

Article

Soil Is a Net Source of Methane in Tropical African Forests

Simona Castaldi ^{1,2,*}, Teresa Bertolini ¹, Giacomo Nicolini ³ and Riccardo Valentini ^{2,3,4}

¹ Dipartimento di Scienze e Tecnologie Ambientali, Biologiche e Farmaceutiche, Università degli Studi della Campania “Luigi Vanvitelli”, via Vivaldi, 43, 81100 Caserta, Italy; teresa.bertolini@gmail.com

² Department of Landscape Design and Sustainable Ecosystems, Agrarian-Technological Institute, RUDN University, Miklukho-Maklaya str., 6, 117198 Moscow, Russia; rik@unitus.it

³ CMCC–Centro euro-Mediterraneo sui Cambiamenti Climatici, IAFES Division, viale Trieste, 127, 01100 Viterbo, Italy; giacomo.nicolini@cmcc.it

⁴ Dipartimento per la Innovazione nei sistemi Biologici, Agroalimentari e Forestali, Università degli Studi della Tuscia, via san Camillo de Lellis, snc, 01100 Viterbo, Italy

* Correspondence: simona.castaldi@unicampania.it; Tel.: +39-0823-274-646

Received: 15 September 2020; Accepted: 29 October 2020; Published: 30 October 2020



Abstract: *Research Highlights:* Monitoring of soil CH₄ fluxes in African tropical forest conducted run for almost two years, contributing to the scant information on greenhouse gas (GHG) fluxes from forests available from this region. Data showed that the forest soil acted as a net yearly source of CH₄. Hotspots of CH₄ emissions were measured both in upland and lowland areas of the forest, and on an annual basis they overcame the soil CH₄ sink during drier periods or in well-drained areas. *Background and Objectives:* Atmospheric studies indicate that tropics are a strong CH₄ source. Regional budgets attribute the majority of this source to wetland ecosystems and flooded lowland forests, whereas un-flooded forests are considered net CH₄ sinks, although few studies in tropical forests, in particular in Africa, are available. The present work aims to contribute to this knowledge gap. *Materials and Methods:* Monitoring campaigns were conducted along the year in the tropical forest of the Ankasa National Park, Ghana, in two contrasting environments, uphill and downhill, using close static chambers coupled with gas chromatography. *Results:* The uphill area was a net weak CH₄ sink with mean daily fluxes ranging from -1.29 to 0.44 mg CH₄ m⁻² d⁻¹. The downhill area was a significant CH₄ source with mean daily fluxes ranging from -0.67 to 188.09 mg CH₄ m⁻² d⁻¹ and with peaks up to 1312 mg CH₄ m⁻² d⁻¹ in the wet season. *Conclusions:* The net annual soil CH₄ budget for the Ankasa Park, normalizing the proportion of downhill areas over the whole park surface, was a source of about 3.3 kg CH₄ ha⁻¹ yr⁻¹. Overlooking such areas might lead to underestimates of the total CH₄ source strength of forested areas.

Keywords: forest ecosystem; CH₄ sink; CH₄ production; CH₄ emissions; lowland; tropical soil

1. Introduction

Methane (CH₄) is the second most relevant biogenic greenhouse gas, after carbon dioxide, in terms of radiative forcing on the climate [1,2]. The CH₄ atmospheric concentration depends on many natural and anthropogenic sources and sinks [2–4] and it has been increasing, in the last decades, at variable rates, the strongest being reached in 2014 (12.5 ppb CH₄ yr⁻¹) [2,4]. On a global basis, tropical areas are considered a key source area of CH₄ production [2–4]). However, as top-down estimates cannot disentangle sources and sinks of CH₄ when more processes occur simultaneously, global CH₄ estimates have been quantified in combination with bottom-up approaches, mainly process-based modelling and/or inventory [2–4]. This detailed analysis indicated that wetlands and forested, flooded or saturated

lowlands (permanent and ephemeral) were the most prominent CH₄ source in the tropics. This source, however, is also characterized by significant uncertainty [3]. A key feature of CH₄ producing systems is the occurrence of anaerobic conditions in soils and sediments, either saturated by high water table or under flooded conditions. As a consequence, the correct quantification of the surface of inundated or saturated soils is a critical factor in determining wetland CH₄ source strength and extension and it greatly contributes to the high uncertainty associated to this CH₄ source [5]. In land surface models, the extent of flooded land is computed from remote sensing data or by using hydrological models [3–5]. Both approaches are characterized by high uncertainty as remote sensing signal deteriorates over dense vegetation, which typically occurs in tropical forested areas, and because the microwave signals can only detect water above or at the soil surface [6], leaving a “black hole” in the quantification of sub-saturated or saturated soil in forested areas. On the other hand, hydrogeological models must rely on complex sets of information often not available for extended areas of tropical regions [5]. Where macroscopic evidence of water inundation fails due to the technical limits of its detection, the CH₄ budget of tropical forests is hence computed by land surface models, treating forested areas mostly as net CH₄ sinks [2–4]. Aerobic soils can provide moderate and transient sources of CH₄, due to methanogenic activity generated in anoxic microsites within an aerobic soil matrix. This phenomenon has been hypothesized in order to explain the relatively small net CH₄ emissions observed from upland and aerobic soils across a range of continents and ecosystems, ranging from well-drained systems to tropical mountain grasslands [7–15]. However, also unaccounted for is subterranean termite activity, particularly in tropical soils, which has been proposed as a possible explanation for observed hotspots of CH₄ emissions in aerobic tropical soils [7,8,15]. Although, in some cases CH₄ emissions as high as those in wetlands have been reported [16]; the majority of the abovementioned net CH₄ emissions observed in un-flooded forests are small compared with inundated areas [2], but their relevance as a global CH₄ source is due to on the dramatic extension of these tropical and subtropical natural areas. The strength and the extension of the CH₄ source from these areas is, however, still high uncertain [2]. Moreover, the availability of only few and short time series of in situ CH₄ measurements, makes tropical areas some of the least constrained regions for inversions using surface measurements [3].

