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1 **Large emissions from floodplain trees close the Amazon methane budget**

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38 **Keywords:** tropical wetlands, methane, tree stem methane emissions, Amazon wetlands.

39 **Wetlands are the largest global source of atmospheric methane (CH₄)¹, a potent greenhouse gas.**
40 **However, methane emission inventories from the Amazon floodplain^{2,3}, the largest natural**
41 **geographic source of CH₄ in the tropics, consistently underestimate the atmospheric burden of CH₄**
42 **determined via remote sensing and inversion modelling^{4,5}, pointing to a major gap in our**
43 **understanding of the contribution of these ecosystems to CH₄ emissions. Here we report CH₄**
44 **fluxes from the stems of 2357 individual Amazonian floodplain trees from 13 locations across the**
45 **central Amazon basin. We find that egress of soil gas through wetland trees is the dominant**
46 **source of regional CH₄ emissions. Amazon tree stem fluxes were up to 150-200 times larger than**
47 **emissions reported for temperate wet forests⁶ and tropical peat swamp forests⁷, representing the**
48 **largest non-ebullitive wetland fluxes observed. Tree emissions had an average $\delta^{13}\text{C-CH}_4$ value of -**
49 **$66.2 \pm 6.4\%$ consistent with a soil biogenic origin. We estimate that floodplain trees emit 15.1 ± 1.8**
50 **to 21.2 ± 2.5 Tg CH₄ yr⁻¹, in addition to 20.5 ± 5.3 Tg CH₄ yr⁻¹ emitted regionally from other sources.**
51 **Furthermore, we provide a top-down regional estimate of CH₄ emissions of 42.7 ± 5.6 Tg CH₄ yr⁻¹ for**
52 **the Amazon basin based on regular vertical lower troposphere CH₄ profiles covering the period**
53 **2010-13. We find close agreement between our 'top-down' and combined 'bottom-up' estimates,**
54 **indicating that large CH₄ emissions from trees adapted to permanent or seasonal inundation can**
55 **account for the missing emission source required to close the Amazon CH₄ budget.**

56 Wetlands are the single largest global source of atmospheric methane (CH₄), emitting an estimated
57 160 to 210 Tg of CH₄ each year to the troposphere¹. Wetlands are concentrated globally in two
58 broad latitudinal bands; one rich in peatlands spanning the boreal and subarctic zones and a second
59 in the tropics and sub-tropics containing vast swamps and seasonally inundated floodplains¹. Low
60 latitude wetlands are notably prolific sources of CH₄ because of their substantial net primary
61 productivity (NPP) and high seasonal temperatures². However, relative to northern wetlands, flux
62 measurements from Amazon floodplain ecosystems are comparatively sparse and have focussed
63 mainly on soil and water surfaces, and gas exchange mediated by aquatic macrophytes^{8,9}.

64 Integration of these emission sources across the lowland Amazon basin based upon remotely sensed
65 wetland distributions, yields an estimated flux of 26 to 29 Tg CH₄ yr^{-1 2,3}. In contrast, estimates
66 derived from atmospheric transport inversion modelling using *in-situ* CH₄ concentrations measured
67 at surface sites remote from Amazonia and satellite greenhouse gas measurements (the so-called
68 'top-down' approaches) are considerably greater at 44 to 52 Tg yr^{-1 4,10} and consistent with estimates
69 of CH₄ flux determined from modelling heterotrophic anaerobic respiration of regional NPP¹⁰.

70 Results of these global inversions should be treated with some caution. This is because the surface

71 air sampling sites are minimally sensitive to the Amazon and the number of total column CH₄
72 estimates from space likely suffer from both temporal sampling bias (data are concentrated in the
73 early dry season between seasons of smoke and clouds) and measurement biases¹¹. In contrast *in-*
74 *situ* measured vertical profile data capture directly the surface flux signals and discern the boundary
75 layer signal from the free troposphere signal¹². New measurements are therefore required to resolve
76 the discrepancy between bottom-up inventories and top-down estimates which cannot be
77 reconciled via contributions from other currently reported CH₄ sources from the Amazon region e.g.,
78 biomass burning, termites and ruminants^{5,13} nor UV-induced aerobic emissions from plants¹⁴ and
79 tank bromeliads¹⁵. Further, the regional stable carbon isotope composition (i.e., ¹³C/¹²C ratio
80 expressed as a δ¹³C value) of atmospheric CH₄ indicates unequivocally that the ‘missing’ Amazonian
81 CH₄ source is derived from microbial metabolism of C₃ photosynthate¹⁶. Consequently, the most
82 likely scenario is that surface-based flux measurements have either missed intense but perhaps
83 spatially disaggregated CH₄ emission sources or they have overlooked an important pathway for
84 egress of soil-produced CH₄.

85 Trees subjected to permanent or periodic inundation develop adaptive features such as enlarged
86 lenticels and hollow aerenchyma tissue to enhance oxygenation of their root systems^{17,18}. The
87 internal conduits that enable air to move downwards also facilitate upward escape of soil CH₄ to the
88 atmosphere^{7,17,18}. Tree-mediated gas emission has been shown to dominate ecosystem CH₄
89 emissions in tropical peat swamp forest where aerobic CH₄-oxidizing bacteria form a highly effective
90 barrier to diffusive flux through peat soil⁷. Total CH₄ emission rates are relatively modest in Borneo
91 peat swamps^{1,7}; however, the capacity for trees to emit CH₄ at higher rates is determined largely by
92 rates of soil CH₄ production and supply¹⁸. Tree-mediated transport of CH₄ has not been investigated
93 to date in the seasonally flooded, dense forests of the Amazon floodplains although ongoing efforts
94 continue to extend the database of flux measurements quantifying CH₄ emission from soil, emergent
95 macrophytes^{8,9}, and open water^{8,19,20}.

