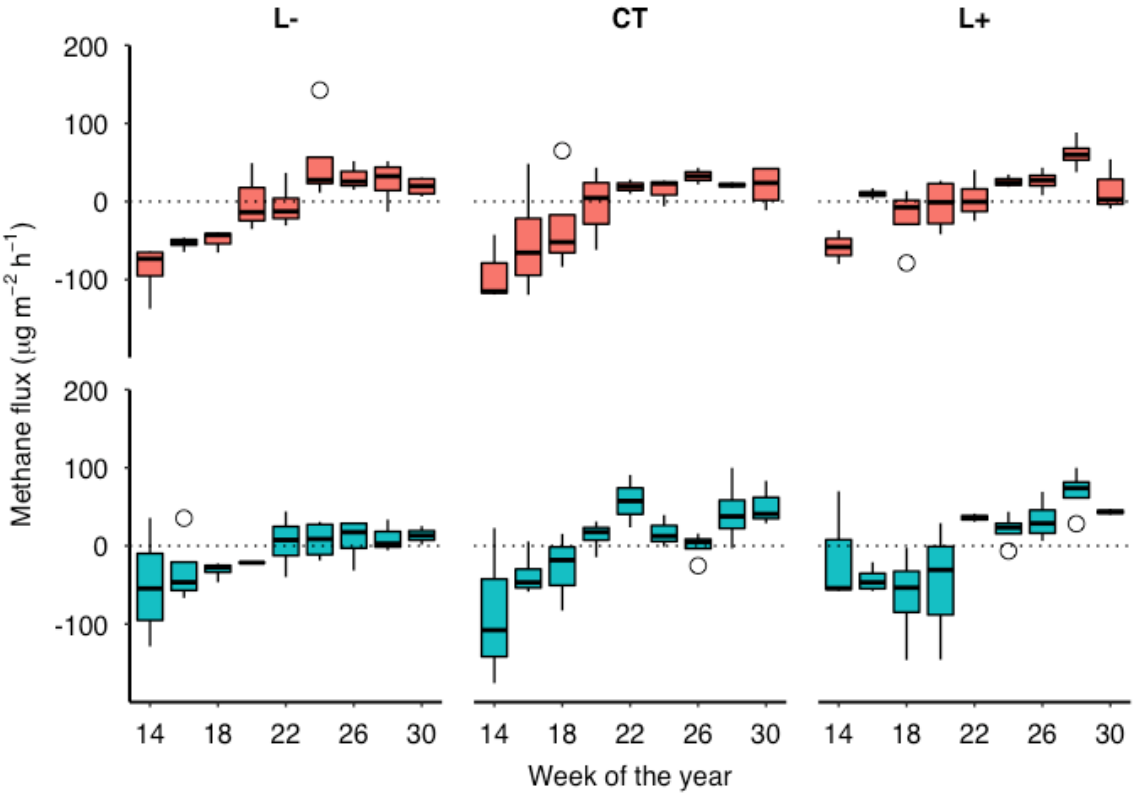


**Figure 3.** Soil methane ($CH_4$) fluxes under individuals of two common tree species: *Heisteria concinna* (top panels) and *Simarouba amara* (bottom panels) in experimental litter removal (L-), control (CT) and litter addition (L+) treatments in a lowland tropical forest on upland soil in Panama, Central America, measured weekly during the transition from the dry season (weeks 14-19) to the wet season (weeks 20-30); ranges (boxes and whiskers) and median lines are shown for *n* = 4 individuals per species and treatment.

248 The range of soil $CH_4$ fluxes under individuals of *Heisteria* (-190 - 539 µg $m^{-2}$ $hr^{-1}$) was
249 greater than under individuals of *Simarouba* (-89.4 - 450 µg $m^{-2}$ $hr^{-1}$). Consequently, the mean
250 soil $CH_4$ flux beneath *Heisteria* individuals was slightly more negative than that beneath
251 *Simarouba* (-2.8 ±5.1 µg $m^{-2}$ $hr^{-1}$ and -2.3 ±5.5 µg $m^{-2}$ $hr^{-1}$ respectively).
252
253 *Species and treatment effects on tree stem fluxes of $CH_4$*
254 Surprisingly, tree stem $CH_4$ fluxes were mostly positive throughout the study period, even
255 though the upland soils at the study site tended to act as a sink for methane during the dry season
256 (Fig. 2). Accordingly, we found no relationship between soil and stem $CH_4$ fluxes. There was
257 a significant species × treatment interaction on stem $CH_4$ fluxes, whereby *Heisteria* stems had
258 higher $CH_4$ fluxes in L+ plots and lower stem fluxes in L- plots compared to *Simarouba* stems
259 ($p < 0.001$, $\chi^2 = 24.5$; Fig. 4). Overall, the median $CH_4$ flux was very similar between the two
260 species, with 72.6 µg $m^{-2}$ $hr^{-1}$ and 75.1 µg $m^{-2}$ $hr^{-1}$ for *Heisteria* and *Simarouba* respectively.
261 Tree stem $CH_4$ fluxes in individuals of *Heisteria* were mostly positive, with a mean flux of 101
262 ±14.9 µg $m^{-2}$ $hr^{-1}$ over the dry-wet season transition, whereas the mean flux for *Simarouba* was
263 lower at 87.7 ±18.5 µg $m^{-2}$ $hr^{-1}$.
264 Stem $CH_4$ fluxes in *Simarouba* displayed greater inter-week variability across all treatments
265 (Fig. 4) and had a greater range throughout the study (-276 µg $m^{-2}$ $hr^{-1}$ to 678 µg $m^{-2}$ $hr^{-1}$) than
266 those from *Heisteria* stems. $CH_4$ stem fluxes from *Heisteria* ranged from -156 to 598 µg $m^{-2}$
267 $hr^{-1}$ and remained relatively constant throughout the study in CT and L- plots but were much
268 more variable in L+ plots (Fig. 4).

