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Large emissions from floodplain trees close the Amazon methane budget

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39 **Wetlands are the largest global source of atmospheric methane (CH_4)[1], a potent greenhouse gas.**

40 **However, methane emission inventories from the Amazon floodplain[2,3], the largest natural**

41 **geographic source of CH_4 in the tropics, consistently underestimate the atmospheric burden of CH_4**

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 CH_4 emissions. Here we report CH_4**

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 CH_4 emissions. Amazon tree stem fluxes were up to 150-200 times larger than**

47 **emissions reported for temperate wet forests[6] and tropical peat swamp forests[7], representing the**

48 **largest non-ebullitive wetland fluxes observed. Tree emissions had an average $\delta^{13}C$-CH_4 value of -**

49 **66.2±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 CH_4 yr^{-1}, in addition to 20.5±5.3 Tg CH_4 yr^{-1} emitted regionally from other sources.**

51 **Furthermore, we provide a top-down regional estimate of CH_4 emissions of 42.7±5.6 Tg CH_4 yr^{-1} for**

52 **the Amazon basin based on regular vertical lower troposphere CH_4 profiles covering the period**

53 **2010-13. We find close agreement between our 'top-down' and combined 'bottom-up' estimates,**

54 **indicating that large CH_4 emissions from trees adapted to permanent or seasonal inundation can**

55 **account for the missing emission source required to close the Amazon CH_4 budget.**

56 Wetlands are the single largest global source of atmospheric methane (CH_4), emitting an estimated

57 160 to 210 Tg of CH_4 each year to the troposphere[1]. 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 floodplains[1]. Low

60 latitude wetlands are notably prolific sources of CH_4 because of their substantial net primary

61 productivity (NPP) and high seasonal temperatures[2]. 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 CH_4 yr^{-1} [2,3]. In contrast, estimates

66 derived from atmospheric transport inversion modelling using *in-situ* CH_4 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 CH_4 flux determined from modelling heterotrophic anaerobic respiration of regional NPP[10].

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 CH_4

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 biases[11]. 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 signal[12]. 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 CH_4 sources from the Amazon region e.g.,

78   biomass burning, termites and ruminants[5,13] nor UV-induced aerobic emissions from plants[14] and

79   tank bromeliads[15]. Further, the regional stable carbon isotope composition (i.e., $^{13}C/^{12}C$ ratio

80   expressed as a $\delta^{13}C$ value) of atmospheric CH_4 indicates unequivocally that the 'missing' Amazonian

81   CH_4 source is derived from microbial metabolism of C3 photosynthate[16]. Consequently, the most

82   likely scenario is that surface-based flux measurements have either missed intense but perhaps

83   spatially disaggregated CH_4 emission sources or they have overlooked an important pathway for

84   egress of soil-produced CH_4.

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 CH_4 to the

88   atmosphere[7,17,18]. Tree-mediated gas emission has been shown to dominate ecosystem CH_4

89   emissions in tropical peat swamp forest where aerobic CH_4-oxidizing bacteria form a highly effective

90   barrier to diffusive flux through peat soil[7]. Total CH_4 emission rates are relatively modest in Borneo

91   peat swamps[1,7]; however, the capacity for trees to emit CH_4 at higher rates is determined largely by

92   rates of soil CH_4 production and supply[18]. Tree-mediated transport of CH_4 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 CH_4 emission from soil, emergent

95   macrophytes[8,9], and open water[8,19,20].

96   We measured CH_4 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 CH_4 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 CH_4. 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 CH_4 emissions from all other pathways in the study plots (Fig. 1b-f; Table

108   1). Moreover, CH_4 emission rates from Amazon floodplain trees were ~150 times larger than stem

109   flux rates reported for southeast Asian peat swamp forests[7] where less CH_4 is released owing to low

110   soil pH, high CH_4 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

3

112   displayed capacity for CH_4 production (Table 2) and stem cores from sampled trees displayed no

113   visual sign of wood rot. These observations suggest that CH_4 emitted from the tree stems originated

114   in the floodplain soil.