96 We measured CH₄ fluxes at 13 floodplain locations in the central Amazon River basin (Fig.1a),
97 quantifying emissions from all known transport pathways, including forested floodplain soil, aquatic
98 surfaces, and floating herbaceous macrophytes as well as stem and leaf surfaces of mature and
99 young trees. At each floodplain site, a 50 × 80 m plot was established that encompassed four
100 transects in which water table depth varied from ~1 m below the soil surface to ~10 m above the soil
101 surface. Nine of the 12 sites sampled in 2014 included an area of exposed floodplain soil in which
102 large hummocks occupied <13.5% of the total surface area. The relative contribution of emissions
103 from individual pathways was determined relative to total ecosystem CH₄ flux (Table 1). Methane
104 emissions from tree stems and aquatic surfaces were the dominant egress pathways (Fig. 1; Table 1).

105 All trees studied released substantial quantities of CH₄. Emission rates for mature and young trees
106 ranged from 0.33 to 337 mg m⁻² stem h⁻¹ and 0.39 to 581 mg m⁻² stem h⁻¹, respectively. Methane flux
107 from tree stems exceeded CH₄ emissions from all other pathways in the study plots (Fig. 1b-f; Table
108 1). Moreover, CH₄ emission rates from Amazon floodplain trees were ~150 times larger than stem
109 flux rates reported for southeast Asian peat swamp forests⁷ where less CH₄ is released owing to low
110 soil pH, high CH₄ oxidation rates and recalcitrant carbon impeding rates of methanogenesis. Fewer
111 than 4% of wood cores extracted from tree stems at 20 and 130 cm above the soil or water surface

112 displayed capacity for CH₄ production (Table 2) and stem cores from sampled trees displayed no
113 visual sign of wood rot. These observations suggest that CH₄ emitted from the tree stems originated
114 in the floodplain soil.

115 The $\delta^{13}\text{C}$ values of tree-mediated CH₄ flux ranged from -76.3 to -59.1‰, averaging $-66.2 \pm 6.4\%$ (n =
116 18; Table 3) consistent with the stable carbon isotope composition of CH₄ in soil water (range -70.8
117 to -54.5‰; Table 3) in the study plots. The $\delta^{13}\text{C}$ values are typical for wetland CH₄ albeit more
118 negative than values generally attributed to tropical wetlands²¹.

119 Young tree leaves emitted small but significant quantities of CH₄ (Fig.1b-f; Table 1). Methane
120 emission from mature leaves, if present, was below the instrument detection limit of c. 2 ppbv.
121 Similar to temperate⁶ and other tropical⁷ trees, stem CH₄ flux rates decreased either linearly or
122 exponentially with increasing stem height sampling position.

123 We pursued two approaches to scaling fluxes to the entire Amazon basin. Firstly, the measured CH₄
124 emission rates and areas of emission surfaces (Supplementary Table 3) were used to estimate the
125 contribution of each transport pathway to total ecosystem CH₄ flux estimated for each 50 × 80 m
126 study plot and then averaged for the river type. Emissions from tree stems and leaves collectively
127 were the dominant source of CH₄ evasion from Amazon floodplain soil (44 to 65 %; Table 1). The
128 contribution from aquatic surfaces was the second most significant source, accounting for 27 to 41%
129 of total CH₄ flux. Soil surfaces, which were corrected for tree basal areas, emitted 2.5 to 15.7% of
130 ecosystem CH₄ flux (Table 1). Conservative scaling of stem emission (considering only 0-140 cm of
131 tree stem emissions) to the central Amazon basin²² yields an annual source strength of $15.1 \pm 1.8 \text{ Tg}$
132 $\text{CH}_4 \text{ yr}^{-1}$ for tree-mediated flux (Table 4). Inclusion of tree emissions to 2.3-5 m stem height,
133 estimated using the relationship between stem CH₄ flux and stem height intervals, yields an annual
134 source strength of $21.2 \pm 2.5 \text{ Tg CH}_4 \text{ yr}^{-1}$, which is equivalent to current bottom-up inventories of
135 total CH₄ emissions for Amazonian wetlands ($26.2 \pm 9.8 \text{ Tg yr}^{-12,3}$; Table 4) that exclude tree
136 emissions. Further, while recent evidence suggests the potential for non-wetland trees to emit CH₄²³⁻
137 ²⁵, no robust measurements of upland tree emission have been reported in the region and those few
138 flux measurements reported elsewhere have been several orders of magnitude smaller than our
139 wetland tree observations, so in keeping with our conservative approach to regional upscaling we
140 have excluded upland tree fluxes pending further evidence.

141 Secondly, during the period 2010 to 2013 we also established top-down regional estimates of CH₄
142 emissions based upon novel regularly measured *in-situ* atmospheric CH₄ profiles from the surface to
143 4.5 km height above sea level using an air-column budgeting approach. Profiles were measured at
144 four locations in the Amazon basin (Alta Floresta (ALF), Rio Branco (RBA), Santarém (SAN) and
145 Tabatinga (TAB)). Flux estimates determined using this approach integrate CH₄ emissions from
146 regions upwind of the sampling sites, covering an increasing area the farther west a site is located in
147 the basin. Based on the envelope of back-trajectory ensembles we estimate the regions of influence
148 to be 2.53 million km² for TAB, 3.67 million km² for RBA, 0.59 million km² for SAN and 1.31 million
149 km² for ALF. The total Amazon basin area is 6.7 million km². The upwind regions of all four sites
150 during all four years were a significant source of CH₄ to the atmosphere with emission rates varying
151 from 11.4 ± 4.5 to $15.9 \pm 2.2 \text{ mg CH}_4 \text{ m}^{-2} \text{ day}^{-1}$ at ALF, 11.4 ± 1.6 to $15.4 \pm 3.2 \text{ mg CH}_4 \text{ m}^{-2} \text{ day}^{-1}$ at RBA,