To gather further evidence on the contribution of tropical forests to CH₄ soil–atmosphere exchange, a study was conducted in a humid equatorial tropical forest in the Ankasa National Park, Ghana. Several campaigns to quantify the CH₄ soil–atmosphere gas exchange were carried out along one year in two contrasting environments representative of the main extreme conditions for CH₄ production and consumption we might typically find in these areas, uphill and downhill, and the evaluation of the overall annual net flux of the Ankasa forest was provided.

2. Materials and Methods

2.1. The Study Site

The study site was a mix moist semi-deciduous and wet evergreen forest (05°16′11.2″ N; 02°41′41.55″ W, 104 m a.s.l.) located in the Ankasa Conservation Area in Ghana [17]. The mean annual temperature is about 25 °C and the mean annual precipitation is 1500–2000 mm, mainly concentrated from the end of March to mid-July and from September to November with a lower intensity [18]. A dry period extends from December to March and a shorter dry period occurs in August [18]. The landscape is characterized by the presence of hills (average elevation 90 m a.s.l.), alternating with narrow valleys. Considering the whole surface of the Ankasa Park, the downhill area was estimated to be around 20% of total Park surface (data derived by SRTM DEM at 90 m resolution, CGIAR–CSI free source). The soils are classified as Oxisols [19]. They developed on coherent biotite-rich granites of the Cape Coast complex to form the Ankasa association consisting of “Abenia” series, the most widespread series on top of the hills, which alternates with the “Ankasa” series, along the slopes. Soils are deeply weathered and highly acidic with a pH in the upland areas varying between 3.6 at 0–5 cm depth and 4.5 at 15–30 cm depth. Alluvial soils occupy the bottom of the fluvial valleys, presenting a pH between 4.3 (0–5 cm

depth) and 5.0 (30–50 cm depth). Soil bulk density varies little within the soil profile when comparing top and bottom hill areas (range 1.30 to 1.36 Mg m^{-3} [17]). Contents of sand, silt and clay along the soil profile in the two areas are reported in Castaldi et al. [17]; overall the sand was the predominant soil mineral fraction. In the top-hill, soil C was highly concentrated in the top 5 cm (average C value $9.8 \pm 1.12 \text{ g kg}^{-1}$, average total N content $0.66 \pm 0.21 \text{ g N kg}^{-1}$), decreasing to $1.15 \pm 0.51 \text{ g C kg}^{-1}$ at 5–15 cm depth and to $0.78 \pm 0.19 \text{ g C kg}^{-1}$ between 30–50 cm depth. In the downhill areas, C content varied between 2.74 and 2.44 g C kg^{-1} in the top 15 cm, reaching $1.10 \pm 0.05 \text{ g C kg}^{-1}$ at 30–50 cm depth [17]. Surface litter C in the top-hill area was estimated around $15 \pm 9 \text{ Mg C ha}^{-1}$ while C stock in the mineral soil, to a depth of 1 m ($151 \pm 20 \text{ Mg C ha}^{-1}$), was estimate to be in the same order of magnitude of the aboveground biomass C ($138\text{--}170 \text{ Mg C ha}^{-1}$) including live and dead wood [20].

2.2. Experimental Set up

Fluxes were measured over an area of about half a hectare in two contrasting environments, uphill and downhill, using eight chambers (replicates) in each site per each day of sampling. The first monitoring site to be established was the top-hill area, as measurements started as part of a wider monitoring experiment aimed at quantifying the forest GHG exchange, which also included CO_2 gas exchange measurements by eddy covariance. Top-hill measurement campaigns of soil–atmosphere gas exchange were conducted from April 2009 to November 2010. The measurements in the downhill area started in May 2010 and lasted until November 2010, covering the wet season period, when CH_4 emissions were hypothesized to occur, and the short dry season of August. Campaigns to monitor gas fluxes were conducted for six consequent days, with few exceptions, each month. Chamber position was modified at each sampling event so as to improve the coverage of the spatial variability over the study. However, in order to avoid the “soil disturbance effect” due to chamber base insertion in the soil, bases were positioned in the ground the evening before sampling [17,21]. In the wet season, during the periods of most intense rainfall, ephemeral ponds (around 50 m^2 , max water depth around 20 cm) occurred in the lowest locations of the downhill area. The pond water drained within a week or two, depending on the rainfall duration and intensity. The monitoring campaigns were not continuous, but concentrated over few days per each month, therefore it was not possible to estimate the total area of forest soil which was oversaturated along the year. However, in order to have an idea of the order of magnitude of the CH_4 concentration in the water, and of the magnitude of the diffusive flux which might derive from these ponds, in one of the campaigns, water was sampled from the pond closest to the chamber sampling area over three consecutive days, while the water was slowly draining away.

2.3. Determination of Gas Exchange between Atmosphere and Soil or Water

Soil–atmosphere gas exchange was measured using closed static PVC chambers ($20 \text{ cm h} \times 15 \text{ cm } \emptyset$) which were placed on bases inserted in the ground ($7 \text{ cm h} \times 15 \text{ cm } \emptyset$) [17]. Gas fluxes were withdrawn at time 0, 30 and 60 min after closure and gas was stored in slightly overpressured, pre-evacuated gas-tight vials (20 mL), which were then sealed with thermal glue and shipped at the end of each six day campaign to Italy for gas chromatographic (GC) analysis. The analytical set up of the GC used for gas analyses is described in detail in Castaldi and others [17]. Flux rates were determined via linear regression of the three sampling points (0, 30, 60 min) for each chamber and by applying a temperature and pressure correction. The minimum detectable flux over a period of 60 min was $0.07 \text{ mg CH}_4 \text{ m}^{-2} \text{ d}^{-1}$. The analytical precision of the GC for standards at ambient concentration was approximately 1.5%, using one standard deviation as a measure of mean error. Any flux below these reported detection limits was assigned a value of zero. At each sampling event, soil temperature (HI93510 thermometer, Hanna Instruments Canada Inc., Laval, Quebec) and volumetric soil water content (ThetaProbe ML2, Delta-T Device Ltd., Cambridge, UK) were hand-measured close by each chamber (0–5 cm depth), 5 cm from the chamber edge [17].