115   The δ^{13}C values of tree-mediated CH_4 flux ranged from -76.3 to -59.1‰, averaging -66.2 ± 6.4‰ (n =

116   18; Table 3) consistent with the stable carbon isotope composition of CH_4 in soil water (range -70.8

117   to -54.5‰; Table 3) in the study plots. The δ^{13}C values are typical for wetland CH_4 albeit more

118   negative than values generally attributed to tropical wetlands[21].

119   Young tree leaves emitted small but significant quantities of CH_4 (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 temperate[6] and other tropical[7] trees, stem CH_4 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 CH_4

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 CH_4 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 CH_4 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 CH_4 flux. Soil surfaces, which were corrected for tree basal areas, emitted 2.5 to 15.7% of

130   ecosystem CH_4 flux (Table 1). Conservative scaling of stem emission (considering only 0-140 cm of

131   tree stem emissions) to the central Amazon basin[22] yields an annual source strength of 15.1 ± 1.8 Tg

132   CH_4 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 CH_4 flux and stem height intervals, yields an annual

134   source strength of 21.2 ± 2.5 Tg CH_4 yr^{-1}, which is equivalent to current bottom-up inventories of

135   total CH_4 emissions for Amazonian wetlands (26.2 ± 9.8 Tg yr^{-1}[2,3]; Table 4) that exclude tree

136   emissions. Further, while recent evidence suggests the potential for non-wetland trees to emit CH_4[23-

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 CH_4

142   emissions based upon novel regularly measured *in-situ* atmospheric CH_4 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 CH_4 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 km^2 for TAB, 3.67 million km^2 for RBA, 0.59 million km^2 for SAN and 1.31 million

149   km^2 for ALF. The total Amazon basin area is 6.7 million km^2. The upwind regions of all four sites

150   during all four years were a significant source of CH_4 to the atmosphere with emission rates varying

151   from 11.4 ± 4.5 to 15.9 ± 2.2 mg CH_4 m^{-2} day^{-1} at ALF, 11.4 ± 1.6 to 15.4 ± 3.2 mg CH_4 m^{-2} day^{-1} at RBA,

4

152   11.1 ± 4.7 to 18.9 ± 3.2 mg CH_4 m^{-2} day^{-1} at TAB and 48.4 ± 7.6 to 60.9 ± 6.3 mg CH_4 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 CH_4 emission rates within our 13 floodplain study

155   plots. The SAN area of influence includes the Tapajós River where we measured the largest CH_4

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    $(F = \overline{F} \times A_{basin}$ with $\overline{F} = \sum_{i=1}^{4} (\frac{A_i}{\sum_{n=1}^{4} A_n}) \times F_i$, $A_{basin} = 6.7 \times 10^6 \ km^2)$ yields a mean total CH_4 flux of

159   42.7 ± 5.6 Tg CH_4 yr^{-1} for the four-year period, which is the equivalent of ~8% of global CH_4

160   emissions. The uncertainty of 5.6 Tg CH_4 yr^{-1} is the standard deviation (1σ) of the four annual

161   emission estimates. In an earlier study[26], we used the 2010-2011 vertical profile data and a simple

162   Bayesian synthesis inversion approach constrained by both prior flux estimates and atmospheric

163   profile data to obtain a net flux estimate of 37 ± 5.9 Tg yr^{-1}. For all inversions and periods considered,

164   the estimated fluxes exceeded the prior flux estimates with wetland prior fluxes based either on the

165   JULES land surface model or the model of Bloom *et al.*[2]. While these earlier estimates are somewhat

166   smaller than the estimates reported here, this is expected because the presence of the prior flux

167   estimates biases the estimates low. The combinations of floodplain tree emissions (15.1 ± 1.8 - 21.2

168   ± 2.5 Tg CH_4 yr^{-1}) and CH_4 emission from other transport pathways (20.5 ± 5.3 Tg yr^{-1}) yields a total

169   that agrees well with our estimate of regional CH_4 emissions determined from inversion modelling of

170   atmosphere CH_4 profiles. Thus, inclusion of tree-mediated CH_4 fluxes reconciles current disparities

171   between 'bottom-up' and 'top down' approaches effectively closing the Amazonian CH_4 budget.