152 11.1 ± 4.7 to 18.9 ± 3.2 mg CH₄ m⁻² day⁻¹ at TAB and 48.4 ± 7.6 to 60.9 ± 6.3 mg CH₄ m⁻² day⁻¹ at SAN.
 153 We observed substantially larger mean annual fluxes at SAN relative to the other three sites, which
 154 is consistent with spatial differences observed in CH₄ emission rates within our 13 floodplain study
 155 plots. The SAN area of influence includes the Tapajós River where we measured the largest CH₄
 156 fluxes from trees and other sources among the 13 floodplain study plots (T10, T11, T12; Fig. 1a).

157 Extrapolation of inversion results to the whole of the Amazon basin using an area-weighted average

158 $(F = \bar{F} \cdot A_{basin} \text{ with } \bar{F} = \sum_{i=1}^4 (\frac{A_i}{\sum_{n=1}^4 A_n}) \cdot F_i, A_{basin} = 6.7 \cdot 10^6 \text{ km}^2)$

159 $(F = \bar{F} \times A_{basin} \text{ with } \bar{F} = \sum_{i=1}^4 (\frac{A_i}{\sum_{n=1}^4 A_n}) \times F_i, A_{basin} = 6.7 \times 10^6 \text{ km}^2)$ yields a mean total CH₄ flux of

160 42.7 ± 5.6 Tg CH₄ yr⁻¹ for the four-year period, which is the equivalent of ~8% of global CH₄
 161 emissions. The uncertainty of 5.6 Tg CH₄ yr⁻¹ is the standard deviation (1σ) of the four annual
 162 emission estimates. In an earlier study²⁶, we used the 2010-2011 vertical profile data and a simple
 163 Bayesian synthesis inversion approach constrained by both prior flux estimates and atmospheric
 164 profile data to obtain a net flux estimate of 37 ± 5.9 Tg yr⁻¹. For all inversions and periods considered,
 165 the estimated fluxes exceeded the prior flux estimates with wetland prior fluxes based either on the
 166 JULES land surface model or the model of Bloom *et al.*². While these earlier estimates are somewhat
 167 smaller than the estimates reported here, this is expected because the presence of the prior flux
 168 estimates biases the estimates low. The combinations of floodplain tree emissions (15.1 ± 1.8 - 21.2
 169 ± 2.5 Tg CH₄ yr⁻¹) and CH₄ emission from other transport pathways (20.5 ± 5.3 Tg yr⁻¹) yields a total
 170 that agrees well with our estimate of regional CH₄ emissions determined from inversion modelling of
 171 atmosphere CH₄ profiles. Thus, inclusion of tree-mediated CH₄ fluxes reconciles current disparities
 172 between ‘bottom-up’ and ‘top down’ approaches effectively closing the Amazonian CH₄ budget.

173 Our results demonstrate that exceptionally large emissions from Amazon floodplain trees alone are
 174 equivalent in size to the entire Arctic CH₄ source and account for ~15% of the global wetland CH₄
 175 source. Together with already understood emission pathways, our findings demonstrate that the
 176 Amazon, in contributing up to a third of the global wetland CH₄ source, is a far larger source of CH₄
 177 than inventories previously acknowledged and is therefore likely to exert greater influence over
 178 global atmospheric CH₄ concentration variability than was previously thought. Given this increased
 179 influence over atmospheric CH₄ there is a need to quantify the controls on soil CH₄ production and
 180 tree emission variability within the biodiverse, hydrologically dynamic and geochemically
 181 heterogeneous Amazon basin while re-appraising representation of CH₄ transport mechanisms in
 182 process-based wetland models if global models are to possess the capacity to accurately predict
 183 changes in CH₄ flux resulting from climate change or other human perturbations such as the planned
 184 construction of hydroelectric dams across the basin²⁷. Finally, given that tropical forested wetlands
 185 spanning the Congo and southeast Asia experience either seasonal or permanent inundation,
 186 wetland-adapted trees may be responsible for a similar proportion of CH₄ flux in those regions,
 187 pointing to potential gross underestimates in bottom-up CH₄ inventories across globally important
 188 regions using current approaches that exclude trees.

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241 **Supplementary information**

242 This file contains supplementary tables (1-5) and supplementary figures (1-2).

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263 **Author contributions**

264 SRP, VG, AP and DB conceived and designed the bottom-up measurement study. The Brazil
265 expeditions (bottom-up measurements) in 2013 and 2014 were planned and organised by AP, OM,
266 WRB, RBP and SRP, which was carried out by SRP, RBP, HMR, LSBC and WRB. EH was responsible for
267 $\delta^{13}\text{C}$ -CH₄ analysis and interpretation of those data. LSBC and CMS identified the tree species in the
268 2014 Brazil expedition. The top down measurement study was designed and carried out by LSB, LVG,
269 JM and EG. VG coordinated integration of the various elements of the study. SRP, VG, LB, EG, DB, EH
270 and AP all contributed to writing of the manuscript.

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273 competing financial interests. Correspondence and requests for materials should be addressed to
274 SRP (Sunitha.Pangala@open.ac.uk) and VG (Vincent.Gauci@open.ac.uk).

275 **Main table legends**

276 **Table 1:** Methane fluxes and estimated ecosystem contributions from five major rivers in the central
277 Amazon basin.
278 **Table 2:** Methane production potentials measured from the wood cores extracted.
279 **Table 3:** $\delta^{13}\text{C}$ values of tree CH_4 flux and porewater CH_4 .
280 **Table 4:** Estimated annual CH_4 emissions from the Amazon basin using bottom up and top down methods.