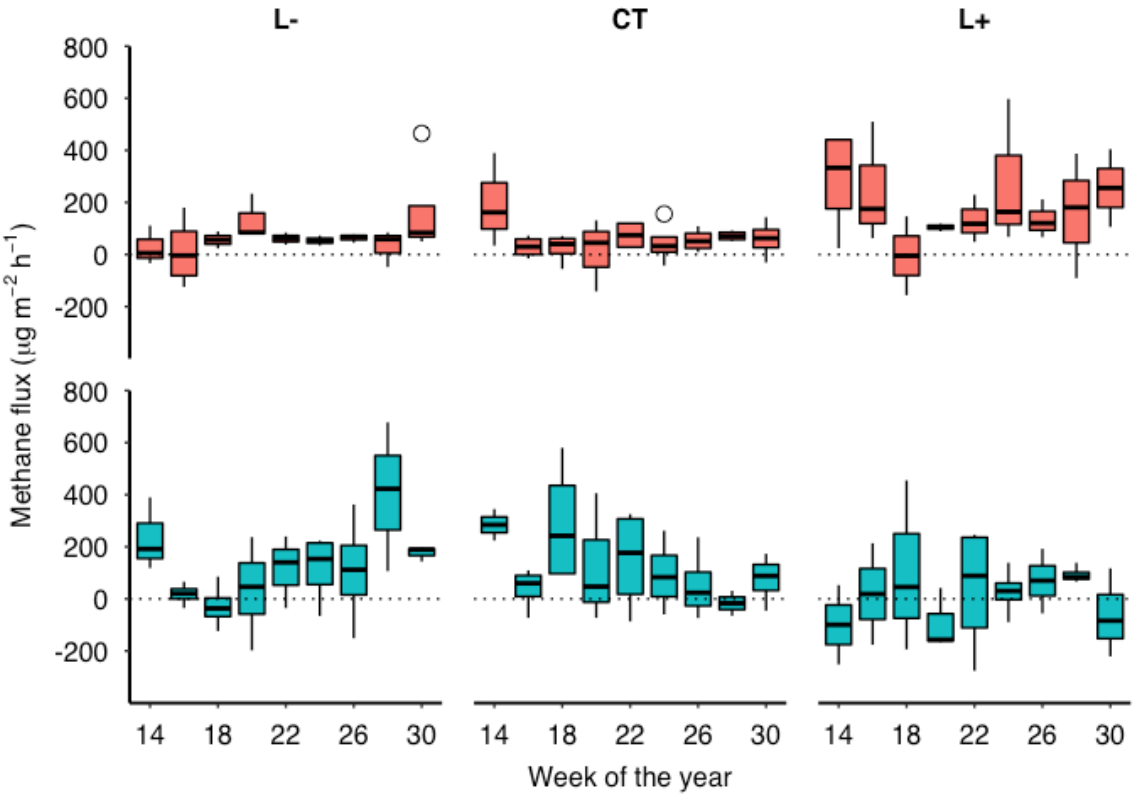


**Figure 4.** Methane ($CH_4$) fluxes from tree stems of *Heisteria concinna* (top panels) and *Simarouba amara* (bottom panels) in experimental litter removal (L-), control (CT) and litter addition (L+) treatments in a lowland tropical forest on upland soil in Panama, Central America; fluxes were measured weekly at 0.3-m height during the transition from the dry season (weeks 14-19) to the wet season (weeks 20-30); ranges (boxes and whiskers) and median lines are shown for *n* = 4 individuals per species and treatment.



*Seasonal variation in $N_2O$ fluxes*

There was no clear seasonal pattern in soil or tree stem $N_2O$ fluxes during the wet season (Fig. 5) and no effect of soil water content. However, we were unable to quantify $N_2O$ fluxes during weeks 14-19, indicating very limited $N_2O$ production during the dry season.

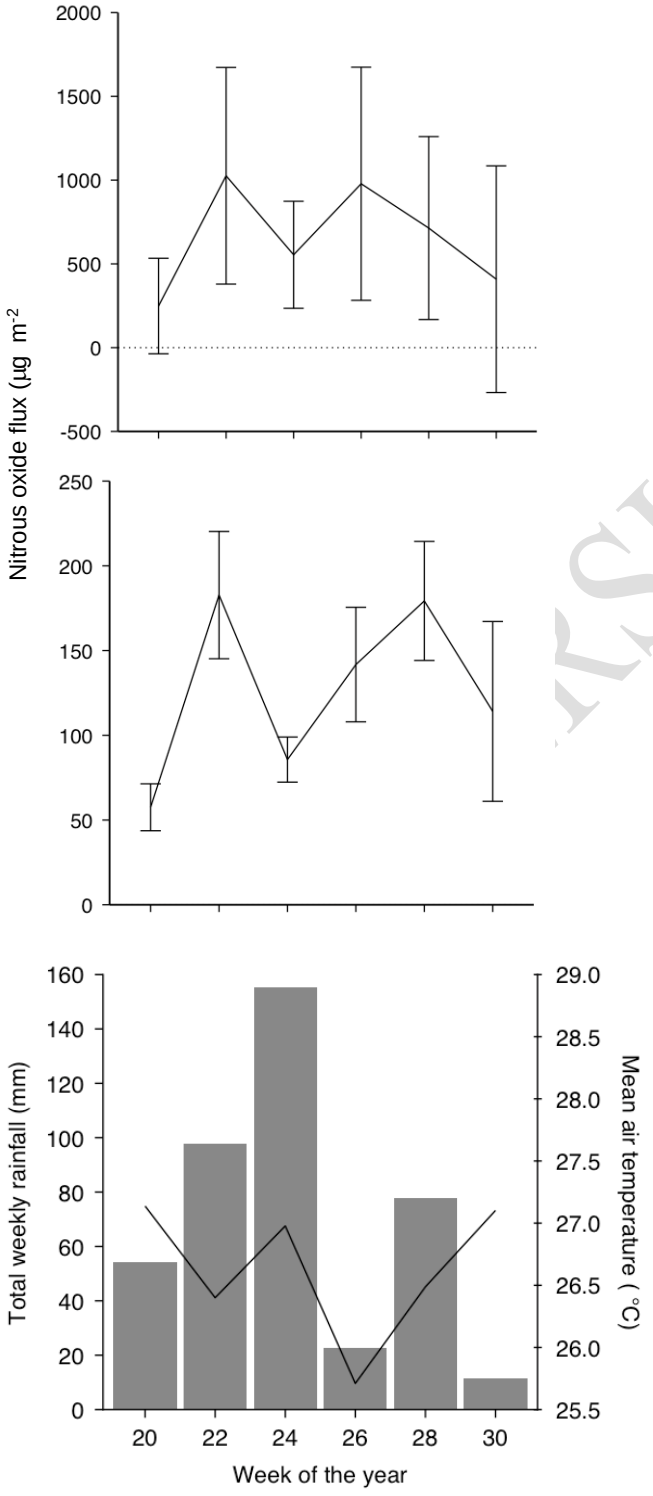


**Figure 5** Nitrous oxide ($N_2O$) fluxes from tree stems at 0.3-m height (top panel) and soil (centre panel) in a lowland tropical forest on upland soil in Panama, Central America; means and standard errors are given for *n* = 4; measurements were made weekly during wet season (bottom panel) showing total rainfall (bars) and temperature (line).