172   Our results demonstrate that exceptionally large emissions from Amazon floodplain trees alone are

173   equivalent in size to the entire Arctic CH_4 source and account for ~15% of the global wetland CH_4

174   source. Together with already understood emission pathways, our findings demonstrate that the

175   Amazon, in contributing up to a third of the global wetland CH_4 source, is a far larger source of CH_4

176   than inventories previously acknowledged and is therefore likely to exert greater influence over

177   global atmospheric CH_4 concentration variability than was previously thought. Given this increased

178   influence over atmospheric CH_4 there is a need to quantify the controls on soil CH_4 production and

179   tree emission variability within the biodiverse, hydrologically dynamic and geochemically

180   heterogeneous Amazon basin while re-appraising representation of CH_4 transport mechanisms in

181   process-based wetland models if global models are to possess the capacity to accurately predict

182   changes in CH_4 flux resulting from climate change or other human perturbations such as the planned

183   construction of hydroelectric dams across the basin[27]. Finally, given that tropical forested wetlands

184   spanning the Congo and southeast Asia experience either seasonal or permanent inundation,

185   wetland-adapted trees may be responsible for a similar proportion of CH_4 flux in those regions,

186   pointing to potential gross underestimates in bottom-up CH_4 inventories across globally important

187   regions using current approaches that exclude trees.

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240   **Supplementary information**

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

262   **Author contributions**

263   SRP, VG, AP and DB conceived and designed the bottom-up measurement study. The Brazil
264   expeditions (bottom-up measurements) in 2013 and 2014 were planned and organised by AP, OM,
265   WRB, RBP and SRP, which was carried out by SRP, RBP, HMR, LSBC and WRB. EH was responsible for
266   δ^{13}C-CH_4 analysis and interpretation of those data. LSBC and CMS identified the tree species in the
267   2014 Brazil expedition. The top down measurement study was designed and carried out by LSB, LVG,
268   JM and EG. VG coordinated integration of the various elements of the study. SRP, VG, LB, EG, DB, EH
269   and AP all contributed to writing of the manuscript.

274   **Main table legends**

275 **Table 1:** Methane fluxes and estimated ecosystem contributions from five major rivers in the central
276 Amazon basin.
277 **Table 2:** Methane production potentials measured from the wood cores extracted.
278 **Table 3:** δ^{13}C values of tree CH$_4$ flux and porewater CH$_4$.
279 **Table 4:** Estimated annual CH$_4$ emissions from the Amazon basin using bottom up and top down methods.

280 **Main figure legends**
281 **Figure 1: Sampling site locations and CH$_4$ flux distributions.** a) Map showing the location of the 13
282 sampling sites within the central Amazon River basin, Brazil. (×) and (●) represent the sites sampled
283 in 2013 and 2014, respectively. Sampling sites are labelled: S1, S2 (River Solimões); N3, N4, N5, N6
284 (River Negro); A7, A8, A9 (River Amazon); T10, T11, T12 (River Tapajós) and M13 (River Madeira).
285 Box and whisker plots showing the distribution of CH$_4$ fluxes measured from all CH$_4$ emitting
286 pathways from river b) Negro, c) Madeira, d) Amazon, e) Solimões and f) Tapajós. Box plots
287 represents CH$_4$ fluxes measured from mature tree stem surfaces (M.stems), young tree stem
288 surfaces (Y.stems), young tree leaf surfaces × 10^{-2} (Y.leaves), emergent macrophytes (MAC), aquatic
289 surfaces where the water table was 0-10 m above the soil surface and soil surfaces where the water
290 table was 0-1 m below the soil surfaces. Stem CH$_4$ fluxes for mature trees were measured at four 30
291 cm intervals between 20 and 140 cm and young trees at 10 cm intervals between 15 and 135 cm.
292 The box plot represents the averaged flux value between the 20 to 140 cm stem portion for mature
293 trees and 15 to 135 cm for young trees. CH$_4$ fluxes (mg m^{-2} hr^{-1}) are expressed per unit area of the
294 CH$_4$ emitting surface measured.

