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Large emissions from floodplain trees close the Amazon methane budget

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Wetlands are the largest global source of atmospheric methane (CH_4)[1], a potent greenhouse gas. However, methane emission inventories from the Amazon floodplain[2,3], the largest natural geographic source of CH_4 in the tropics, consistently underestimate the atmospheric burden of CH_4 determined via remote sensing and inversion modelling[4,5], pointing to a major gap in our understanding of the contribution of these ecosystems to CH_4 emissions. Here we report CH_4 fluxes from the stems of 2357 individual Amazonian floodplain trees from 13 locations across the central Amazon basin. We find that egress of soil gas through wetland trees is the dominant source of regional CH_4 emissions. Amazon tree stem fluxes were up to 150-200 times larger than emissions reported for temperate wet forests[6] and tropical peat swamp forests[7], representing the largest non-ebullitive wetland fluxes observed. Tree emissions had an average $\delta^{13}C$-CH_4 value of -66.2±6.4‰ consistent with a soil biogenic origin. We estimate that floodplain trees emit 15.1 ± 1.8 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. Furthermore, we provide a top-down regional estimate of CH_4 emissions of 42.7±5.6 Tg CH_4 yr^{-1} for the Amazon basin based on regular vertical lower troposphere CH_4 profiles covering the period 2010-13. We find close agreement between our 'top-down' and combined 'bottom-up' estimates, indicating that large CH_4 emissions from trees adapted to permanent or seasonal inundation can account for the missing emission source required to close the Amazon CH_4 budget.

Wetlands are the single largest global source of atmospheric methane (CH_4), emitting an estimated 160 to 210 Tg of CH_4 each year to the troposphere[1]. Wetlands are concentrated globally in two broad latitudinal bands; one rich in peatlands spanning the boreal and subarctic zones and a second in the tropics and sub-tropics containing vast swamps and seasonally inundated floodplains[1]. Low latitude wetlands are notably prolific sources of CH_4 because of their substantial net primary productivity (NPP) and high seasonal temperatures[2]. However, relative to northern wetlands, flux measurements from Amazon floodplain ecosystems are comparatively sparse and have focussed mainly on soil and water surfaces, and gas exchange mediated by aquatic macrophytes[8,9]. Integration of these emission sources across the lowland Amazon basin based upon remotely sensed wetland distributions, yields an estimated flux of 26 to 29 Tg CH_4 yr^{-1} [2,3]. In contrast, estimates derived from atmospheric transport inversion modelling using *in-situ* CH_4 concentrations measured at surface sites remote from Amazonia and satellite greenhouse gas measurements (the so-called 'top-down' approaches) are considerably greater at 44 to 52 Tg yr^{-1} [4,10] and consistent with estimates of CH_4 flux determined from modelling heterotrophic anaerobic respiration of regional NPP[10]. Results of these global inversions should be treated with some caution. This is because the surface air sampling sites are minimally sensitive to the Amazon and the number of total column CH_4 estimates from space likely suffer from both temporal sampling bias (data are concentrated in the early dry season between seasons of smoke and clouds) and measurement biases[11]. In contrast *in-situ* measured vertical profile data capture directly the surface flux signals and discern the boundary layer signal from the free troposphere signal[12]. New measurements are therefore required to resolve the discrepancy between bottom-up inventories and top-down estimates which cannot be reconciled via contributions from other currently reported CH_4 sources from the Amazon region e.g., biomass burning, termites and ruminants[5,13] nor UV-induced aerobic emissions from plants[14] and tank bromeliads[15]. Further, the regional stable carbon isotope composition (i.e., $^{13}C/^{12}C$ ratio expressed as a $\delta^{13}C$ value) of atmospheric CH_4 indicates unequivocally that the 'missing' Amazonian CH_4 source is derived from microbial metabolism of C3 photosynthate[16]. Consequently, the most likely scenario is that surface-based flux measurements have either missed intense but perhaps spatially disaggregated CH_4 emission sources or they have overlooked an important pathway for egress of soil-produced CH_4.

Trees subjected to permanent or periodic inundation develop adaptive features such as enlarged lenticels and hollow aerenchyma tissue to enhance oxygenation of their root systems[17,18]. The internal conduits that enable air to move downwards also facilitate upward escape of soil CH_4 to the atmosphere[7,17,18]. Tree-mediated gas emission has been shown to dominate ecosystem CH_4 emissions in tropical peat swamp forest where aerobic CH_4-oxidizing bacteria form a highly effective barrier to diffusive flux through peat soil[7]. Total CH_4 emission rates are relatively modest in Borneo peat swamps[1,7]; however, the capacity for trees to emit CH_4 at higher rates is determined largely by rates of soil CH_4 production and supply[18]. Tree-mediated transport of CH_4 has not been investigated to date in the seasonally flooded, dense forests of the Amazon floodplains although ongoing efforts continue to extend the database of flux measurements quantifying CH_4 emission from soil, emergent macrophytes[8,9], and open water[8,19,20].