*Species and treatment effects on soil $N_2O$ fluxes*

There was a marginally significant additive effect of species and litter treatment for soil $N_2O$ fluxes ($p = 0.092$, $\chi^2 = 6.45$), whereby soil $N_2O$ fluxes measured beneath *Heisteria* individuals were greater in L+ plots compared to L- plots and tended to be higher than soil $N_2O$ fluxes measured beneath *Simarouba* (means of 138 ±21.7 µg $m^{-2}$ $h^{-1}$ and 114 ±15.7 µg $m^{-2}$ $h^{-1}$, respectively; Fig. 6). Wet season soil $N_2O$ fluxes beneath individuals of *Heisteria* ranged from -190 to 539 µg $m^{-2}$ $h^{-1}$ (median: 110 µg $m^{-2}$ $h^{-1}$) and $N_2O$ fluxes under *Simarouba* trees ranged from -89.4 to 450 µg $m^{-2}$ $h^{-1}$ (median: 87.9 µg $m^{-2}$ $h^{-1}$).

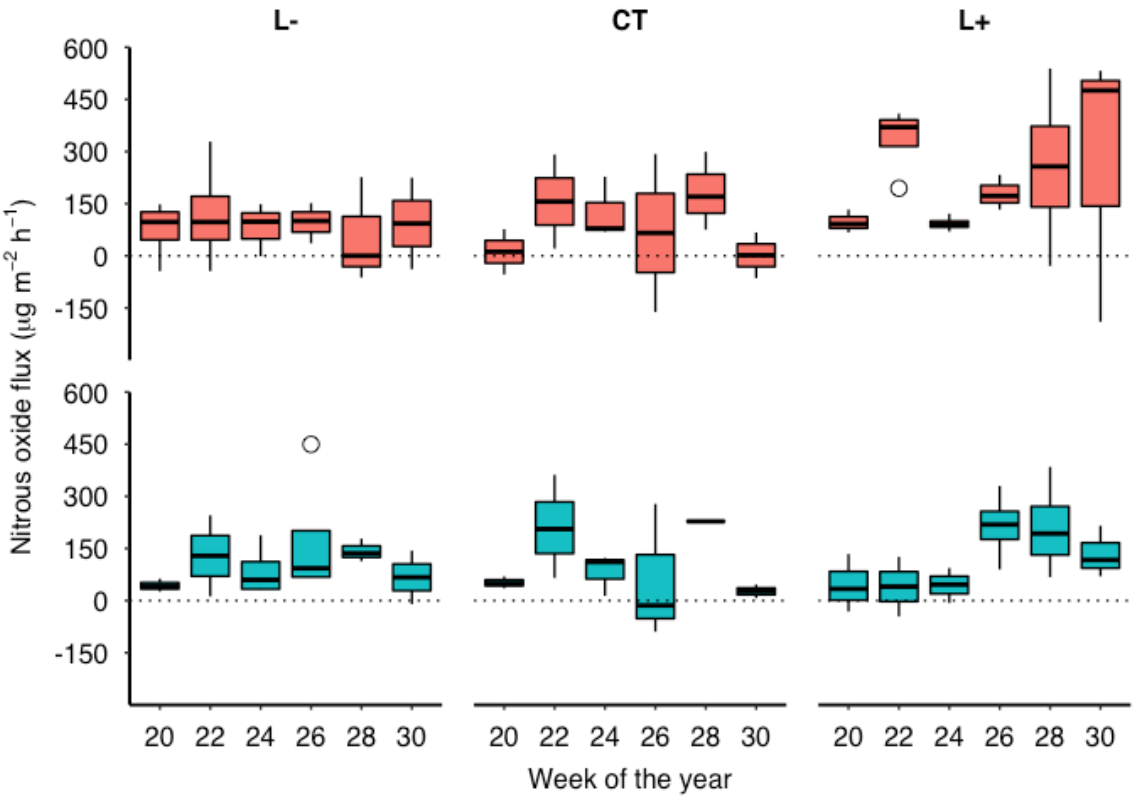


**Figure 6.** Soil nitrous oxide ($N_2O$) fluxes under individuals of *Heisteria concinna* (top panels) and *Simarouba amara* (bottom panels) in experimental litter removal (L-), control (CT) and litter addition (L+) treatments in a lowland tropical forest on upland soil in Panama, Central America, measured weekly during the wet season; ranges (boxes and whiskers) and median lines are shown for $n = 4$ individuals per species and treatment.

*Species and treatment effects on tree stem fluxes of $N_2O$*

There was a significant additive effect of species and litter treatment on stem $N_2O$ fluxes ($\chi^2$ = 9.66, $p$ = 0.022), whereby fluxes from *Simarouba* stems were greater than those from *Heisteria* in L+ and CT plots, but not in L- plots (Fig. 7). Overall, median $N_2O$ fluxes from *Heisteria* were much lower than those from *Simarouba* (101 µg $m^{-2}$ $h^{-1}$ and 1001 µg $m^{-2}$ $h^{-1}$, respectively) over the course of the study. $N_2O$ fluxes from *Heisteria* stems were less variable (range: -2857 to 4270 µg $m^{-2}$ $h^{-1}$) than *Simarouba* (range: -3770 to 8361 µg $m^{-2}$ $h^{1}$). Overall, a greater proportion of stem fluxes in *Simarouba* were positive and the mean stem flux from *Heisteria* was 80 ±234 µg $m^{-2}$ $h^{-1}$ compared to 1193 ±361 µg $m^{-2}$ $h^{-1}$ for *Simarouba* (Fig. 7).