Methods

Ecosystem scale measurements

Thirteen temporary plots (50 × 80 m) were set up in the floodplains (várzeas and Igapó) of the five major rivers of the central Amazon basin, Brazil. During 2013, sampling was conducted at the Cuniã ecological field station (Rondônia) a floodplain fed by the River Madeira (Fig. 1). During 2014, all sampling locations (n = 12) were within the 1.77 million km^2 reference quadrant of the central Amazon basin previously characterised in detail with Synthetic Aperture Radar (SAR) imagery[3,28]. The 12 sampling locations consisted of four sampling locations in River Negro (black water), two in River Solimões (white water), three in River Amazon (white water), and three in River Tapajós (clear water). Methane sampling was conducted in the flooded forests (Supplementary Table 1) and sample locations S1, S2, A7, A8 and M13 were comprised of várzeas with white waters, neutral pH, and high sediment load from the Andean and pre-Andean regions. Sample plots N3, N4, N5, N6, T10, T11 and T12 consisted of igapós with black water (N3, N4, N5 and N6) or clear water (T10, T11 and T12), having a pH ranging from 4 to 5.5 and 4.4 to 7, respectively. Our measurements across the 13 sites ensured that any differences between the distinct water types (clear, white and black) characteristic of the Amazon River and attributed mostly to its channel morphology and geology were captured.

Within each study plot, stem CH$_4$ flux from mature trees (diameter at breast height; DBH = 6-74 cm; tree height = 5-22 m; n = 1759 trees; Supplementary Table 2) was measured at 30 cm intervals between 20 and 140 cm height and for young trees (tree height ≤ 5 m; DBH ≤ 6 cm; n = 598 trees) at 10 cm intervals between 15 and 135 cm above the soil/water surface. CH$_4$ emissions from young and mature trees were measured across the plot, split into four transects within which the water table depths ranged from wet (0-10 m above the soil surface) to dry (0 – 1 m below the soil surface) conditions. Methane emissions from stems of mature and young trees were measured using static chambers as described by Pangala *et al.*[7,18] and Siegenthaler *et al.*[29]. Methane emissions (n = 207) were measured from aquatic surfaces within each plot, inside the flooded forests using floating chambers (Supplementary Figure 1) deployed for 24 hours as described by Bastviken *et al.*[30]. Floating chambers were deployed in four transects within each plot, where the water table depths ranged from 0 to 10 m above the soil surface. These transects also extended into the raised hummocks where the water-table was below the soil surface and in these areas soil CH$_4$ fluxes (n = 380) were measured using cylindrical static chambers (30 × 30 cm; diameter × height; Supplementary Figure 1). 'Aquatic surfaces' refers to the water body within the flooded forest and does not include 'open waters' outside the flooded forest with no vegetation.

Floating chambers (1 × 1 × 1.5 m; height × width × length) were used to measure CH$_4$ emissions from emergent floating macrophytes (n = 80). The chambers were constructed of gas-impermeable fluorinated ethylene propylene film (Adtech Ltd., Gloucestershire, UK) wrapped around a pipe frame. Floats were attached to the bottom of the frame. Emergent macrophytes were absent in study locations in the River Negro catchment probably due to low nutrient concentrations in the acidic black waters. Due to receding water table levels, floating macrophytes were absent in River Madeira. Therefore, CH$_4$ fluxes from emergent floating macrophytes were measured only in Rivers