281 **Main figure legends**

282 **Figure 1: Sampling site locations and CH_4 flux distributions.** a) Map showing the location of the 13
283 sampling sites within the central Amazon River basin, Brazil. (×) and (●) represent the sites sampled
284 in 2013 and 2014, respectively. Sampling sites are labelled: S1, S2 (River Solimões); N3, N4, N5, N6
285 (River Negro); A7, A8, A9 (River Amazon); T10, T11, T12 (River Tapajós) and M13 (River Madeira).
286 Box and whisker plots showing the distribution of CH_4 fluxes measured from all CH_4 emitting
287 pathways from river b) Negro, c) Madeira, d) Amazon, e) Solimões and f) Tapajós. Box plots
288 represents CH_4 fluxes measured from mature tree stem surfaces (M.stems), young tree stem
289 surfaces (Y.stems), young tree leaf surfaces $\times 10^{-2}$ (Y.leaves), emergent macrophytes (MAC), aquatic
290 surfaces where the water table was 0-10 m above the soil surface and soil surfaces where the water
291 table was 0-1 m below the soil surfaces. Stem CH_4 fluxes for mature trees were measured at four 30
292 cm intervals between 20 and 140 cm and young trees at 10 cm intervals between 15 and 135 cm.
293 The box plot represents the averaged flux value between the 20 to 140 cm stem portion for mature
294 trees and 15 to 135 cm for young trees. CH_4 fluxes ($\text{mg m}^{-2} \text{hr}^{-1}$) are expressed per unit area of the
295 CH_4 emitting surface measured.

296 **Methods**

297 **Ecosystem scale measurements**

298 Thirteen temporary plots (50 × 80 m) were set up in the floodplains (várzeas and Igapó) of the five
299 major rivers of the central Amazon basin, Brazil. During 2013, sampling was conducted at the Cuniã
300 ecological field station (Rondônia) a floodplain fed by the River Madeira (Fig. 1). During 2014, all
301 sampling locations ($n = 12$) were within the 1.77 million km² reference quadrant of the central
302 Amazon basin previously characterised in detail with Synthetic Aperture Radar (SAR) imagery^{3,28}. The
303 12 sampling locations consisted of four sampling locations in River Negro (black water), two in River
304 Solimões (white water), three in River Amazon (white water), and three in River Tapajós (clear
305 water). Methane sampling was conducted in the flooded forests (Supplementary Table 1) and
306 sample locations S1, S2, A7, A8 and M13 were comprised of várzeas with white waters, neutral pH,
307 and high sediment load from the Andean and pre-Andean regions. Sample plots N3, N4, N5, N6, T10,
308 T11 and T12 consisted of igapós with black water (N3, N4, N5 and N6) or clear water (T10, T11 and
309 T12), having a pH ranging from 4 to 5.5 and 4.4 to 7, respectively. Our measurements across the 13
310 sites ensured that any differences between the distinct water types (clear, white and black)
311 characteristic of the Amazon River and attributed mostly to its channel morphology and geology
312 were captured.

313 Within each study plot, stem CH₄ flux from mature trees (diameter at breast height; DBH = 6-74 cm;
314 tree height = 5-22 m; $n = 1759$ trees; Supplementary Table 2) was measured at 30 cm intervals
315 between 20 and 140 cm height and for young trees (tree height ≤ 5 m; DBH ≤ 6 cm; $n = 598$ trees) at
316 10 cm intervals between 15 and 135 cm above the soil/water surface. CH₄ emissions from young and
317 mature trees were measured across the plot, split into four transects within which the water table
318 depths ranged from wet (0-10 m above the soil surface) to dry (0 – 1 m below the soil surface)
319 conditions. Methane emissions from stems of mature and young trees were measured using static
320 chambers as described by Pangala *et al.*^{7,18} and Siegenthaler *et al.*²⁹. Methane emissions ($n = 207$)
321 were measured from aquatic surfaces within each plot, inside the flooded forests using floating
322 chambers (Supplementary Figure 1) deployed for 24 hours as described by Bastviken *et al.*³⁰. Floating
323 chambers were deployed in four transects within each plot, where the water table depths ranged
324 from 0 to 10 m above the soil surface. These transects also extended into the raised hummocks
325 where the water-table was below the soil surface and in these areas soil CH₄ fluxes ($n = 380$) were
326 measured using cylindrical static chambers (30 × 30 cm; diameter × height; Supplementary Figure 1).
327 'Aquatic surfaces' refers to the water body within the flooded forest and does not include 'open
328 waters' outside the flooded forest with no vegetation.

329 Floating chambers (1 × 1 × 1.5 m; height × width × length) were used to measure CH₄ emissions from
330 emergent floating macrophytes ($n = 80$). The chambers were constructed of gas-impermeable
331 fluorinated ethylene propylene film (Adtech Ltd., Gloucestershire, UK) wrapped around a pipe
332 frame. Floats were attached to the bottom of the frame. Emergent macrophytes were absent in
333 study locations in the River Negro catchment probably due to low nutrient concentrations in the
334 acidic black waters. Due to receding water table levels, floating macrophytes were absent in River
335 Madeira. Therefore, CH₄ fluxes from emergent floating macrophytes were measured only in Rivers