Gas samples to determine the concentration of CH_4 dissolved in water were obtained using the headspace technique [22]. 150 mL of water was sampled just below the water surface of the pond,

taking 10 replicates of water each day for 3 consecutive days, when the water level measured on average 10, 8 and 6 cm above soil level, respectively. The dimension of the ephemeral pond was in the order of about 50 m² and water samples were taken circling the pond and extending the sampling towards the center of the pond using a support, so as to avoid pressing the soil with boots and releasing the CH₄ trapped in the soil close to the sampling point. The water was gently transferred inside a 325 mL glass bottle which was immediately capped with butyl rubber septa and closed with a screw cap for air-tight closure. The cap was provided with a circular hole made with a needle, to allow gas sampling through the septa using a syringe. The water was vigorously shaken for about one minute to allow the gas phase of the headspace and water to achieve equilibrium. Then a sample from the bottle headspace was transferred into a 20 mL air tight vial using a double needle system and by replacing the displaced air volume with water. The temperature of the water was measured at the same height of the water sample just after water sampling. To have a reference concentration of the CH₄ concentration in the headspace at T₀, before the equilibration, a sample of air was taken just at the surface of the water and stored in a 20 mL vial as described for the other gas samples. Vials were shipped to Italy for GC analysis. CH₄ partial pressure was converted into concentration in water using Henry's constant, water temperature, the measured gas partial pressures in air, and taking into account the volume of water and headspace inside the bottle [23].

Gas saturation was calculated as the ratio between the measured gas concentration and that expected if the water sample was in equilibrium with the atmosphere. The hourly flux was determined using an ensemble of three different modelling approaches, as none of the commonly used approaches for water–gas exchange are generally tested and applied in conditions such as those presented in this paper, i.e., under a forest canopy, but they refer to open wetlands and lake ecosystems. The first two models estimated the gas flux using the gas concentration in water and the gas transfer velocity K₆₀₀ [24,25]. In both models, which used wind velocity as a key variable, the latter was set at a value of <0.15 m s⁻¹ (2 m height), based on measures done in the same area below the canopy during eddy covariance campaigns (data not shown). The third model is a steady-state diffusion model based on Fick's law, which doesn't take into account the wind factor but could still be considered appropriate for the conditions of very low wind under the forest canopy of Ankasa.

2.4. Statistic Analysis

Simple linear regression and non-linear regression analysis was performed to find the relationship between independent and dependent variables. Data were tested for normality distribution (Shapiro–Wilk test) and constant variance (Sigma Stat, Jandel Scientific).

3. Results

Fluxes of CH₄ were measured uphill for almost two consecutive years between April 2009 and November 2010, during 19 campaigns for a total of 98 days of sampling. The daily mean flux (Figure 1) varied between −1.29 and 0.44 mg CH₄ m⁻² d⁻¹.

Only for eight of the 98 days of monitoring was the average CH₄ flux above zero (net CH₄ source), and these days mainly occurred in the wet season 2010 (Figure 2). The average net CH₄ source was due to a few chambers characterized by high CH₄ emissions among chambers showing almost zero flux, as can be noted by the very high standard error for these days of positive fluxes (Figure 1). These single chamber hotspots, however, never exceeded 8 mg CH₄ m⁻² d⁻¹. The average daily flux for the whole period of observation was -0.61 ± 0.35 (1 SD) mg CH₄ m⁻² d⁻¹; while considering only one year of observation, from December 2009 to November 2010 the annual average flux was -0.42 ± 0.31 (1 SD) mg CH₄ m⁻² d⁻¹.

The water-filled pore space (WFPS) is a key control parameter for soil gas diffusivity, which in turn strongly controls the rate of soil CH₄ uptake. In Figure 3, CH₄ fluxes measured in the top-hill area were plotted against the values of WFPS%, with each data point representing the daily average value of gas and WFPS ($n = 8$). Figure 3 shows that there was a trend of reduction in CH₄ uptake rates

for increasing water-filled pore space values, which followed a sigmoidal shape, with the steepest reduction between 50% and 60% WFPS. This range is generally regarded as a limit above which the rate of aerobic processes slows down, due to gas diffusion limitation. The small net CH₄ emissions observed were recorded only above 50% WFPS, but overall WFPS values rarely exceeded 60%.

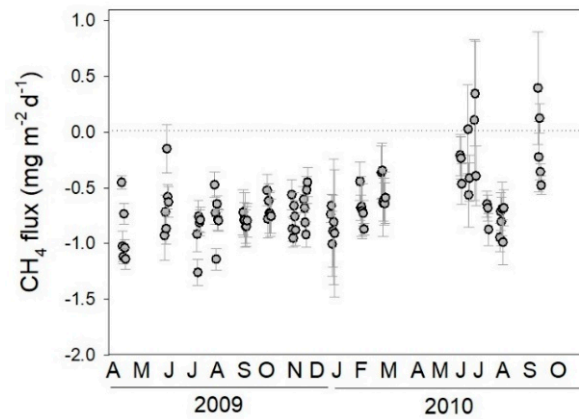


Figure 1. Average daily CH₄ fluxes (± 1 SE, $n = 8$) measured in the top-hill site of Ankasa Park from April 2009 to October 2010.

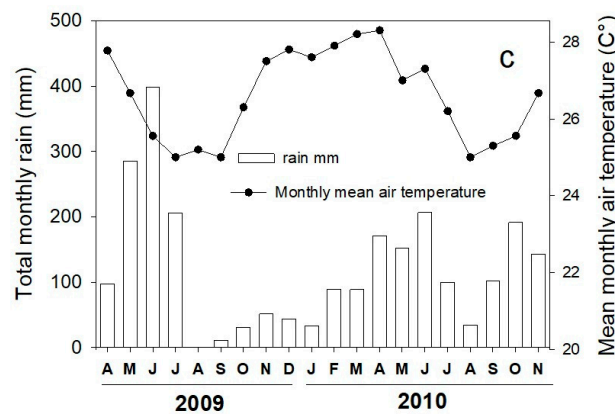


Figure 2. Total monthly rainfall (mm) and average mean monthly temperature estimated for the site from the TRMM database (Tropical Rainfall Measuring Mission, NASA).