We measured CH_4 fluxes at 13 floodplain locations in the central Amazon River basin (Fig.1a), quantifying emissions from all known transport pathways, including forested floodplain soil, aquatic surfaces, and floating herbaceous macrophytes as well as stem and leaf surfaces of mature and young trees. At each floodplain site, a 50 × 80 m plot was established that encompassed four transects in which water table depth varied from ~1 m below the soil surface to ~10 m above the soil surface. Nine of the 12 sites sampled in 2014 included an area of exposed floodplain soil in which large hummocks occupied <13.5% of the total surface area. The relative contribution of emissions from individual pathways was determined relative to total ecosystem CH_4 flux (Table 1). Methane emissions from tree stems and aquatic surfaces were the dominant egress pathways (Fig. 1; Table 1).

All trees studied released substantial quantities of CH_4. Emission rates for mature and young trees 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 from tree stems exceeded CH_4 emissions from all other pathways in the study plots (Fig. 1b-f; Table 1). Moreover, CH_4 emission rates from Amazon floodplain trees were ~150 times larger than stem flux rates reported for southeast Asian peat swamp forests[7] where less CH_4 is released owing to low soil pH, high CH_4 oxidation rates and recalcitrant carbon impeding rates of methanogenesis. Fewer than 4% of wood cores extracted from tree stems at 20 and 130 cm above the soil or water surface displayed capacity for CH_4 production (Table 2) and stem cores from sampled trees displayed no visual sign of wood rot. These observations suggest that CH_4 emitted from the tree stems originated in the floodplain soil.

The $\delta^{13}C$ values of tree-mediated CH_4 flux ranged from -76.3 to -59.1‰, averaging -66.2 ± 6.4‰ (n = 18; Table 3) consistent with the stable carbon isotope composition of CH_4 in soil water (range -70.8 to -54.5‰; Table 3) in the study plots. The $\delta^{13}C$ values are typical for wetland CH_4 albeit more negative than values generally attributed to tropical wetlands[21].

Young tree leaves emitted small but significant quantities of CH_4 (Fig.1b-f; Table 1). Methane emission from mature leaves, if present, was below the instrument detection limit of *c.* 2 ppbv. Similar to temperate[6] and other tropical[7] trees, stem CH_4 flux rates decreased either linearly or exponentially with increasing stem height sampling position.

We pursued two approaches to scaling fluxes to the entire Amazon basin. Firstly, the measured CH_4 emission rates and areas of emission surfaces (Supplementary Table 3) were used to estimate the contribution of each transport pathway to total ecosystem CH_4 flux estimated for each 50 × 80 m study plot and then averaged for the river type. Emissions from tree stems and leaves collectively were the dominant source of CH_4 evasion from Amazon floodplain soil (44 to 65 %; Table 1). The contribution from aquatic surfaces was the second most significant source, accounting for 27 to 41% of total CH_4 flux. Soil surfaces, which were corrected for tree basal areas, emitted 2.5 to 15.7% of ecosystem CH_4 flux (Table 1). Conservative scaling of stem emission (considering only 0-140 cm of tree stem emissions) to the central Amazon basin[22] yields an annual source strength of 15.1 ± 1.8 Tg CH_4 yr^{-1} for tree-mediated flux (Table 4). Inclusion of tree emissions to 2.3-5 m stem height, estimated using the relationship between stem CH_4 flux and stem height intervals, yields an annual source strength of 21.2 ± 2.5 Tg CH_4 yr^{-1}, which is equivalent to current bottom-up inventories of total CH_4 emissions for Amazonian wetlands (26.2 ± 9.8 Tg yr^{-1}[2,3]; Table 4) that exclude tree emissions. Further, while recent evidence suggests the potential for non-wetland trees to emit CH_4[23-25], no robust measurements of upland tree emission have been reported in the region and those few flux measurements reported elsewhere have been several orders of magnitude smaller than our wetland tree observations, so in keeping with our conservative approach to regional upscaling we have excluded upland tree fluxes pending further evidence.