336 Solimões, Amazon and Tapajós. Rooted macrophytes were absent in all sampling locations during
337 our study period.

338 Leaf emissions were measured from leaf surfaces of young trees ($n = 260$ trees) and mature trees
339 (when accessible; $n = 180$ trees) using static chambers as described by Pangala *et al.*¹⁸. The
340 chambers, which enclosed four different branches per tree, were deployed for 10 minutes during
341 each flux measurement. In the 2014 campaign, we measured CH₄ emissions from tree stem and leaf
342 surfaces in the flooded forest and emergent macrophytes in real-time by cavity-ring down laser
343 spectroscopy as described in Pangala *et al.*¹⁸. However, on days with heavy rainfall, gas sampling
344 and analysis were conducted as described in Pangala *et al.*⁷ i.e. collection via syringes and later
345 analysis for CH₄ content. Methane emissions from tree stems and leaf surfaces from trees with
346 water table below the soil surface in the 2014 campaign and all measurements in the 2013
347 campaigns were performed as described in Siegenthaler *et al.*²⁹ and Pangala *et al.*⁷, respectively.
348 Gas samples from chambers enclosing soil and aquatic surfaces were extracted using a syringe and
349 then transferred to glass vials for CH₄ analysis by modified cavity ring down laser spectroscopy^{6,7}. CH₄
350 fluxes are expressed per unit surface area enclosed within the corresponding static chambers and
351 fluxes therefore reported as mg m⁻² h⁻¹ correspond to mg m⁻² soil h⁻¹ for soil fluxes, mg m⁻² stem h⁻¹
352 for mature and young stem fluxes, mg m⁻² leaf h⁻¹ for leaf fluxes, mg m⁻² aquatic h⁻¹ for aquatic fluxes
353 and mg m⁻² MAC h⁻¹ for macrophytes fluxes. Two sets of wood cores were extracted diagonally at 20
354 and 130 cm stem height above the forest floor/water surface for 67% and 73%, respectively, of
355 mature trees investigated for stem CH₄ fluxes. The wood cores were incubated to investigate CH₄
356 production potential as described by Covey *et al.*²³.

357 Gas samples were collected from flux chambers and porewater (head space equilibration method)
358 for $\delta^{13}\text{C}$ -CH₄ analysis using gas-tight syringes and then transferred to evacuated (10^{-3} bar) 125 ml
359 Wheaton® vials fitted with Bellco® stoppers and crimp seals. Vials were over-pressured by ~ 0.5 bar
360 to ensure ingress of air did not occur as a result of pressure or temperature changes during transport
361 to the laboratory. The $\delta^{13}\text{C}$ values of CH₄ were measured using a ThermoFinnigan® Delta XP stable
362 isotope ratio mass spectrometer. Methane in the glass vials was purified and combusted to CO₂
363 using a ThermoFinnigan PreCon®, which was modified to house a 6.4 mm stainless steel combustion
364 reactor containing palladium on quartz wool heated to 780°C³¹ and a Sofnocat® reagent trap
365 operated at room temperature to remove carbon monoxide. The instrument was calibrated using
366 BOC alpha-gravimetric and Isometric Ltd standards (ISO-B, ISO-H, ISO-L and ISO-T)³². Analysis
367 precision based upon replicate measurements of standards containing 2 ppmv CH₄ was $\pm 0.1\%$. The
368 $\delta^{13}\text{C}$ values and mixing ratios of CH₄ in the chamber headspace measured either three or four times
369 during each 30 minute deployment were used to determine the $\delta^{13}\text{C}$ value of CH₄ flux via Keeling
370 regression analysis.

371 The locations of trees were mapped in each of the 13 study plots along with the area occupied by
372 emergent macrophytes and water-table depths (measured within 1 m of all trees) along the
373 boundary of the plot and within four internal transects. Tree height, DBH, stem diameter at 10 cm
374 intervals between 0 and 200 cm stem height, and basal diameter were measured for all trees in each
375 plot. The floodplain on River Madeira site sampled in 2013 was comprised of non-flooded forest
376 because of receding water-table levels. Várzeas in the region had shrunk to small ponds with trees

377 around the edges, which were subjected to water-table levels at or below the soil surface. In all the
378 study plots, the edge of the floodplain where floating macrophytes ceased to exist was regarded as
379 the plot boundary and open water beyond that point, which contained no vegetation, was excluded
380 from the ecosystem contribution estimations but was later included in the regional upscaling using
381 the literature values⁸. Nine of the 12 sites investigated during 2014 contained both flooded and non-
382 flooded portions (<13.5%) of floodplain, three sites were fully flooded. Area occupied by aquatic
383 surfaces, soil surfaces and mature and young trees were mapped for each study site and the
384 corresponding surface areas were calculated.