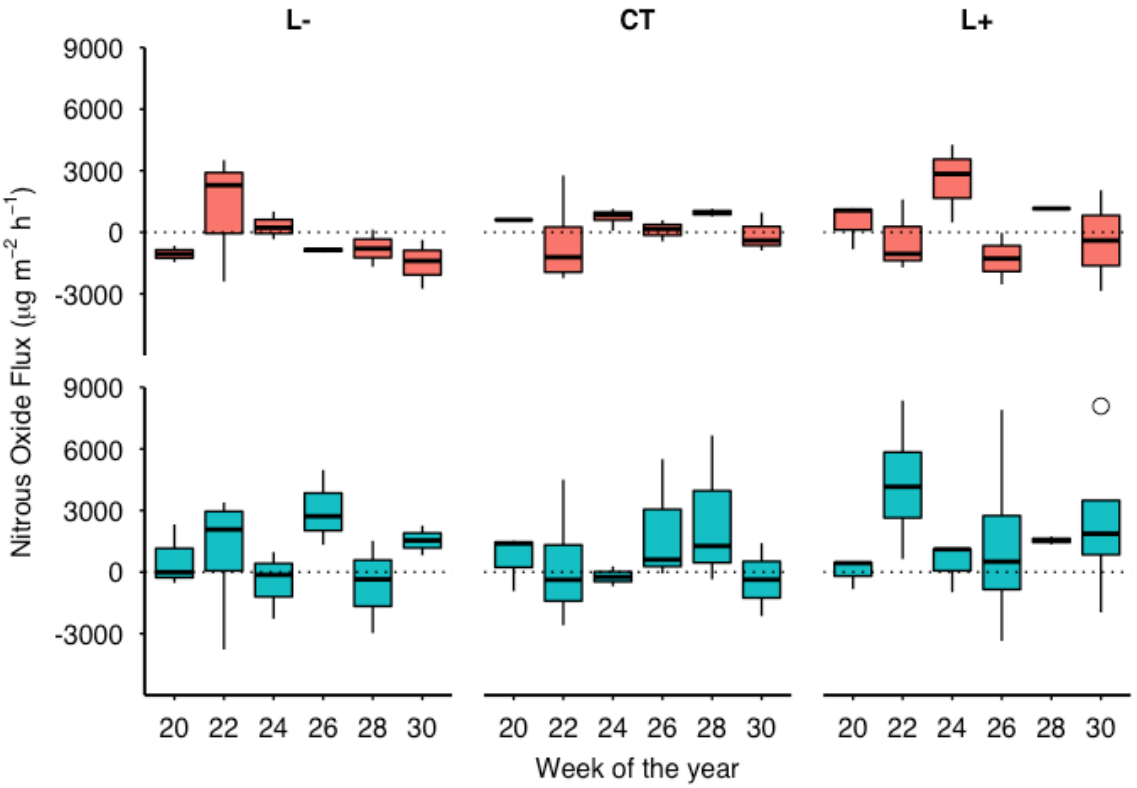


**Figure 7.** Nitrous oxide ($N_2O$) fluxes from tree stems of *Heisteria concinna* (top panels) and *Simarouba amara* (bottom panels) in experimental litter removal (L-), control (CT) and litter addition (L+) treatments in a lowland tropical forest on upland soil in Panama, Central America; fluxes were measured weekly at 0.3-m height during the wet season (weeks 20-30); ranges (boxes and whiskers) and median lines are shown for $n$ = 4 individuals per species and treatment.

## Discussion

We demonstrate that upland soils in this seasonal tropical forest represented a sink for $CH_4$ during the dry season but became a source of $CH_4$ and $N_2O$ during the transition to the wet season. Importantly, tree stems consistently emitted $CH_4$ during both the wet and dry seasons, which may offset the dry season soil sink. Tree stem fluxes of $N_2O$ were only detectable in the wet season but were also mostly positive, indicating that tropical tree stems on upland soils could be a hitherto unaccounted for source of $N_2O$.

### *Seasonal patterns of soil $CH_4$ and $N_2O$ fluxes*

Soil trace GHG fluxes were strongly seasonal. The soil acted as a $CH_4$ sink during the dry season, when upper horizons of the soil profile have low soil water content and are well aerated and methanogenic archaea are probably dormant. With the onset of the wet season at the beginning of May (week 20; Fig. 2b), soil $CH_4$ fluxes shifted from strongly negative towards positive values, i.e. net $CH_4$ emissions. The linear relationship between soil $CH_4$ emissions and soil water content (Fig. 1) is typical of tropical and subtropical swamp forest and rainforest sites (Yu et al., 2008; Rowlings et al., 2012; Hall et al., 2013), and indicates that the higher soil water content during the wet season at our site created conditions that were more favourable for methanogenesis but less so for methanotrophy.

$N_2O$ emissions usually increase with soil water content as the more productive anaerobic process of denitrification becomes dominant (Keller and Reiners, 1994) but we observed no relationship between soil water content and soil $N_2O$ emissions in our study. However, we were unable to statistically assess seasonal patterns in $N_2O$ fluxes because fluxes were not quantifiable for the majority of dry season measurements from the soil or tree stem bases. The lack of measurable $N_2O$ production during the dry season at our study site is nonetheless

316 consistent with strong seasonality in $N_2O$ fluxes, as the low variability in soil water content
317 during the wet season explains why there was no relationship with $N_2O$. Alternatively, the
318 timing and frequency of measurements may have been insufficient to detect relationships
319 between soil water content and $N_2O$ fluxes, as soil water saturation at our study site occurred
320 as a result of heavy rainfall, rather than rising water table depth or flooding, and mesocosm
321 studies have demonstrated that $N_2O$ emissions were greatest 24 hours (Machacova et al. 2013)
322 or ~45 hours (Liengaard et al. 2014) after rewetting but then declined rapidly.