Secondly, during the period 2010 to 2013 we also established top-down regional estimates of CH_4 emissions based upon novel regularly measured *in-situ* atmospheric CH_4 profiles from the surface to 4.5 km height above sea level using an air-column budgeting approach. Profiles were measured at four locations in the Amazon basin (Alta Floresta (ALF), Rio Branco (RBA), Santarém (SAN) and Tabatinga (TAB)). Flux estimates determined using this approach integrate CH_4 emissions from regions upwind of the sampling sites, covering an increasing area the farther west a site is located in the basin. Based on the envelope of back-trajectory ensembles we estimate the regions of influence 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 km^2 for ALF. The total Amazon basin area is 6.7 million km^2. The upwind regions of all four sites during all four years were a significant source of CH_4 to the atmosphere with emission rates varying 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, 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. We observed substantially larger mean annual fluxes at SAN relative to the other three sites, which is consistent with spatial differences observed in CH_4 emission rates within our 13 floodplain study plots. The SAN area of influence includes the Tapajós River where we measured the largest CH_4 fluxes from trees and other sources among the 13 floodplain study plots (T10, T11, T12; Fig. 1a).

Extrapolation of inversion results to the whole of the Amazon basin using an area-weighted average ($F = \overline{F} \times A_{basin}$ 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 42.7 ± 5.6 Tg CH_4 yr^{-1} for the four-year period, which is the equivalent of ~8% of global CH_4 emissions. The uncertainty of 5.6 Tg CH_4 yr^{-1} is the standard deviation (1σ) of the four annual emission estimates. In an earlier study[26], we used the 2010-2011 vertical profile data and a simple Bayesian synthesis inversion approach constrained by both prior flux estimates and atmospheric profile data to obtain a net flux estimate of 37 ± 5.9 Tg yr^{-1}. For all inversions and periods considered, the estimated fluxes exceeded the prior flux estimates with wetland prior fluxes based either on the JULES land surface model or the model of Bloom *et al.*[2]. While these earlier estimates are somewhat smaller than the estimates reported here, this is expected because the presence of the prior flux estimates biases the estimates low. The combinations of floodplain tree emissions (15.1 ± 1.8 - 21.2 ± 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 that agrees well with our estimate of regional CH_4 emissions determined from inversion modelling of atmosphere CH_4 profiles. Thus, inclusion of tree-mediated CH_4 fluxes reconciles current disparities between ‘bottom-up’ and ‘top down’ approaches effectively closing the Amazonian CH_4 budget.

Our results demonstrate that exceptionally large emissions from Amazon floodplain trees alone are equivalent in size to the entire Arctic CH_4 source and account for ~15% of the global wetland CH_4 source. Together with already understood emission pathways, our findings demonstrate that the Amazon, in contributing up to a third of the global wetland CH_4 source, is a far larger source of CH_4 than inventories previously acknowledged and is therefore likely to exert greater influence over global atmospheric CH_4 concentration variability than was previously thought. Given this increased influence over atmospheric CH_4 there is a need to quantify the controls on soil CH_4 production and tree emission variability within the biodiverse, hydrologically dynamic and geochemically heterogeneous Amazon basin while re-appraising representation of CH_4 transport mechanisms in process-based wetland models if global models are to possess the capacity to accurately predict changes in CH_4 flux resulting from climate change or other human perturbations such as the planned construction of hydroelectric dams across the basin[27]. Finally, given that tropical forested wetlands spanning the Congo and southeast Asia experience either seasonal or permanent inundation, wetland-adapted trees may be responsible for a similar proportion of CH_4 flux in those regions, pointing to potential gross underestimates in bottom-up CH_4 inventories across globally important regions using current approaches that exclude trees.

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Supplementary information

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

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Author contributions

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

Author information

Reprints and permissions information is available at www.nature.com/reprints. Authors declare no competing financial interests. Correspondence and requests for materials should be addressed to SRP (Sunitha.Pangala@open.ac.uk) and VG (Vincent.Gauci@open.ac.uk).

Main table legends

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

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

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

Table 4: Estimated annual CH_4 emissions from the Amazon basin using bottom up and top down methods.

Main figure legends

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

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

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

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

The locations of trees were mapped in each of the 13 study plots along with the area occupied by emergent macrophytes and water-table depths (measured within 1 m of all trees) along the boundary of the plot and within four internal transects. Tree height, DBH, stem diameter at 10 cm intervals between 0 and 200 cm stem height, and basal diameter were measured for all trees in each plot. The floodplain on River Madeira site sampled in 2013 was comprised of non-flooded forest because of receding water-table levels. Várzeas in the region had shrunk to small ponds with trees around the edges, which were subjected to water-table levels at or below the soil surface. In all the study plots, the edge of the floodplain where floating macrophytes ceased to exist was regarded as the plot boundary and open water beyond that point, which contained no vegetation, was excluded from the ecosystem contribution estimations but was later included in the regional upscaling using the literature values[8]. Nine of the 12 sites investigated during 2014 contained both flooded and non-flooded portions (<13.5%) of floodplain, three sites were fully flooded. Area occupied by aquatic surfaces, soil surfaces and mature and young trees were mapped for each study site and the corresponding surface areas were calculated.