385 Using ArcGIS, a polygon map for each of the sampling sites was developed, which contained water
386 table depth information and locations of trees across the transects. A spatial distribution model
387 developed from the information collected during the campaign was used to estimate macrophyte
388 surface area, aquatic surface area and soil surface areas after deducting tree basal area
389 (Supplementary Table 3). Methane fluxes from soil and water surfaces, and macrophytes were
390 estimated using CH₄ emission rates measured during the campaign and emission surfaces estimated
391 using the spatial distribution model. The leaf surface area of the young trees were estimated using
392 the methods described by Santiago *et al.*³³ which was multiplied by measured leaf CH₄ flux rates to
393 determine total ecosystem leaf CH₄ emissions. Using the stem diameter measured between 20 and
394 140 cm stem height, stem surface area was estimated and multiplied by the corresponding stem CH₄
395 flux rate to obtain stem emissions for each tree. Stem CH₄ emissions for individual trees measured
396 along the length of trees were then estimated based upon relationships between stem CH₄ flux rates
397 and stem sampling position at 30 cm tree stem height intervals. Approximately 42% of trees
398 measured displayed a linear relationship ($R^2 > 0.95$; $P < 0.0001$) between stem sampling height and
399 stem CH₄ flux rate. Trees exhibiting such a relationship had stem CH₄ flux rates equal to zero at stem
400 height between 2.3 and 3.5 m. The remaining trees studied exhibited an exponential relationship
401 between stem CH₄ flux rate and stem height. Although regression models based on exponential
402 relationships suggested the possibility of the entire tree emitting CH₄, we set stem CH₄ emissions to
403 zero when the percentage difference between the ratios of stem CH₄ flux at two consecutive 30 cm
404 stem height intervals was $\geq 0.1\%$. In such cases, stem CH₄ flux rate was equal to zero at stem heights
405 ranging between 3.8 and 5 m. Using the stem diameter measured at 10 cm intervals between 20 and
406 200 cm stem height, a relationship was established (exponential and/or power function relationship)
407 to estimate stem circumference and surface area for each tree up to 5 m. Total CH₄ emission up to
408 2.3 - 5 m length of the individual trees based upon the relationship each tree followed, was
409 estimated by multiplying measured and/or estimated CH₄ flux rates and corresponding stem surface
410 areas (Supplementary Table 3). Average stem CH₄ flux per tree was estimated by dividing total stem
411 emissions measured by the number of trees studied, within each study plot. The average flux rate
412 per tree subsequently was multiplied by the total number of trees within each plot to obtain total
413 ecosystem CH₄ contribution from trees for each study site.

414 To estimate total annual CH₄ contributions from the entire lowland Amazon basin, we averaged CH₄
415 emissions across 13 sites for each individual pathways studied, assumed the estimated fluxes are
416 representative of basin-wide fluxes and then applied the fluxes to the entire Amazon basin area,
417 which was estimated using surface area data obtained from Melack *et al.*³⁴ and Hess *et al.*²²
418 (Supplementary Table 5). Monthly area coverage for open water, flooded forest and macrophytes in

419 1.77 million km² of the central Amazon basin were obtained from Melack *et al.*³⁴ and the percentage
 420 decrease in water-table depths relative to October data (lowest water-table month reported for
 421 most land cover classes by Melack *et al.*³⁴) and percentage increase in water-table depths relative to
 422 May data (highest water-table month reported for most land cover classes in Melack *et al.*³⁴) was
 423 estimated. The percentage increases/decreases were applied to the high and low water surface area
 424 for flooded forest, open water and macrophyte area within the Amazon basin wetland area (8.4 ×
 425 10⁵ km²) reported in Hess *et al.*²² and surface areas for the remaining months were estimated. Soil
 426 surface area at the peak of the wet season was considered to be zero and for the remaining 11
 427 months, soil surface area was estimated by subtracting the subsequent month flooded-forest
 428 surface area and tree basal area from the flooded forest area during the peak of the wet season. Our
 429 work suggests that up to 13.5% of the flooded forest was comprised of exposed soil and raised
 430 hummocks in May, hence it is estimated that the soil surface area reached zero in June and
 431 thereafter the water table receded. This observation was applied to soil surface area calculations.
 432 Aquatic surface area was estimated by subtracting tree basal area from flooded-forest area.
 433 Estimated monthly surface areas are listed in Supplementary Table 5. Tree-mediated CH₄ flux, similar
 434 to other CH₄ emission pathways, was averaged across all 13 sites and was estimated to be 1350 ±
 435 553 g ha⁻¹ d⁻¹ and 98 ± 47 g ha⁻¹ d⁻¹ for mature and young tree stem emissions between 0-140 cm
 436 stem heights above the forest floor/water surface. However, when 0 to 5 m stem height was
 437 considered the fluxes increased to 1927 ± 793 g ha⁻¹ d⁻¹ and 104 ± 49 g ha⁻¹ d⁻¹ for mature and young
 438 trees, respectively. Open water CH₄ fluxes outside/beyond the edges of the flooded-forest were not
 439 measured in our study. Fluxes from macrophytes were measured in some plots but the macrophytes
 440 tended to be floating at the edges rather than inside the flooded-forest. Rooted macrophytes were
 441 absent in all the plots. Thus CH₄ flux data for open water and macrophytes from Devol *et al.*⁸ were
 442 used to estimate these components for the entire Amazon basin. Uncertainties expressed as
 443 standard deviation (SD) of means in CH₄ fluxes from all pathways were estimated using a
 444 bootstrapping method (10,000 iterations).

445 Aircraft measurements

446 To estimate CH₄ fluxes (F) based on atmospheric CH₄ vertical profile measurements we apply a
 447 simple air column budgeting technique following Miller *et al.*³⁵:

$$448 \quad F = \int_{z=0 \text{ (agl)}}^{4.4 \text{ km}} \frac{\Delta CH_4(z)}{t(z)} dz$$

$$449 \quad F = \int_{z=0 \text{ (agl)}}^{4.4 \text{ km}} \frac{CH_4(z')}{t(z')} dz'$$

450 where $\Delta CH_4 = CH_{4,site} - CH_{4,bg}$ is the difference between CH₄ mass per volume measured *in situ* at a site
 451 inside the basin and background (*bg*) air entering the basin from the Atlantic, z is height above
 452 ground (*agl*) and $t(z)$ air-mass trajectory travel time from the coast to height z at the site. The CH₄
 453 concentration of background air is estimated from atmospheric SF₆ measured at the site and
 454 compared with NOAA background stations Barbados (*RGB*, 7.92°S, 14.42°W) and Ascension (*ASC*,
 455 7.92°S, 14.42°W) respectively, using a linear mixing model:

$$456 \quad CH_{4,bg} = f_{ASC} \cdot CH_{4,ASC} + (1 - f_{ASC}) \cdot CH_{4,RPB} \quad \text{with } f_{ASC} = \frac{SF_{6,site} - SF_{6,RPB}}{SF_{6,ASC} - SF_{6,RPB}}$$

$$CH_{4,bg} = f_{ASC} \times CH_{4,ASC} + (1 - f_{ASC}) \times CH_{4,RPB} \quad \text{with } f_A = \frac{S_{6,S} F_{S_{6,R}} I}{S_{6,A} F_{S_{6,R}} I}$$

SF₆ is suited for this purpose because it has virtually no sources in the Amazon Basin and atmospheric SF₆ concentration is substantially higher in the northern compared to the southern hemisphere. Air mass travel times are estimated using back trajectories calculate d using the HYSPLIT model³⁶ (http://ready.arl.noaa.gov/HYSPLIT_traj.php).