323 We focussed primarily on soil water content as a control of trace GHG fluxes because
324 temperature differences have a more profound effect on trace GHG fluxes when temperatures
325 drop below 15ºC (Castro *et al*., 1995), and soil temperature was consistently >24ºC in our study.
326 Other factors such as diffusion rate (affected by e.g. soil density; Le Mer and Roger, 2001; Liu
327 et al., 2007) and drought effects (Davidson et al., 2008; Itoh et al., 2010) become relatively
328 more important at higher temperatures. It is also worth noting that the 2014 El Niño event
329 resulted in a strong dry season in Panama, with lower rainfall than average, and seasonal effects
330 in the present study may therefore have been exaggerated by the more severe dry season
331 compared with other years.

332

333 *Trace GHG fluxes from tree stems*

334 Despite substantial changes in soil trace GHG fluxes between the dry and the wet season,
335 there was no clear seasonal pattern in trace GHG fluxes from tree stems and therefore no support
336 for our hypothesis of a relationship between $CH_4$ and $N_2O$ fluxes from the soil and adjacent tree
337 stems. Emissions of $CH_4$ and $N_2O$ from tree stems in floodplain and wetland systems reflect the
338 composition of soil trace GHG concentrations (Terazawa et al., 2007; Purvaja et al., 2004;
339 Pangala et al., 2013) because the majority of $CH_4$ and $N_2O$ emitted via the plant pathway
340 originates from methanogenic consortia and denitrifying communities in the soil. However,

variation in tree water uptake and trace GHG production with soil depth could explain why we found no relationship between soil and tree stem $CH_4$ or $N_2O$ fluxes. Previous work at the study site demonstrated that soil water content and $N_2O$ concentrations increase with depth, whereas $CH_4$ concentrations were highest at *c.* 20-cm depth and then decreased with soil depth to *c.* 1.25-m (Koehler et al., 2012). Accordingly, we would expect higher $CH_4$ fluxes from stems when trees source water from shallower soil horizons, and higher $N_2O$ fluxes when trees access water from deeper in the soil profile. Hence, the relationship between soil and tree stem GHG fluxes is likely to be influenced by the location of $CH_4$ or $N_2O$ production in the soil profile, the rooting architecture of the tree species, and the preferential water uptake patterns of individual trees. Further, there is increasing evidence that $CH_4$ is also produced within tree stems (Wang et al., 2016) and methanogens can predominate in heartwood microbial communities (Yip et al., 2018), which could also contribute to $CH_4$ emissions from tree stems even when the soils were acting as a sink.

The majority of $CH_4$ and $N_2O$ fluxes from tree stems were positive throughout the study (Figs. 4 & 7) and, unlike soil trace GHG fluxes, the rates did not change significantly between seasons. Consistent trace GHG emissions from tree stem bases has also been observed in temperate upland forests (Wang et al. 2016; Pitz & Megonigal, 2017; Warner et al., 2017) and suggests that GHGs may be produced within the tree stem (Covey et al. 2012; Wang et al. 2016), or that the transport of trace GHGs through tree stems bypassed the oxygenated surface horizons, where the majority of $CH_4$ oxidation (Teh et al., 2005; Wolf et al., 2012) and more complete denitrification (Koehler et al., 2012; Wieder et al., 2011) occurs. Hence, the production or transport of trace GHGs in tree stems could represent a large and currently unaccounted for source of $N_2O$ and $CH_4$ emissions from tropical forests on upland soils. The median tree stem fluxes of $CH_4$ (~ 74 μg $m^{-2}$ $h^{-1}$) and $N_2O$ (~99 μg $m^{-2}$ $h^{-1}$) we measured during the transition from the dry to the rainy season are comparable to fluxes measured in temperate

upland trees (Machacova et al., 2013; Wang et al., 2016; Wen et al., 2017; Pitz and Megonigal 2018) and our tree stem $CH_4$ fluxes also lie within the range of tropical peatland forests (17-185 μg $m^{-2}$ $h^{-1}$; Pangala et al., 2013).

We measured trace GHG fluxes from the stems of two common tree species with distinct life history strategies and wood densities because stem GHG fluxes are also likely to vary by species' physiological traits (Pangala et al., 2013). In our study, both $CH_4$ and $N_2O$ fluxes were generally higher from stems of the fast-growing pioneer *Simarouba* (Fig. 4 & 7) except in the litter addition plots, where stem $CH_4$ fluxes were greater from the slow-growing shade-tolerant tree *Heisteria*. Higher stem emissions from the canopy species *Simarouba* could possibly be explained by greater rates of evapotranspiration and lower wood density compared to the subcanopy species *Heisteria*. Mesocosm studies of two temperate tree species common to forests on upland soils found that fast-growing species created channels of greater gas diffusivity because they had higher fine root density and greater maximum root depth (Fender et al., 2013). Further, higher rates of net primary productivity in canopy species may result in greater root exudation, which could stimulate $CH_4$ and $N_2O$ production (Butterbach-Bahl et al., 2002). The potential influence of different tree species traits on stem trace GHG fluxes makes it challenging to assess ecosystem-level fluxes in highly diverse tropical forests, especially as $CH_4$ and $N_2O$ concentrations within soil pore gas and water are also influenced tree diversity (Machacova et al., 2013; Warner et al., 2017). However, we demonstrate that tree species with distinct life history strategies and ecological niches emit $CH_4$ and $N_2O$, from their stems, which suggests that trace GHG fluxes from tree stems could be widespread in tropical forests on upland soils.

Interestingly, tree stems also acted as a sink for trace GHG gases in this study (Figs. 4 and 7). It is conceivable that changes in $CH_4$ and $N_2O$ concentrations in soil water at different depths could generate a diffusion gradient from the atmosphere into tree stems, thus resulting in tree