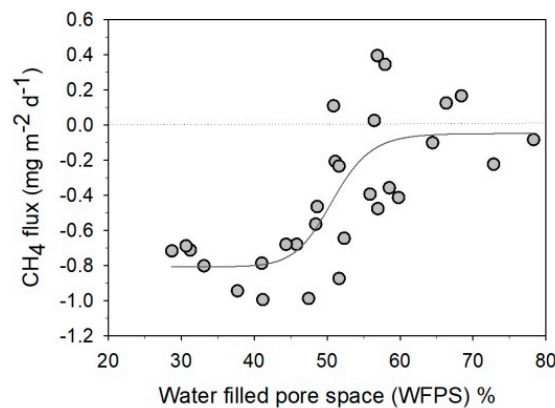


Figure 3. Upland site, average ($n = 8$) daily CH₄ fluxes plotted vs. soil water-filled pore space (%) estimated from volumetric water content measured inside the chambers at the end of gas sampling. Equation of the fitting curve: $y = -0.81 + 0.76/[1 + (x/-50.78)^{-19.23}]$, $R^2 = 0.58$, $p < 0.0001$.

Soil CH₄ fluxes were measured in the downhill site of Ankasa Park from May to November 2010. Figure 4 shows that the daily average CH₄ fluxes were significantly higher than those observed in the top-hill area, varying from a minimum of $-0.67 \text{ mg CH}_4 \text{ m}^{-2} \text{ d}^{-1}$ to $188.09 \text{ mg CH}_4 \text{ m}^{-2} \text{ d}^{-1}$, with an average flux of $26.8 \text{ mg CH}_4 \text{ m}^{-2} \text{ d}^{-1}$ for the whole observation period.

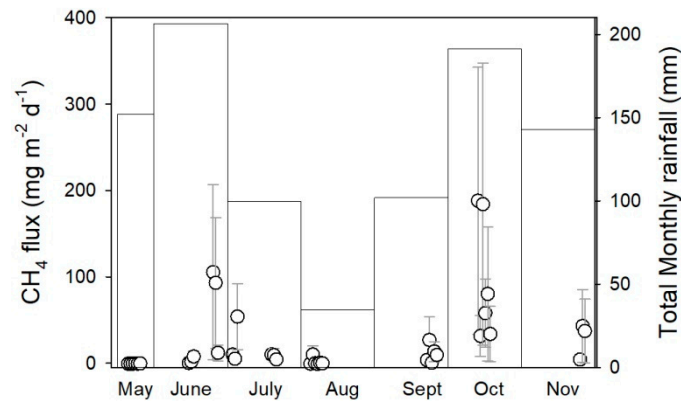


Figure 4. Average daily CH₄ fluxes (± 1 SE, $n = 8$) measured in the downhill area of Ankasa Reserve from May to November 2010. The bars (reference y-axis on the right) represent the total monthly rainfall (mm) from the TRMM database (Tropical Rainfall Measuring Mission, NASA).

Taking into account all the measured fluxes over the 38 days of sampling from eight chambers, 47% of measured fluxes were above $0 \text{ mg CH}_4 \text{ m}^{-2} \text{ d}^{-1}$, i.e., net CH₄ emission, and about 13% of positive fluxes were above $10 \text{ mg CH}_4 \text{ m}^{-2} \text{ d}^{-1}$, reaching values as high as $1312 \text{ mg CH}_4 \text{ m}^{-2} \text{ d}^{-1}$ (Table 1, Figure 5).

Table 1. Frequency distribution of CH₄ fluxes measured from each single chamber in the downhill area from May to November 2010 ($n = 304$).

Flux Class $\text{mg CH}_4 \text{ m}^{-2} \text{ d}^{-1}$	Counts	Frequency %
<0	162	53.3
0	17	5.6
0–1	40	13.2
1–10	44	14.5
10–50	19	6.2
50–100	9	3.0
100–1000	13	3.6
>1000	2	0.7

The highest emissions were reached during the period of most intense rainfall, which corresponded to the months of June and October (Figure 4). These were also the months when the highest spatial variability was observed (Figure 5).

As also noted in the top-hill site, fluxes started to significantly increase when the WFPS was above 40% (Figure 6). However, while the top-hill site remained on average a net sink of CH₄ even at higher values of WFPS, the downhill site became a very strong CH₄ source (fluxes $> 100 \text{ mg CH}_4 \text{ m}^{-2} \text{ d}^{-1}$) above 60% WFPS. Moreover, when the WFPS was between 20% and 40%, a significant number of chambers had a flux between 1 and $100 \text{ mg CH}_4 \text{ m}^{-2} \text{ d}^{-1}$. The probe measuring the water content was 0–7 cm deep, hence, it cannot be excluded that at lower depth a higher water content was reached compared to the topsoil centimeters, due to an increase in the water table during the rainy season.

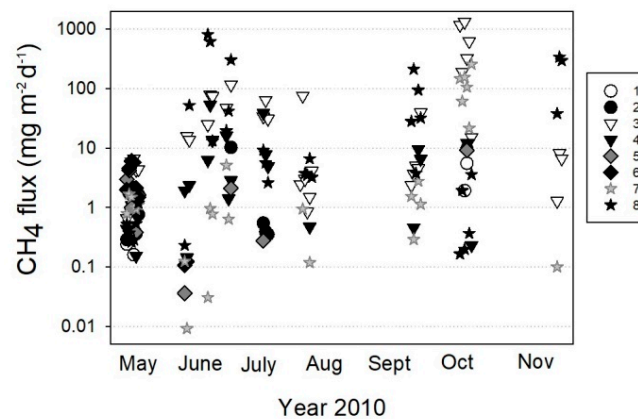


Figure 5. Net emissions of CH_4 reported for each chamber used in the downhill area. Symbols numbered in the legend from 1 to 8 refer to the 8 positions of the chambers in the field.