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

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

1.77 million km^2 of the central Amazon basin were obtained from Melack *et al.*[34] and the percentage decrease in water-table depths relative to October data (lowest water-table month reported for most land cover classes by Melack *et al.*[34]) and percentage increase in water-table depths relative to May data (highest water-table month reported for most land cover classes in Melack *et al.*[34]) was estimated. The percentage increases/decreases were applied to the high and low water surface area for flooded forest, open water and macrophyte area within the Amazon basin wetland area (8.4 × 10^5 km^2) reported in Hess *et al.*[22] and surface areas for the remaining months were estimated. Soil surface area at the peak of the wet season was considered to be zero and for the remaining 11 months, soil surface area was estimated by subtracting the subsequent month flooded-forest surface area and tree basal area from the flooded forest area during the peak of the wet season. Our work suggests that up to 13.5% of the flooded forest was comprised of exposed soil and raised hummocks in May, hence it is estimated that the soil surface area reached zero in June and thereafter the water table receded. This observation was applied to soil surface area calculations. Aquatic surface area was estimated by subtracting tree basal area from flooded-forest area. Estimated monthly surface areas are listed in Supplementary Table 5. Tree-mediated CH_4 flux, similar to other CH_4 emission pathways, was averaged across all 13 sites and was estimated to be 1350 ± 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 stem heights above the forest floor/water surface. However, when 0 to 5 m stem height was considered the fluxes increased to 1927 ± 793 g ha^{-1} d^{-1} and 104 ± 49 g ha^{-1} d^{-1} for mature and young trees, respectively. Open water CH_4 fluxes outside/beyond the edges of the flooded-forest were not measured in our study. Fluxes from macrophytes were measured in some plots but the macrophytes tended to be floating at the edges rather than inside the flooded-forest. Rooted macrophytes were absent in all the plots. Thus CH_4 flux data for open water and macrophytes from Devol *et al.*[8] were used to estimate these components for the entire Amazon basin. Uncertainties expressed as standard deviation (SD) of means in CH_4 fluxes from all pathways were estimated using a bootstrapping method (10,000 iterations).

Aircraft measurements

To estimate CH_4 fluxes (F) based on atmospheric CH_4 vertical profile measurements we apply a simple air column budgeting technique following Miller *et al.*[35]:

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

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 inside the basin and background (*bg*) air entering the basin from the Atlantic, *z* is height above ground (*agl*) and *t(z)* air-mass trajectory travel time from the coast to height *z* at the site. The CH_4 concentration of background air is estimated from atmospheric SF_6 measured at the site and compared with NOAA background stations Barbados (*RGB,* 7.92°S, 14.42°W) and Ascension (*ASC,* 7.92°S, 14.42°W) respectively, using a linear mixing model:

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

SF_6 is suited for this purpose because it has virtually no sources in the Amazon Basin and atmospheric SF_6 concentration is substantially higher in the northern compared to the southern hemisphere. Air mass travel times are estimated using back trajectories calculated using the HYSPLIT model[36] (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 CH_4 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 CH_4 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

Our aircraft CO_2 and CH_4 measurement data is available at http://www.ccst.inpe.br/projetos/lagee/. CH_4 flux data from the bottom up study are available from SRP on request.

Supplementary table legends

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

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

Table 3: Surface area (m^2) used to estimate ecosystem contributions from all CH_4 emitting pathways in each sampling plot.

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

Table 5: Estimated surface areas for the entire lowland Amazon basin (km^2)[a].

Supplementary figure legends

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

Figure 2: Frequency distribution of stem CH_4 fluxes from 20-50 cm of stem height from mature trees 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.

	River Negro		**River Madeira**		**River Amazon**		**River Solimões**		**River Tapajós**	
Methane emitting pathways	**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 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 ($\mu g\ CH_4\ h^{-1}\ m^{-3}$ 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: $\delta^{13}C$ values of tree CH_4 flux and porewater CH_4.

	Flux			**Porewater**	
	$\delta^{13}C(CH_4)$[a] (‰)	**SD (‰)**	**n[b]**	**$\delta^{13}C(CH_4)$[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 $\delta^{13}C$ values are reported for CH_4 flux; [b] n represents one chamber deployment from which three or four pairs of CH_4 concentration and $\delta^{13}C(CH_4)$ values were used to determine a $\delta^{13}C$ value for CH_4 flux via Keeling regression analysis; [c] The range of $\delta^{13}C$ 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].