391 stem uptake of trace GHGs, possibly as a result of active consumption by epiphytic and
392 endophytic methanotrophs. This intriguing possibility merits further attention.
393
394 *Links between trace GHG fluxes, seasonality, and decomposition processes*
395 The decomposition of plant litter plays a role in methanogenesis and nitrous oxide emissions
396 because it releases the labile carbon that supplies electron donors for methanogens (Megonigal
397 and Guenther 2008) and the nitrate for denitrification (Teh et al., 2008). In upland tropical forest
398 soils, the combined spatial variability in soil water content, decomposition processes, and
399 electron donors may represent a greater control on trace GHG uptake or emission than any
400 single factor. At our study site, a large proportion of the annual litterfall occurs during the dry
401 season (Sayer & Tanner 2010), and the rapid increase in soil fluxes of $CH_4$ and $N_2O$ at the start
402 of the wet season are likely to result from a combination of increased water-filled pore space
403 and the decomposition of the thick litter that accumulates during the dry season (Wieder and
404 Wright, 1995). Enhanced decomposition immediately after wetting (Vasconcelos *et al*., 2007)
405 could lead to spikes in $N_2O$ fluxes that coincide with rainfall (e.g. weeks 24 and 28 in Fig. 5).
406 Correspondingly, the low rates of decomposition could also partly explain why we were unable
407 to detect $N_2O$ fluxes in the majority of dry season measurements.
408 Given the influence of decomposition processes on trace GHG production, we hypothesised
409 that both $CH_4$ and $N_2O$ emissions would be higher in litter addition plots as a result of greater
410 availability in substrate for soil microorganisms. Although there were no consistent effects of
411 the litter treatments on trace GHG emissions, higher $N_2O$ emissions in litter addition plots
412 towards the end of the study period (weeks 24-30; Fig. 6 & 7) and the additive or interactive
413 effect of litter addition and species on tree stem $N_2O$ and $CH_4$ fluxes (Fig. 4) suggest that there
414 is a connection between trace GHG fluxes and litter quantity. However, increased litter inputs
415 are only likely to result in increased $N_2O$ emissions where there are sufficiently large

communities of denitrifying bacteria that can respond quickly to wetting events, creating hotspots and 'hot moments' (McClain et al. 2003). The high inter-week variation in tree stem $N_2O$ fluxes in the present study (Fig. 7) could therefore also be explained by the timing and presence of such hotspots within the rooting zone.

Overall, the discrepancies between soil and stem trace GHG fluxes, and the apparent stronger effect of litter manipulation on fluxes of $CH_4$ and $N_2O$ from *Heisteria* tree stems compared to *Simarouba* stems (Fig. 4 and 7) may be attributed to differences in species' rooting depths and the gradients of trace GHG production with soil depth. The possible role of rooting depth as a control of trace GHG fluxes from tree stems on upland soils is another avenue of investigation that requires further attention.

### 3.5 Conclusions

Our results suggest that tropical tree stems on upland soils may represent a hitherto unaccounted for conduit of trace GHG emissions, which likely originate from deeper soil horizons. Together, litter addition and tree species identity influenced stem $CH_4$ and $N_2O$ fluxes, which suggests that tree uptake and emissions of trace GHGs is influenced both by decomposition processes and species traits. Given that tropical forests on upland soils cover a larger area than tropical wetland forests, the mechanisms underlying the fluxes of $CH_4$ and $N_2O$ from tree stems, and their contribution to global trace GHG budgets, merits further examination.

### Acknowledgements

We thank M. Peacock and P. Rafferty for assistance with laboratory analyses, L. Bréchet, F. Valdez and B Castro for help with fieldwork, and E.V.J. Tanner for discussions about the litter manipulation experiment; BW was supported by a studentship from the Natural Environment Research Council, UK; VG acknowledges support from the AXA Research Fund and NERC

441 grant NE/J010928/1; EJS was supported by funding from the European Research Council under
442 the European Union's Seventh Framework Programme (FP/2007-2013), ERC Grant Agreement
443 No. 307888. The authors have no conflict of interest to declare.

444

445 **References**

446 Alm, J., Shurpali, N.J., Tuittila, E.S., Laurila, T., Maljanen, M., Saarnio, S. & Minkkinen, K.
447 (2007). Methods for Determining Emission Factors for the Use of Peat and Peatlands - Flux
448 Measurements and Modelling. *Boreal Environment Research*, 12:85-100.

449 Baird, A.J., Stamp, I., Heppell, C.M. & Green, S.M. (2010). $CH_4$ flux from peatlands: A new
450 measurement method. *Ecohydrology*, 3, 360-367.

451 Bates, D., Mächler, M., Bolker, B. & Walker, S. (2015). Fitting Linear Mixed Effects Models
452 Using lme4. *Journal of Statistical Software*, 67, 1-48.

453 Bouwman, A. V., Van der Hoek, K. W., & Olivier, J. G. J. (1995). Uncertainties in the global
454 source distribution of nitrous oxide. *Journal of Geophysical Research: Atmospheres*, 100,
455 2785-2800.

456 Butterbach-Bahl, K. & Papen, H. (2002). Four years continuous record of CH4-exchange
457 between the atmosphere and untreated and limed soil of a N-saturated spruce and beech
458 forest ecosystem in Germany. *Plant and Soil*, 240, 77-90.

459 Carmichael, M.J., Bernhardt, E.S., Bräuer, S.L. & Smith, W.K. (2014). The role of vegetation
460 in methane flux to the atmosphere: Should vegetation be included as a distinct category in
461 the global methane budget? *Biogeochemistry*, 119, 1-24.

462 Castro, M.S., Steudler, P.A., Melillo, J.M., Aber, J.D. & Bowden, R.D. (1995). Factors
463 controlling atmospheric methane consumption by temperate forest soils. *Global*
464 *Biogeochemical Cycles*, 9, 1-10.

465 Cavelier, J. (1992). Fine-root biomass and soil properties in a semideciduous and a lower

montane rain forest in Panama. *Plant and Soil*, 142, 187-201.

Condit, R., Windsor, D.M. & Hubbell, S.P. (2013). NPP Tropical Forest: Barro Colorado, Panama, 1969-2000, R1. Data set. Available at: http://daac.ornl.gov.

Cooper, M.D., Evans, C.D., Zielinski, P., Levy, P.E., Gray, A., Peacock, M., Norris, D., Fenner, N. & Freeman, C. (2014). Infilled ditches are hotspots of landscape methane flux following peatland re-wetting. *Ecosystems*, 17, 1227-1241.

Covey, K.R., Wood, S.A., Warren, R.J., Lee, X. and Bradford, M.A. (2012). Elevated methane concentrations in trees of an upland forest. *Geophysical Research Letters*, 39, 15.

Davidson, E.A., Nepstad, D.C., Ishida, F.Y. & Brando, P.M (2008). Effects of an experimental drought and recovery on soil emissions of carbon dioxide, methane, nitrous oxide, and nitric oxide in a moist tropical forest. *Global Change Biology*, 14, 2582-2590.