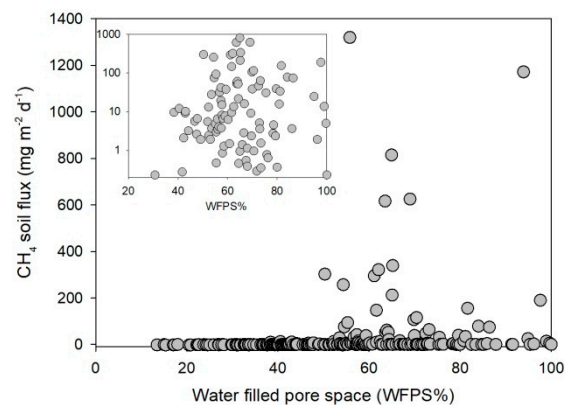


Figure 6. CH_4 fluxes plotted vs. soil water-filled pore space (%) measured inside the chambers after the gas sampling, in the downhill area. In the small box, fluxes are reported on a log scale to evidence the density of CH_4 fluxes in the range of values 1–100 $\text{mg CH}_4 \text{ m}^{-2} \text{ d}^{-1}$.

Indeed, CH_4 fluxes did not correlate with the short-term cumulative rainfall, i.e., the cumulative rainfall of the two days prior the gas flux measurement, which might mainly influence the topsoil water content. On the contrary, CH_4 fluxes significantly correlated with the mid and long term cumulative rainfall, i.e., cumulative rainfall of 10 and 30 days prior gas sampling, respectively (Figure 7). The two latter relationships were fitted by non-linear curves, the best fit being the one related to the cumulative rainfall of 10 days prior gas flux measurement ($p < 0.0001$). Overall, the steepest increase in CH_4 positive fluxes per unit of rainfall increase was found for the cumulative rainfall in the 10 days preceding the flux measurements.

During the periods of most intense rainfall, ephemeral ponds occurred. The lifetime of these ponds was quite variable, from a few days to a couple of weeks, depending on the rainfall regime. The estimates of the CH_4 concentration in the water and gas saturation %, determined as described in the methods section, are reported in Table 2, together with the estimates of the average daily diffusive flux from the ephemeral ponds, calculated with three different modelling approaches. Results showed that the concentration of CH_4 and its percentage saturation in water were very high at day one of sampling, increased the next day and started to slightly decrease on the third day of sampling, while the water level continuously decreased over the observation period. The estimated diffusive fluxes, considering the single replicates, ranged from a minimum of 5.34 $\text{mg CH}_4 \text{ m}^{-2} \text{ d}^{-1}$ (model 1) to a maximum of 127.36 $\text{mg CH}_4 \text{ m}^{-2} \text{ d}^{-1}$ (Fick's model). The average of the ensemble of the three models varied between a minimum of 9.73 $\text{mg CH}_4 \text{ m}^{-2} \text{ d}^{-1}$ to a maximum of 77.13 $\text{mg CH}_4 \text{ m}^{-2} \text{ d}^{-1}$. Considering these sole diffusive fluxes, the CH_4 source strength of the analyzed pond was comparable

to the 90th percentile distribution of the fluxes measured with soil chambers (Table 1) for days 2 and 3, and above the 50th percentile for day 1.

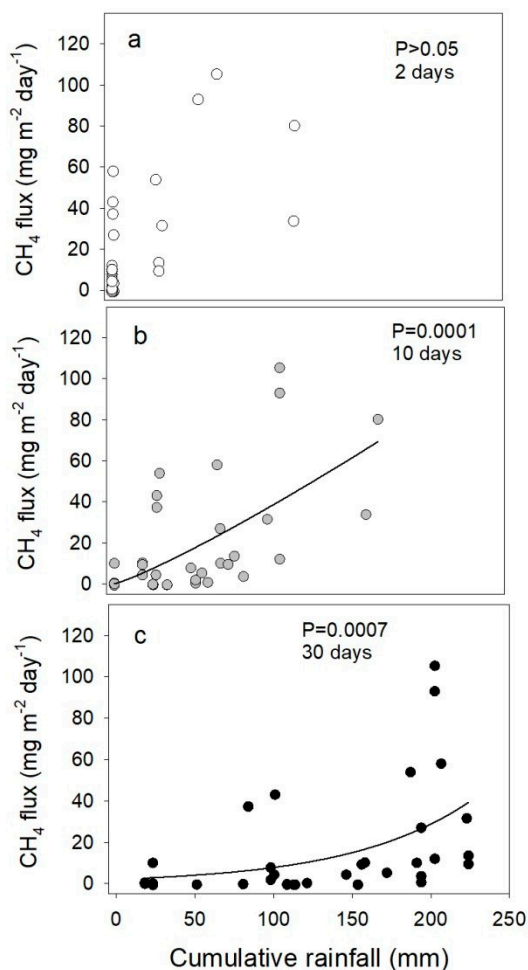


Figure 7. Mean daily values of CH₄ fluxes plotted vs. cumulative rainfall calculated considering 2 days (a), 10 days (b) or 30 days (c) preceding the date of gas sampling. P represents the statistical significance of the fitting curve (solid line). Equation panel b: $y = 0.18 \times x^{1.16}$; equation panel c: $y = 40.2 + 16.7/(1 + e^{-(x-94.4)/6.08})$.

Table 2. Estimates (± 1 SD, $n = 10$) of CH₄ concentration in pond water, % saturation of CH₄ in water, CH₄ diffusive fluxes determined with three different models, average (± 1 SD) of the ensemble of the three models’ results. Data were calculated for three consecutive days of sampling in June 2010.

	Day 1	Day 2	Day 3
Water height (cm) above soil surface	10	8	6
CH₄ concentration in water (M)	0.06 \pm 0.03	0.51 \pm 0.13	0.43 \pm 0.09
% CH₄ saturation \pm 1SD (10)	(6.39 \pm 3.16) $\times 10^4$	(4.65 \pm 1.24) $\times 10^5$	(4.57 \pm 0.01) $\times 10^5$
Diffusive CH₄ fluxes (mg CH₄ m⁻² d⁻¹ \pm 1 SD)			
Model 1 [24]	5.34 \pm 2.65	42.41 \pm 11.32	36.02 \pm 7.30
Model 2 [25]	7.77 \pm 3.85	61.72 \pm 13.87	52.43 \pm 10.63
Model 3 (Fick’s model)	16.08 \pm 9.97	127.26 \pm 33.96	108.39 \pm 22.00
Mean of CH₄ flux model ensemble			
mg CH ₄ m ⁻² d ⁻¹ \pm 1 SD	9.73 \pm 5.63	77.13 \pm 44.47	65.61 \pm 37.94