We applied this method to vertical air profiles sampled roughly bi-weekly from 2010 to 2013 at four sites in the Brazilian Amazon located along the main airstream: at Alta Floresta (ALF; 8.80°S, 56.75°W), Rio Branco (RBA; 9.38°S, 67.62°W), Santarém (SAN; 2.86°S; 54.95°W) and Tabatinga (TAB; 5.96°S, 70.06°W). Concomitantly, carbon monoxide (CO) also was measured which allowed us to determine the CH₄ component derived from fires during the dry season of each site. Air samples were collected using a two-component portable semi-automatic collection system, consisting of a first unit with two compressors and rechargeable batteries and a second unit with 17 (at SAN) and 12 (at ALF, RBA and TAB) 700 mL boro-silicate glass flasks connected by tubing and valves, which are opened and closed by a microprocessor. The samples were generally taken between noon and 1 PM local time, when the boundary layer tends to be well mixed. After sampling, the unit containing the air flasks was transported to the high-precision greenhouse gas laboratory at IPEN (Instituto de Pesquisas Energeticas e Nucleares) in Sao Paulo, where CH₄ and CO concentrations in air were quantified. The accuracy and precision (1.5 ppb) of our greenhouse gas analysis system in Brazil is similar to the system of the bottom up of NOAA (National Oceanic and Atmospheric Administration, USA)³⁵.

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Data availability statement

499 Our aircraft CO₂ and CH₄ measurement data is available at <http://www.ccst.inpe.br/projetos/lagee/>.
500 CH₄ flux data from the bottom up study are available from SRP on request.

501 **Supplementary table legends**

502 **Table 1:** Additional information for all sampling sites (50 × 80 m) in this study.

503 **Table 2:** Tree species identified within our 13 plots across the central Amazon basin.

504 **Table 3:** Surface area (m²) used to estimate ecosystem contributions from all CH₄ emitting pathways in each
505 sampling plot.

506 **Table 4:** Coefficient of variation (%) for surface areas used in the ecosystem contribution
507 estimations.

508 **Table 5:** Estimated surface areas for the entire lowland Amazon basin (km²)^a.

509 **Supplementary figure legends**

510 **Figure 1:** Photographs depicting one of the study sites, a typically inundated flooded forest (a), soil
511 flux (b), mature tree stem flux (c) and aquatic flux (d) measurements.

512 **Figure 2:** Frequency distribution of stem CH₄ fluxes from 20-50 cm of stem height from mature trees
513 measured from river a) Negro, b) Madeira, c) Amazon, d) Solimões and e) Tapajós.

Table 1: Methane fluxes and estimated ecosystem contributions from five major rivers in the central Amazon basin.

Methane emitting pathways	River Negro		River Madeira		River Amazon		River Solimões		River Tapajós	
	Fluxes ± SD ^a	Ecosystem contribution	Fluxes ± SD	Ecosystem contributions	Fluxes ± SD	Ecosystem contributions	Fluxes ± SD	Ecosystem contributions	Fluxes ± SD	Ecosystem contributions
	mg m ⁻² h ⁻¹	g ha ⁻¹ d ⁻¹ (%)	mg m ⁻² h ⁻¹	g ha ⁻¹ d ⁻¹ (%)	mg m ⁻² h ⁻¹	g ha ⁻¹ d ⁻¹ (%)	mg m ⁻² h ⁻¹	g ha ⁻¹ d ⁻¹ (%)	mg m ⁻² h ⁻¹	g ha ⁻¹ d ⁻¹ (%)
Mature tree stem emissions^b	474 ± 151 (58.3)		836±323 (52.3)		823±214 (43.6)		1874±477 (53)		2866±759 (41.5)	
20-50 cm	30.2 ± 20.7		33.2±26		46.4 ± 33.7		83.2±42.8		141±71.4	
50-80 cm	22.2 ± 15.3		27.5±23.1		34.5 ± 25.6		62.4±32.4		106±54.5	
80-110 cm	15.4 ± 10.7		24.8±22.7		24.5 ± 18.3		44.2±23.1		73.5±38.4	
110-140 cm	10.7 ± 7.6		20.1± 19.4		16.7 ± 13.1		31.9±17.2		51.8±29.1	
Young tree stem emissions^b	47.4±11 (5.8)		83±33.2 (5.2)		50.3±13.3 (2.7)		157±40.5 (4.4)		181±56.1 (2.6)	
15-45 cm	59±28.2		50.2±32.9		103±44.9		150±67.4		271±109	
45-75 cm	41.9±20.2		42.5±32.3		73.5±32.8		108±49.9		180±74.1	
75-105 cm	29.1±14.1		35.4±31.7		50.6±23.4		77.6±36.2		125±54.1	
105-135 cm	18.9±9.7		28.5±25.7		32.8±16.4		49.1±24.2		77.83±38.3	
Young tree leaf emissions^c	0.016±0.04	3.86±4.6 (0.5)	0.019±0.04	5.07±4.8 (0.317)	0.038±0.07	5.93±7.3 (0.3)	0.051±0.09	13.5±13.1 (0.4)	0.09±0.11	17.3±15.7 (0.2)
Macrophytes	-		-		7.29±10.8	190±745 (10)	6.62±8.9	134±261 (3.8)	39±41.9	966±2105 (13.9)
Aquatic emissions	1.51±3.2	219±544 (27)	7.34±2.59	423±148 (26.5)	6.1±14.7	768±1792 (40.7)	4.37±5.77	1269±1111 (35.9)	25.7±29.8	2426±2898 (35.1)
Soil emissions	1.06±0.8	67.7±56 (8.3)	1.33±1.57	251±289 (15.7)	2.73±2.62	49±179 (2.6)	4.27±4.3	88.6±108 (2.5)	10.6±7.7	456±564 (6.6)