Fender, A.C., Gansert, D., Jungkunst, H.F., Fiedler, S., Beyer, F., Schützenmeister, K., Thiele, B., Valtanen, K., Polle, A. & Leuschner, C. (2013). Root-induced tree species effects on the source/sink strength for greenhouse gases ($CH_4$, $N_2O$ and $CO_2$) of a temperate deciduous forest soil. *Soil Biology and Biochemistry*, 57, 587-597.

Gauci, V., Gowing, D.J., Hornibrook, E.R., Davis, J.M. & Dise, N.B. (2010). Woody stem methane emission in mature wetland alder trees. *Atmospheric Environment*, 44, 2157-2160.

Graffmann, K., Grosse, W., Junk, W.J. & Parolin, P. (2008). Pressurized gas transport in Amazonian floodplain trees. *Environmental and Experimental Botany*, 62, 371–375

Hall, S.J., McDowell, W.H. & Silver, W.L. (2013). When wet gets wetter: Decoupling of moisture, redox biogeochemistry, and greenhouse gas fluxes in a humid tropical forest soil. *Ecosystems*, 16, 576-589.

Houghton, J.T., Ding, Y., Griggs, D.J., Noguer, M., Van Der Linden, P.J., Dai, X., Maskell,

K. & Johnson, C.A. (2001). *Climate change 2001: the scientific basis. Contribution of working group I to the third assessment report of the International Panel on Climate Change*. Cambridge University Press, UK, 36, p.44.

IPCC (2007). *Climate Change 2007 - The Physical Science Basis: Working Group I Contribution to the Fourth Assessment Report of the IPCC*, Cambridge University Press, Cambridge, UK and New York, USA.

IPCC (2013). *Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge University Press, Cambridge, UK and New York, USA.

Itoh, M., Kosugi, Y., Takanashi, S., Hayashi, Y., Kanemitsu, S., Osaka, K., Tani, M. & Nik, A.R. (2010). Temporal and spatial variations of soil carbon dioxide , methane , and nitrous oxide fluxes in a Southeast Asian tropical rainforest. *Biogeosciences Discussions*, 7, 6847-6887.

Keller, M. & Reiners, W.A. (1994). Soil-atmosphere exchange of nitrous oxide, nitric oxide, and methane under secondary succession of pasture to forest in the Atlantic lowlands of Costa Rica. *Global Biogeochemical Cycles*, 8, 399-404.

Koehler, B., Corre, M.D., Steger, K., Well, R., Zehe, E., Sueta, J.P. & Veldkamp, E. (2012). An in-depth look into a tropical lowland forest soil: Nitrogen-addition effects on the contents of $N_2O$, $CO_2$ and $CH_4$ and $N_2O$ isotopic signatures down to 2-m depth. *Biogeochemistry*, 111, 695-713.

Kreuzwieser, J., Buchholz, J. & Rennenberg, H. (2003). Emission of methane and nitrous oxide by Australian mangrove ecosystems. *Plant Biology*, 5, 423-431.

Krithika, K., Purvaja, R. & Ramesh, R. (2008). Fluxes of methane and nitrous oxide from an Indian mangrove. *Current Science*, 94, 218-224.

Le Mer, J. & Roger, P. (2001). Production, oxidation, emission and consumption of methane

by soils: a review. *European Journal of Soil Biology*, 37, 25-50.

Leigh, E.G. (1999). *Tropical Forest Ecology : A View from Barro Colorado Island*. Oxford University Press, Oxford, UK.

Li, C.S., Aber, J., Stange, F., Butterbach-Bahl, K. & Papen, H. (2000), A process-oriented model of $N_2O$ and NO emissions from forest soils: 1. Model development, *Journal of Geophysical Research*, 105, 4369-4384.

Liengaard, L., Figueiredo, V., Markfoged, R., Revsbech, N.P., Nielsen, L.P., Prast, A.E. & Kühl, M. (2014). Hot moments of $N_2O$ transformation and emission in tropical soils from the Pantanal and the Amazon (Brazil). *Soil Biology and Biochemistry*, 75, 26-36.

Liu, C., Holst, J., Brüggemann, N., Butterbach-Bahl, K., Yao, Z., Yue, J., Han, S., Han, X., Krümmelbein, J., Horn, R. & Zheng, X. (2007). Winter-grazing reduces methane uptake by soils of a typical semi-arid steppe in Inner Mongolia, China. *Atmospheric Environment*, 41, 5948-5958.

Machacova, K., Papen, H., Kreuzwieser, J. & Rennenberg, H. (2013). Inundation strongly stimulates nitrous oxide emissions from stems of the upland tree *Fagus sylvatica* and the riparian tree *Alnus glutinosa*. *Plant and Soil*, 364, 287-301.

Machacova, K., Bäck, J., Vanhatalo, A., Halmeenmäki, E., Kolari, P., Mammarella, I., Pumpanen, J., Acosta, M., Urban, O. & Pihlatie, M. (2016). *Pinus sylvestris* as a missing source of nitrous oxide and methane in boreal forest. *Scientific Reports*, 6, 23410.

McClain, M.E., Boyer, E.W., Dent, C.L., Gergel, S.E., Grimm, N.B., Groffman, P.M., Hart, S.C., Harvey, J.W., Johnston, C.A., Mayorga, E. & McDowell, W.H. (2003). Biogeochemical hot spots and hot moments at the interface of terrestrial and aquatic ecosystems. *Ecosystems*, 6, 301-312.

Megonigal, J.P. & Guenther, A.B. (2008). Methane emissions from upland forest soils and

vegetation. *Tree Physiology*, 28, 491-498.

Pan, Y., Birdsey, R.A., Phillips, O.L. & Jackson, R.B. (2013). The structure, distribution, and biomass of the world's forests. *Annual Review of Ecology, Evolution, and Systematics, 44,* 593-622

Pangala, S.R., Moore, S., Hornibrook, E.R. & Gauci, V. (2013). Trees are major conduits for methane egress from tropical forested wetlands. *New Phytologist*, 197, 524-531.

Pangala, S.R., Gowing, D.J., Hornibrook, E.R. & Gauci, V. (2014), Controls on methane emissions from *Alnus glutinosa* saplings. *New Phytologist*, 201: 887-896.