4. Discussion

Atmospheric studies have demonstrated high atmospheric CH₄ concentrations over tropical areas [26]. The main source of this CH₄ has been attributed to permanent and ephemeral wetlands, combining top-down and bottom-up approaches [2–4]. Methanogenesis is the final step of a chain of anaerobic reactions, where microbial groups that utilize a variety of inorganic terminal electron acceptors (TEAs) for their respiration process [27,28] compete for organic substrates, which leads to CH₄ production. Strong reducing conditions are needed for CH₄ production until TEAs have been consumed; drops in water table levels might quickly re-oxidize reduced forms of TEAs, reducing methanogenic activity [29,30]. For this reason, sediments of permanently flooded areas are considered as the most important environment contributing to CH₄ emissions in natural ecosystems. For the same reason, CH₄ fluxes scaled up to regional level for forest areas classified as “upland forests” or “unflooded forests” are computed as net CH₄ sinks, assuming that aerobic conditions and methanotrophic activity dominates the net soil–atmosphere CH₄ exchange [3,4]. To what extent this assumption can be considered valid over the whole area of tropical forests is not clear, as a number of studies have shown small to moderate, and rarely high, temporal hotspots of CH₄ emissions from unsaturated tropical soils. A mechanism similar to N₂O production in aerobic soils at unsaturated conditions [31] has been hypothesised to occur in anoxic microsites of CH₄ production [7–15]. The geomorphology of many tropical forested areas presents a very variable landscape, made by a mosaic of hills and downhill areas, where, during the period of most intense rainfall, a transient strong accumulation of water in the soil system occurs, leading to soil profile saturation and short-lived (hours to a few days) waterlogged areas. While the latter might be highly ephemeral in the dry season, disappearing within few days, during the wet season the continuous and intense rainfall might saturate a significant part of the soil profile in the downhill areas, making the water table quite superficial. This condition of saturation of the sub-surface soil layers might co-occur with significant amounts of carbon, which typically characterizes the soil profile of the tropical soil, thanks to the continuous organic inputs of the highly productive tropical forests. In the studied downhill area of the Ankasa Park, the analysis of the organic C distribution along the soil profile [17] showed a quite uniform C concentration down to 30 cm depth, varying from 2.77 g C kg⁻¹ soil at 0–5 cm depth to 2.32 g C kg⁻¹ soil at 15–30 cm. Between 30–50 cm depth, the C concentration of soil decreased but it was still around 1.1 g C kg⁻¹. The combination of good availability of electron donors, soil saturation, and high rates of soil respiration typical of tropical forests [17,32], might create a favorable environment for the complex sequence of microbial syntrophic interactions between fermentative bacteria and methanogenic archaea, which leads to significant rates of CH₄ production, as observed in wetland ecosystems [27,28]. Isotopic studies have demonstrated that the fraction of labile C transferred through methanogenesis is a better predictor of net CH₄ fluxes than the simple plant productivity rates [14,33]. Moreover, 14 C-labeling studies suggested that, in soil, carbon produced as root exudates might act as a direct substrate for CH₄ production [34,35], or indirectly influence CH₄ production by stimulating the decomposition of recalcitrant soil organic matter [36]. Topographic differences in CH₄ fluxes, linked to local differences in hydrologic and redox conditions at different landscape positions, have been reported in previous studies [11,37–39]. Transitional states, where soil shifts from being a CH₄ sink to a CH₄ source, in function of rainfall and topography, could be very rapid, thus requiring a quite complex model to incorporate an explicit simulation of transient water content increase, inhibition of oxygen diffusion, and switch of CH₄ production [2,39].

Our observations showed that in the top-hill area, there was indeed a moderate number of cases when the soil acted as a net CH₄ source, and these cases occurred mostly when the WFPS was above 40% in accordance with previous observations [7–15,40]. Considering that the top-hill soil was well drained and at a height of about 20 m above the downhill level, it might be assumed that the observed small net CH₄ emissions might be generated in anaerobic hotspots of microbial activity, within an aerobic soil, as previously reported for other upland soils in tropical areas during the wet season. These hotspots, even if not sufficient to transform the upland areas of Ankasa into a net CH₄ source,

might contribute to reducing the strength of the net CH₄ sink over the year. This, together with the strong and transient net CH₄ emissions observed in the downhill area in the wettest months, might result in the Anakasa forest soil being a net CH₄ source over a whole year cycle.

CH₄ fluxes in the order of 100 mg CH₄ m⁻² d⁻¹, and above, were observed in the downhill area when the topsoil was not saturated (WFPS just above 60%), and when WFPS was only between 20% and 40% a significant number of fluxes were still between 1 and 10 mg CH₄ m⁻² d⁻¹. These observations, and the correlation between CH₄ positive fluxes and rainfall intensity in the 10 days preceding the gas flux measurements, might suggest a sub-surface CH₄ source. Among ephemeral flooded areas, studies in rice paddies showed that several days of flooded conditions were needed for the onset of methanogenesis to take place, i.e., when available oxygen and TEAs are reduced, usually at a redox potential more negative than about 150 to 200 mV [41,42]. The transient nature of the water table was also shown by the small ponds, which after reaching their maximum size drained quite quickly after the rain stopped, accompanied by a rapid decline in the CH₄ flux emissions and water CH₄ concentration.