9

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

337 Leaf emissions were measured from leaf surfaces of young trees (*n* = 260 trees) and mature trees
338 (when accessible; *n* = 180 trees) using static chambers as described by Pangala *et al.*[18]. The
339 chambers, which enclosed four different branches per tree, were deployed for 10 minutes during
340 each flux measurement. In the 2014 campaign, we measured CH_4 emissions from tree stem and leaf
341 surfaces in the flooded forest and emergent macrophytes in real-time by cavity-ring down laser
342 spectroscopy as described in Pangala *et al.*[18]. However, on days with heavy rainfall, gas sampling
343 and analysis were conducted as described in Pangala *et al.*[7] i.e. collection via syringes and later
344 analysis for CH_4 content. Methane emissions from tree stems and leaf surfaces from trees with
345 water table below the soil surface in the 2014 campaign and all measurements in the 2013
346 campaigns were performed as described in Siegenthaler *et al.*[29] and Pangala *et al.*[7], respectively.
347 Gas samples from chambers enclosing soil and aquatic surfaces were extracted using a syringe and
348 then transferred to glass vials for CH_4 analysis by modified cavity ring down laser spectroscopy[6,7]. CH_4
349 fluxes are expressed per unit surface area enclosed within the corresponding static chambers and
350 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}
351 for mature and young stem fluxes, mg m^{-2} leaf h^{-1} for leaf fluxes, mg m^{-2} aquatic h^{-1} for aquatic fluxes
352 and mg m^{-2} MAC h^{-1} for macrophytes fluxes. Two sets of wood cores were extracted diagonally at 20
353 and 130 cm stem height above the forest floor/water surface for 67% and 73%, respectively, of
354 mature trees investigated for stem CH_4 fluxes. The wood cores were incubated to investigate CH_4
355 production potential as described by Covey *et al.*[23].

356 Gas samples were collected from flux chambers and porewater (head space equilibration method)
357 for δ^{13}C-CH_4 analysis using gas-tight syringes and then transferred to evacuated (10^{-3} bar) 125 ml
358 Wheaton® vials fitted with Bellco® stoppers and crimp seals. Vials were over-pressured by ~0.5 bar
359 to ensure ingress of air did not occur as a result of pressure or temperature changes during transport
360 to the laboratory. The δ^{13}C values of CH_4 were measured using a ThermoFinnigan® Delta XP stable
361 isotope ratio mass spectrometer. Methane in the glass vials was purified and combusted to CO_2
362 using a ThermoFinnigan PreCon®, which was modified to house a 6.4 mm stainless steel combustion
363 reactor containing palladium on quartz wool heated to 780°C[31] and a Sofnocat® reagent trap
364 operated at room temperature to remove carbon monoxide. The instrument was calibrated using
365 BOC alpha-gravimetric and Isometric Ltd standards (ISO-B, ISO-H, ISO-L and ISO-T)[32]. Analysis
366 precision based upon replicate measurements of standards containing 2 ppmv CH_4 was ±0.1‰. The
367 δ^{13}C values and mixing ratios of CH_4 in the chamber headspace measured either three or four times
368 during each 30 minute deployment were used to determine the δ^{13}C value of CH_4 flux via Keeling
369 regression analysis.

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

376 around the edges, which were subjected to water-table levels at or below the soil surface. In all the
377 study plots, the edge of the floodplain where floating macrophytes ceased to exist was regarded as
378 the plot boundary and open water beyond that point, which contained no vegetation, was excluded
379 from the ecosystem contribution estimations but was later included in the regional upscaling using
380 the literature values[8]. Nine of the 12 sites investigated during 2014 contained both flooded and non-
381 flooded portions (<13.5%) of floodplain, three sites were fully flooded. Area occupied by aquatic
382 surfaces, soil surfaces and mature and young trees were mapped for each study site and the
383 corresponding surface areas were calculated.