^a The fluxes are per unit area of the corresponding CH₄ emitting surface area and SD are estimated using bootstrapping methods; ^b Ecosystem contributions from young and mature tree stems were estimated using the measured stem CH₄ fluxes between 15-20 and 135-140 cm stem height above the soil/water surface at 30 cm stem height intervals and multiplied by the corresponding stem surface area. Contributions between 0-20 cm stem height were assumed to be the same as the 20-50 cm stem CH₄ flux and was included in the ecosystem contributions; ^c young tree leaf CH₄ fluxes are the average of four different branches per tree (*n* = 260). No CH₄ emissions were detected from mature tree leaves (*n* = 180).

Table 2: Methane production potentials measured from the wood cores extracted.

No of trees sampled	Percentage trees showing evidence of CH ₄ production potential (%)	CH ₄ production potential rates ± SD (µg CH ₄ h ⁻¹ m ⁻³ vol of wood) ^a
At 20 cm above the soil/water surface		
<i>n</i> = 1232	1.3	158 ± 274
At 130 cm above the soil/water surface		
<i>n</i> = 1343	3.7	440 ± 579

^a CH₄ production potential was measured by incubating the stem cores for 12 hrs in 35 ml Wheaton vials flushed with N₂²³.

Table 3: $\delta^{13}\text{C}$ values of tree CH_4 flux and porewater CH_4 .

	Flux			Porewater	
	$\delta^{13}\text{C}(\text{CH}_4)^{\text{a}}$	SD	n ^b	$\delta^{13}\text{C}(\text{CH}_4)^{\text{c}}$	N
	(‰)	(‰)		(‰)	
River Negro					
N3	-76.3	0.9	4	-	-
N6	-64.6	3.2	5	-	-
River Amazon					
A7	-65.4	2.2	4	-58.5/-54.5	2
A9	-61.8	3.3	3	-70.8/-63.3	3
River Tapajós					
T11	-59.1	0.4	3	-55.6	1

^a Mean $\delta^{13}\text{C}$ values are reported for CH_4 flux; ^b n represents one chamber deployment from which three or four pairs of CH_4 concentration and $\delta^{13}\text{C}(\text{CH}_4)$ values were used to determine a $\delta^{13}\text{C}$ value for CH_4 flux via Keeling regression analysis; ^c The range of $\delta^{13}\text{C}$ values are reported for porewater CH_4 .

Table 4: Estimated annual CH₄ emissions from the Amazon basin using bottom up and top down methods.

Approach: bottom up (BU) top-down (TD)	CH ₄ emitting pathways	CH ₄ fluxes ± SD (g ha ⁻¹ d ⁻¹)	Annual emissions ± SD (Tg CH ₄ yr ⁻¹) ^a	Study
	Mature tree stems	1350 ± 553 - 1927 ± 793 ^b	14 ± 1.8 - 20 ± 2.5 ^b	This study
	Young tree stems	98 ± 46.8 - 104 ± 49.2 ^b	1.02 ± 0.15 - 1.08 ± 0.16 ^b	This study
	Young tree leaf emissions	9.5 ± 15.9	0.099 ± 0.05	This study
BU			15.1 ± 1.8 - 21.2 ± 2.5^b	This study
	Aquatic surfaces	1033 ± 1622	9.7 ± 5.2	This study
	Soil surfaces	170 ± 299	1.1 ± 0.7	This study
	Macrophytes	3245 ± 721 – 1229 ± 334 ^c	8 ± 0.6 ^d	3,8
	Open water	270 ± 80.1	1.2 ± 0.05 ^d	8
	River channel		0.4 - 0.6 ^e	19
BU		Total surface emissions (including trees)	35.6 ± 5.6 – 41.7 ± 5.9^b	This study
BU		Total surface emissions (no trees)	20.5 ± 5.3	This study
BU		Total surface emissions (no trees)	29.4	3
BU		Total surface emissions (no trees)	26.2 ± 9.8	2
TD	Biomass burning (non-wetland source)		4.1 ± 0.7	This study
TD	All		42.7 ± 5.6	This study
TD	All		44 ± 4.8	10
TD	All		40.2 - 52	4
TD	All		37 ± 5.9	26

^a Surface area used to estimate regional CH₄ contributions reported in Supplementary Table 5; ^b The upper range represents the inclusion of stem CH₄ emissions estimated for up to 5 m of the stem height for mature trees and 1.85 m for young trees using the relationship between stem CH₄ flux and stem height positions; ^c Aquatic macrophyte CH₄ emissions from high and low water season estimated and reported by Devol *et al.*⁸ and Melack *et al.*³; ^d CH₄ fluxes to estimate emissions from macrophytes and open water were obtained from Devol *et al.*⁸ and Melack *et al.*³; ^e total annual CH₄ emission estimates from river channels in the Amazon basin obtained from Sawakuchi *et al.*¹⁹.