Overall, on the basis of the data collected in the sampling campaigns, the top-hill area was a weak sink of about -1.5 kg CH₄ ha⁻¹ yr⁻¹ (daily average -0.42 mg CH₄ m⁻² day⁻¹). The downhill area would provide about 23.5 kg CH₄ ha⁻¹ (on average 12.8 mg CH₄ m⁻² d⁻¹) during the period with the most intense rainfall, between June and November. The analysis of CH₄ fluxes measured during the campaign in May indicated a daily average of -0.42 mg CH₄ m⁻² day⁻¹, hence in line with fluxes in the drained upland site. Assuming, therefore, that during the drier season the downhill area would have a net CH₄ exchange similar to the upland, the net annual budget of the downhill area would be around 22.7 kg CH₄ ha⁻¹ yr⁻¹. Over the year, the Anakasa forest would be a mosaic of CH₄ sources and sinks. We hence estimated the overall soil CH₄ flux in the whole Park surface, considering the surface covered by the bottom hill areas and the observed temporal dynamics of fluxes during the year. The flux obtained was 3.3 kg CH₄ ha⁻¹ yr⁻¹, i.e., the forest acted as a net source of CH₄. This source might even be underestimated. In fact, in the ephemeral ponds of downhill areas, bubbling might also contribute to CH₄ emissions into the atmosphere, but our diffusive approach could not capture this source. Another source of CH₄ might be represented by diffusion through plant tissues. Scientists have discovered the significant role of tropical trees as preferential escape routes for CH₄ trapped in waterlogged soils of the Amazon basin [43]. They found that many tree species were provided with lenticels for gas exchanges, similar to those present in rice plants. We have cross-checked the list of species reported by Pangala and others [43] with the species reported in the study area, as part of a parallel study on biodiversity (data not shown) but no more than a couple of genera have been found in common. This does not exclude the fact that some of the African tree species, having roots frequently exposed to soil saturation, might present specific gas exchange routes, but the issue will need further investigation. If this "tree route" of CH₄ degassing would also occur in our study site, in particular in the downhill areas, CH₄ emissions from the forest might be even higher than calculated on the sole basis of soil fluxes.

5. Conclusions

Studies of CH₄ fluxes in tropical forests combining observations in plateaus and valleys areas, using different techniques, have estimated that the forest was a net CH₄ source. The reported average daily source strength varied from 2 to 20 mg CH₄ m⁻² d⁻¹ depending on the study site and time of the year, with the wetter sites showing the highest fluxes [44–46]. The general consensus was that tropical "unflooded" forests might be an underestimated CH₄ source. We confirmed in this study that considering both the hotspots of CH₄ emissions in the upland areas which significantly reduce the annual upland soil CH₄ sink, and the strong emissions measured during wettest periods in downhill areas, the Anakasa forest could be considered a net CH₄ source. Such a CH₄ source is much less strong than what was measured or modeled for wetlands ecosystems and flooded forested areas [2], but considering the vast area covered by tropical forests receiving a significant amount of rainfall

during the wet season and throughout the year in equatorial regions, the underestimated CH₄ source of such ecosystems might deserve a more in-depth analysis in the global CH₄ budget.

Author Contributions: S.C. and R.V. conceived the research and designed the study, T.B. and G.N. contributed to collecting and analyzing data, C.S. wrote the paper with feedback from all authors. All authors have read and agreed to the published version of the manuscript.

Funding: Research supported by the EU project CarboAfrica” (GOCE, 037132) and the EU ERC grant GHG Africa no. 247349. RV supported by the Russian Scientific Foundation Project # 19-77-300-12. SC supported by “RUDN University program 5–100”.

Acknowledgments: We thank JJ Mensah for support in field sampling, Sergio Noce (CMCC) for providing DEM data of the site.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

References

1. Myhre, G.; Shindell, D.; Bréon, F.M.; Collins, W.; Fuglestedt, J.; Huang, J.; Koch, D.; Lamarque, J.F.; Lee, D.; Mendoza, B.; et al. Anthropogenic and Natural Radiative Forcing. In *Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*; Stocker, T.F., Qin, D., Plattner, G.K., Tignor, M., Allen, S.K., Boschung, J., Nauels, A., Xia, Y., Bex, V., Midgley, P.M., Eds.; Cambridge University Press: Cambridge, UK; New York, NY, USA, 2013; pp. 659–740. [[CrossRef](#)]
2. Saunio, M.; Stavert, A.R.; Poulter, B.; Bousquet, P.; Canadell, J.G.; Jackson, R.B.; Raymond, P.A.; Dlugokencky, E.J.; Houweling, S.; Patra, P.K.; et al. The Global Methane Budget 2000–2017. *Earth Syst. Sci. Data* **2020**, *12*, 1561–1623. [[CrossRef](#)]
3. Kirschke, S.; Bousquet, P.; Ciais, P.; Saunio, M.; Canadell, J.G.; Dlugokencky, E.; Bergamaschi, P.; Bergmann, D.; Blake, D.R.; Bruhwiler, L.M.P.; et al. Three decades of global methane sources and sinks. *Nat. Geosci.* **2013**, *6*, 813–823. [[CrossRef](#)]
4. Saunio, M.; Bousquet, P.; Poulter, B.; Peregon, A.; Ciais, P.; Canadell, J.G.; Dlugokencky, E.; Etiope, G.; Bastviken, D.; Houweling, S.; et al. Variability and quasi-decadal changes in the methane budget over the period 2000–2012. *Atmos. Chem. Phys.* **2017**, *17*, 11135–11161. [[CrossRef](#)]
5. Melton, J.R.; Wania, R.; Hodson, E.L.; Poulter, B.; Ringeval, B.; Spahni, R.; Bohn, T.; Avis, C.A.; Beerling, D.J.; Chen, G.; et al. Present state of global wetland extent and wetland methane modelling: Conclusions from a model inter-comparison project (WETCHIMP). *Biogeosciences* **2013**, *10*, 753–788. [[CrossRef](#)]
6. Prigent, C.; Papa, F.; Aires, F.; Rossow, W.B.; Matthews, E. Global inundation dynamics inferred from multiple satellite observations, 1993–2000. *J. Geophys. Res. Atmos.* **2007**, *112*, 112. [[CrossRef](#)]
7. Castaldi, S.; Ermice, A.; Strumia, S. Fluxes of N₂O and CH₄ from soils of savannas and seasonally-dry ecosystems. *J. Biogeogr.* **2006**, *33*, 401–415. [[CrossRef](#)]
8. Castaldi, S.; De Grandcourt, A.; Rasile, A.; Skiba, U.; Valentini, R. CO₂, CH₄ and N₂O fluxes from soil of a burned grassland in Central Africa. *Biogeosciences* **2010**, *7*, 3459–3471. [[CrossRef](#)]
9. Keller, M.; Veldkamp, E.; Weitz, A.; Reiners, W.A. Effect of pasture age on soil trace-gas emissions from a deforested area of Costa Rica. *Nature* **1993**, *365*, 244–246. [[CrossRef](#)]
10. Reiners, W.; Keller, M.; Gerow, K.G. Estimating Rainy Season Nitrous Oxide and Methane Fluxes Across Forest and Pasture Landscapes in Costa Rica. *Water Air Soil Pollut.* **1998**, *105*, 117–130. [[CrossRef](#)]
11. Silver, W.L.; Lugo, A.; Keller, M. Soil oxygen availability and biogeochemistry along rainfall and topographic gradients in upland wet tropical forest soils. *Biogeochemistry* **1999**, *44*, 301–328. [[CrossRef](#)]
12. Teh, Y.A.; Diem, T.; Jones, S.P.; Quispe, L.P.H.; Baggs, E.; Morley, N.; Richards, M.; Smith, P.; Meir, P. Methane and nitrous oxide fluxes across an elevation gradient in the tropical Peruvian Andes. *Biogeosciences* **2014**, *11*, 2325–2339. [[CrossRef](#)]
13. Von Fischer, J.C.; Hedin, L.O. Separating methane production and consumption with a field-based isotope pool dilution technique. *Glob. Biogeochem. Cycles* **2002**, *16*, 8–1–8–13. [[CrossRef](#)]
14. Von Fischer, J.C.; Hedin, L.O. Controls on soil methane fluxes: Tests of biophysical mechanisms using stable isotope tracers. *Glob. Biogeochem. Cycles* **2007**, *21*, 2007. [[CrossRef](#)]