384 Using ArcGIS, a polygon map for each of the sampling sites was developed, which contained water
385 table depth information and locations of trees across the transects. A spatial distribution model
386 developed from the information collected during the campaign was used to estimate macrophyte
387 surface area, aquatic surface area and soil surface areas after deducting tree basal area
388 (Supplementary Table 3). Methane fluxes from soil and water surfaces, and macrophytes were
389 estimated using CH_4 emission rates measured during the campaign and emission surfaces estimated
390 using the spatial distribution model. The leaf surface area of the young trees were estimated using
391 the methods described by Santiago *et al.*[33] which was multiplied by measured leaf CH_4 flux rates to
392 determine total ecosystem leaf CH_4 emissions. Using the stem diameter measured between 20 and
393 140 cm stem height, stem surface area was estimated and multiplied by the corresponding stem CH_4
394 flux rate to obtain stem emissions for each tree. Stem CH_4 emissions for individual trees measured
395 along the length of trees were then estimated based upon relationships between stem CH_4 flux rates
396 and stem sampling position at 30 cm tree stem height intervals. Approximately 42% of trees
397 measured displayed a linear relationship ($R^2 > 0.95$; $P < 0.0001$) between stem sampling height and
398 stem CH_4 flux rate. Trees exhibiting such a relationship had stem CH_4 flux rates equal to zero at stem
399 height between 2.3 and 3.5 m. The remaining trees studied exhibited an exponential relationship
400 between stem CH_4 flux rate and stem height. Although regression models based on exponential
401 relationships suggested the possibility of the entire tree emitting CH_4, we set stem CH_4 emissions to
402 zero when the percentage difference between the ratios of stem CH_4 flux at two consecutive 30 cm
403 stem height intervals was ≥ 0.1%. In such cases, stem CH_4 flux rate was equal to zero at stem heights
404 ranging between 3.8 and 5 m. Using the stem diameter measured at 10 cm intervals between 20 and
405 200 cm stem height, a relationship was established (exponential and/or power function relationship)
406 to estimate stem circumference and surface area for each tree up to 5 m. Total CH_4 emission up to
407 2.3 - 5 m length of the individual trees based upon the relationship each tree followed, was
408 estimated by multiplying measured and/or estimated CH_4 flux rates and corresponding stem surface
409 areas (Supplementary Table 3). Average stem CH_4 flux per tree was estimated by dividing total stem
410 emissions measured by the number of trees studied, within each study plot. The average flux rate
411 per tree subsequently was multiplied by the total number of trees within each plot to obtain total
412 ecosystem CH_4 contribution from trees for each study site.

413 To estimate total annual CH_4 contributions from the entire lowland Amazon basin, we averaged CH_4
414 emissions across 13 sites for each individual pathways studied, assumed the estimated fluxes are
415 representative of basin-wide fluxes and then applied the fluxes to the entire Amazon basin area,
416 which was estimated using surface area data obtained from Melack *et al.*[34] and Hess *et al.*[22]
417 (Supplementary Table 5). Monthly area coverage for open water, flooded forest and macrophytes in

418 1.77 million km^2 of the central Amazon basin were obtained from Melack *et al.*[34] and the percentage

419 decrease in water-table depths relative to October data (lowest water-table month reported for

420 most land cover classes by Melack *et al.*[34]) and percentage increase in water-table depths relative to

421 May data (highest water-table month reported for most land cover classes in Melack *et al.*[34]) was

422 estimated. The percentage increases/decreases were applied to the high and low water surface area

423 for flooded forest, open water and macrophyte area within the Amazon basin wetland area (8.4 ×

424 10^5 km^2) reported in Hess *et al.*[22] and surface areas for the remaining months were estimated. Soil

425 surface area at the peak of the wet season was considered to be zero and for the remaining 11

426 months, soil surface area was estimated by subtracting the subsequent month flooded-forest

427 surface area and tree basal area from the flooded forest area during the peak of the wet season. Our

428 work suggests that up to 13.5% of the flooded forest was comprised of exposed soil and raised

429 hummocks in May, hence it is estimated that the soil surface area reached zero in June and

430 thereafter the water table receded. This observation was applied to soil surface area calculations.