Pangala, S.R., Enrich-Prast, A., Basso, L.S., Peixoto, R.B., Bastviken, D., Hornibrook, E.R., Gatti, L.V., Marotta, H., Calazans, L.S.B., Sakuragui, C.M., Bastos, W.R., Malm, O., Gloor, E., Bharat Miller, J. & Gauci, V. (2017). Large emissions from floodplain trees close the Amazon methane budget. *Nature, 552*, 230.

Pinheiro, J.C. & Bates, D.M., 2000. (2000) Linear Mixed-Effects Models: Basic Concepts and Examples. In: *Mixed-Effects Models in S and S-PLUS*. Statistics and Computing. Springer, New York, NY, pp.3-56.

Pitz, S. and Megonigal, J.P. (2017). Temperate forest methane sink diminished by tree emissions. *New Phytologist*, 214, 1432-1439.

Pitz, S. L., Megonigal, J. P., Chang, C. H., & Szlavecz, K. (2018). Methane fluxes from tree stems and soils along a habitat gradient. *Biogeochemistry*, 137, 307-320.

Purvaja, R., Ramesh, R. & Frenzel, P. (2004). Plant-mediated methane emission from an Indian mangrove. *Global Change Biology*, 10, 1825-1834.

R Core Team (2016). *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna Austria.

Rice, A.L., Butenhoff, C.L., Shearer, M.J., Teama, D., Rosenstiel, T.N. & Khalil, M.A.K. (2010). Emissions of anaerobically produced methane by trees. *Geophysical Research*

*Letters*, 37, 1-6.

Rowlings, D.W., Grace, P.R., Kiese, R. & Weier, K.L. (2012). Environmental factors controlling temporal and spatial variability in the soil-atmosphere exchange of $CO_2$, $CH_4$ and $N_2O$ from an Australian subtropical rainforest. *Global Change Biology*, 18, 726-738.

Rusch, H. & Rennenberg, H. (1998). Black alder (*Alnus glutinosa* (L.) Gaertn.) trees mediate methane and nitrous oxide emission from the soil to the atmosphere. *Plant and Soil*, 201, 1-7.

Sayer, E.J., Tanner, E.V.J. & Cheesman, A.W. (2006). Increased litterfall changes fine root distribution in a moist tropical forest. *Plant and Soil*, 281, 5-13.

Sayer, E.J. & Tanner, E.V.J. (2010). Experimental investigation of the importance of litterfall in lowland semi-evergreen tropical forest nutrient cycling. *Journal of Ecology*, 98, 1052-1062.

Siegenthaler, A., Welch, B., Pangala, S. R., Peacock, M., & Gauci, V. (2016) Technical Note: Semi-rigid chambers for methane gas flux measurements on tree stems, *Biogeosciences*, 13, 1197-1207.

Tang, X., Liu, S., Zhou, G., Zhang, D. & Zhou, C. (2006). Soil-atmospheric exchange of $CO_2$, $CH_4$, and $N_2O$ in three subtropical forest ecosystems in southern China. *Global Change Biology*, 12, 546-560.

Teh, Y.A., Dubinsky, E.A., Silver, W.L. & Carlson, C.M. (2008). Suppression of methanogenesis by dissimilatory Fe(III)- reducing bacteria in tropical rain forest soils: Implications for ecosystem methane flux. *Global Change Biology*, 14, 413-422.

Teh, Y.A., Silver, W.L. & Conrad, M.E. (2005). Oxygen effects on methane production and oxidation in humid tropical forest soils. *Global Change Biology*, 11, 1283-1297.

Terazawa, K., Ishizuka, S., Sakata, T., Yamada, K. & Takahashi, M. (2007). Methane emissions from stems of *Fraxinus mandshurica var. japonica* trees in a floodplain forest.

*Soil Biology and Biochemistry*, 39, 2689-2692.

Vasconcelos, S.S., Zarin, D.J., Da Rosa, M.B.S., de Assis Oliveira, F. & De Carvalho, C.J.R. (2007). Leaf decomposition in a dry season irrigation experiment in eastern Amazonian forest regrowth. *Biotropica*, 39, 593-600.

Wang, Z.P., Gu, Q., Deng, F.D., Huang, J.H., Megonigal, J.P., Yu, Q., Lü, X.T., Li, L.H., Chang, S., Zhang, Y.H. & Feng, J.C. (2016). Methane emissions from the trunks of living trees on upland soils. *New Phytologist*, 211, 429-439.

Warner, D.L., Villarreal, S., McWilliams, K., Inamdar, S. & Vargas, R. (2017). Carbon dioxide and methane fluxes from tree stems, coarse woody debris, and soils in an upland temperate forest. *Ecosystems*, 1-12.

Wen, Y., Corre, M.D., Rachow, C., Chen, L. and Veldkamp, E. (2017). Nitrous oxide emissions from stems of alder, beech and spruce in a temperate forest. *Plant and Soil*, 420, 423-434.

Wieder, R.K. & Wright, S.J. (1995). Tropical forest litter dynamics and dry season irrigation on Barro Colorado Island, Panama. *Ecology*, 76, 1971-1979.

Wieder, W.R., Cleveland, C.C. & Townsend, A.R. (2011). Throughfall exclusion and leaf litter addition drive higher rates of soil nitrous oxide emissions from a lowland wet tropical forest. *Global Change Biology*, 17, 3195-3207.

Wolf, K., Flessa, H. & Veldkamp, E. (2012). Atmospheric methane uptake by tropical montane forest soils and the contribution of organic layers. *Biogeochemistry*, 111, 469-483

Yip, D. Z., Veach, A. M., Yang, Z. K., Cregger, M. A., & Schadt, C. W. (2018). Methanogenic Archaea dominate mature heartwood habitats of Eastern Cottonwood (Populus deltoides). *New Phytologist*, doi: 10.1111/nph.15346.

Yu, K., Faulkner, S.P. & Baldwin, M.J. (2008). Effect of hydrological conditions on nitrous oxide, methane, and carbon dioxide dynamics in a bottomland hardwood forest and its

implication for soil carbon sequestration. *Global Change Biology*, 14, 798-812.

Zhuang, Q., Lu, Y. & Chen, M. (2012). An inventory of global $N_2O$ emissions from the soils of natural terrestrial ecosystems. *Atmospheric Environment*, 47, 66-75.