431 Aquatic surface area was estimated by subtracting tree basal area from flooded-forest area.

432 Estimated monthly surface areas are listed in Supplementary Table 5. Tree-mediated CH$_4$ flux, similar

433 to other CH$_4$ emission pathways, was averaged across all 13 sites and was estimated to be 1350 ±

434 553 g ha^{-1} d^{-1} and 98 ± 47 g ha^{-1} d^{-1} for mature and young tree stem emissions between 0-140 cm

435 stem heights above the forest floor/water surface. However, when 0 to 5 m stem height was

436 considered the fluxes increased to 1927 ± 793 g ha^{-1} d^{-1} and 104 ± 49 g ha^{-1} d^{-1} for mature and young

437 trees, respectively. Open water CH$_4$ fluxes outside/beyond the edges of the flooded-forest were not

438 measured in our study. Fluxes from macrophytes were measured in some plots but the macrophytes

439 tended to be floating at the edges rather than inside the flooded-forest. Rooted macrophytes were

440 absent in all the plots. Thus CH$_4$ flux data for open water and macrophytes from Devol *et al.*[8] were

441 used to estimate these components for the entire Amazon basin. Uncertainties expressed as

442 standard deviation (SD) of means in CH$_4$ fluxes from all pathways were estimated using a

443 bootstrapping method (10,000 iterations).

Aircraft measurements

445 To estimate CH$_4$ fluxes (*F*) based on atmospheric CH$_4$ vertical profile measurements we apply a

446 simple air column budgeting technique following Miller *et al.*[35]:

447 $$F = \int_{z=0\,(agl)}^{4.4\,km} \frac{\Delta CH_4(z')}{t(z')}dz'$$

448 where $\Delta CH_4 = CH_{4,site} - CH_{4,bg}$ is the difference between CH$_4$ mass per volume measured *in situ* at a site

449 inside the basin and background (*bg*) air entering the basin from the Atlantic, z is height above

450 ground (*agl*) and *t(z)* air-mass trajectory travel time from the coast to height z at the site. The CH$_4$

451 concentration of background air is estimated from atmospheric SF$_6$ measured at the site and

452 compared with NOAA background stations Barbados (*RGB,* 7.92°S, 14.42°W) and Ascension (*ASC,*

453 7.92°S, 14.42°W) respectively, using a linear mixing model:

454 $$CH_{4,bg} = f_{ASC} \times CH_{4,ASC} + (1 - f_{ASC}) \times CH_{4,RPB} \quad \text{with} \quad f_A = \frac{S_{6,s} - S_{i,6,R}}{S_{6,A} - S_{6,R}} \quad .$$

455 SF$_6$ is suited for this purpose because it has virtually no sources in the Amazon Basin and

456 atmospheric SF$_6$ concentration is substantially higher in the northern compared to the southern

457 hemisphere. Air mass travel times are estimated using back trajectories calculated using the

458 HYSPLIT model[36] (http://ready.arl.noaa.gov/HYSPLIT_traj.php).

459 We applied this method to vertical air profiles sampled roughly bi-weekly from 2010 to 2013 at four

460 sites in the Brazilian Amazon located along the main airstream: at Alta Floresta (ALF; 8.80°S,

461 56.75°W), Rio Branco (RBA; 9.38°S, 67.62°W), Santarém (SAN; 2.86°S; 54.95°W) and Tabatinga (TAB;

462 5.96°S, 70.06°W). Concomitantly, carbon monoxide (CO) also was measured which allowed us to

463 determine the CH_4 component derived from fires during the dry season of each site. Air samples

464 were collected using a two-component portable semi-automatic collection system, consisting of a

465 first unit with two compressors and rechargeable batteries and a second unit with 17 (at SAN) and

466 12 (at ALF, RBA and TAB) 700 mL boro-silicate glass flasks connected by tubing and valves, which are

467 opened and closed by a microprocessor. The samples were generally taken between noon and 1 PM

468 local time, when the boundary layer tends to be well mixed. After sampling, the unit containing the

469 air flasks was transported to the high-precision greenhouse gas laboratory at IPEN (Instituto de

470 Pesquisas Energeticas e Nucleares) in Sao Paulo, where CH_4 and CO concentrations in air were

471 quantified. The accuracy and precision (1.5 ppb) of our greenhouse gas analysis system in Brazil is

472 similar to the system of the bottom up of NOAA (National Oceanic and Atmospheric

473 Administration, USA)[35].

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494 ## Data availability statement

495 Our aircraft CO_2 and CH_4 measurement data is available at http://www.ccst.inpe.br/projetos/lagee/.

496 CH_4 flux data from the bottom up study are available from SRP on request.

497 ## Supplementary table legends

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

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

500 **Table 3:** Surface area (m^2) used to estimate ecosystem contributions from all CH_4 emitting pathways in each
501 sampling plot.
502 **Table 4:** Coefficient of variation (%) for surface areas used in the ecosystem contribution
503 estimations.
504 **Table 5:** Estimated surface areas for the entire lowland Amazon basin (km^2)[a].

505 **Supplementary figure legends**
506 **Figure 1**: Photographs depicting one of the study sites, a typically inundated flooded forest (a), soil
507 flux (b), mature tree stem flux (c) and aquatic flux (d) measurements.
508 **Figure 2**: Frequency distribution of stem CH_4 fluxes from 20-50 cm of stem height from mature trees
509 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 Fluxes ± SD[a] ($mg\ m^{-2}\ h^{-1}$)	River Negro Ecosystem contribution ($g\ ha^{-1}\ d^{-1}$ (%))	River Madeira Fluxes ± SD ($mg\ m^{-2}\ h^{-1}$)	River Madeira Ecosystem contributions ($g\ ha^{-1}\ d^{-1}$ (%))	River Amazon Fluxes ± SD ($mg\ m^{-2}\ h^{-1}$)	River Amazon Ecosystem contributions ($g\ ha^{-1}\ d^{-1}$ (%))	River Solimões Fluxes ± SD ($mg\ m^{-2}\ h^{-1}$)	River Solimões Ecosystem contributions ($g\ ha^{-1}\ d^{-1}$ (%))	River Tapajós Fluxes ± SD ($mg\ m^{-2}\ h^{-1}$)	River Tapajós Ecosystem contributions ($g\ ha^{-1}\ d^{-1}$ (%))
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 CH_4 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 CH_4 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 CH_4 flux and was included in the ecosystem contributions; [c] young tree leaf CH_4 fluxes are the average of four different branches per tree (n = 260). No CH_4 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		
n = 1232	1.3	158 ± 274
At 130 cm above the soil/water surface		
n = 1343	3.7	440 ± 579

[a] CH_4 production potential was measured by incubating the stem cores for 12 hrs in 35 ml Wheaton vials flushed with N_2[23].

Table 3: δ^{13}C values of tree CH$_4$ flux and porewater CH$_4$.

	Flux			Porewater	
	δ^{13}C(CH$_4$)[a]	SD	n[b]	δ^{13}C(CH$_4$)[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 δ^{13}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 δ^{13}C(CH$_4$) values were used to determine a δ^{13}C value for CH$_4$ flux via Keeling regression analysis; [c] The range of δ^{13}C values are reported for porewater CH$_4$.

Table 4: Estimated annual CH_4 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 CH_4 contributions reported in Supplementary Table 5; [b] The upper range represents the inclusion of stem CH_4 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 CH_4 flux and stem height positions; [c] Aquatic macrophyte CH_4 emissions from high and low water season estimated and reported by Devol et al.[8] and Melack et al.[3]; [d] CH_4 fluxes to estimate emissions from macrophytes and open water were obtained from Devol et al.[8] and Melack et al.[3]; [e] total annual CH_4 emission estimates from river channels in the Amazon basin obtained from Sawakuchi et al.[19].