15. Verchot, L.V.; Davidson, E.A.; Cattânio, J.H.; Ackerman, I.L. Land-Use Change and Biogeochemical Controls of Methane Fluxes in Soils of Eastern Amazonia. *Ecosystems* **2000**, *3*, 41–56. [[CrossRef](#)]
16. Teh, Y.A.; Silver, W.L.; Conrad, M.E. Oxygen effects on methane production and oxidation in humid tropical forest soils. *Glob. Chang. Biol.* **2005**, *11*, 1283–1297. [[CrossRef](#)]
17. Simona, C.; Bertolini, T.; Valente, A.; Chiti, T.; Valentini, R. Nitrous oxide emissions from soil of an African rain forest in Ghana. *Biogeosciences* **2013**, *10*, 4179–4187. [[CrossRef](#)]
18. Fattore, F.; Bertolini, T.; Materia, S.; Gualdi, S.; M'Bou, A.T.; Nicolini, G.; Valentini, R.; De Grandcourt, A.; Tedesco, D.; Castaldi, S. Seasonal trends of dry and bulk concentration of nitrogen compounds over a rain forest in Ghana. *Biogeosciences* **2014**, *11*, 3067–3081. [[CrossRef](#)]
19. Ahn, P.M. *Soils of the Lower Tano Basin, South-Western Ghana*; Ministry of Food and Agriculture, Scientific Services Division, Soil and Land-use Survey Branch: Kumasi, Ghana, 1961.
20. Chiti, T.; Certini, G.; Grieco, E.; Valentini, R. The role of soil in storing carbon in tropical rainforests: The case of Ankasa Park, Ghana. *Plant Soil* **2010**, *331*, 453–461. [[CrossRef](#)]
21. Keller, M.; Weitz, A.M.; Bryan, B.; Rivera, M.M.; Silver, W.L. Soil-atmosphere nitrogen oxide fluxes: Effects of roots disturbance. *J. Geophys. Res.* **2000**, *105*, 17693–17698. [[CrossRef](#)]
22. McAullife, C. Gas Chromatographic determination of solutes by multiple phase equilibration. *Chem. Technol.* **1971**, *1*, 46–51.
23. Sanders, R. Compilation of Henry's Law Constants for Inorganic and Organic Species of Potential Importance in Environmental Chemistry. 1999. Available online: <https://www.ft.unicamp.br/~{}mariaacm/ST405/Lei%20de%20Henry.pdf> (accessed on 10 October 2015).
24. Vachon, D.; Prairie, Y.T. The ecosystem size and shape dependence of gas transfer velocity versus wind speed relationships in lakes. *Can. J. Fish. Aquat. Sci.* **2013**, *70*, 1757–1764. [[CrossRef](#)]
25. Repo, M.E.; Huttunen, J.T.; Naumov, A.V.; Chichulin, A.V.; Lapshina, E.D.; Bleuten, W.; Martikainen, P.J. Release of CO₂ and CH₄ from small wetland lakes in western Siberia. *Tellus B Chem. Phys. Meteorol.* **2007**, *59*, 788–796. [[CrossRef](#)]
26. Bergamaschi, P.; Frankenberg, C.; Meirink, J.F.; Krol, M.; Villani, M.G.; Houweling, S.; Dentener, F.; Dlugokencky, E.J.; Miller, J.B.; Gatti, L.V.; et al. Inverse modeling of global and regional CH₄ emissions using SCIAMACHY satellite retrievals. *J. Geophys. Res. Space Phys.* **2009**, *114*. [[CrossRef](#)]
27. Magonig, J.P.; Hines, M.E.; Visscher, P.T. *Anaerobic Metabolism: Linkages to Trace Gases and Aerobic Processes*; Schlesinger, W.H., Ed.; Elsevier-Pergamon: Oxford, UK, 2004; pp. 317–424.
28. Bridgman, S.D.; Cadillo-Quiroz, H.; Keller, J.K.; Zhuang, Q. Methane emissions from wetlands: Biogeochemical, microbial, and modeling perspectives from local to global scales. *Glob. Chang. Biol.* **2013**, *19*, 1325–1346. [[CrossRef](#)]
29. Deppe, M.; Knorr, K.-H.; McKnight, D.M.; Blodau, C. Effects of short-term drying and irrigation on CO₂ and CH₄ production and emission from mesocosms of a northern bog and an alpine fen. *Biogeochemistry* **2010**, *100*, 89–103. [[CrossRef](#)]
30. Deppe, M.; McKnight, D.M.; Blodau, C. Effects of Short-Term Drying and Irrigation on Electron Flow in Mesocosms of a Northern Bog and an Alpine Fen. *Environ. Sci. Technol.* **2010**, *44*, 80–86. [[CrossRef](#)]
31. Smith, K.A. Anaerobic Zones and Denitrification in Soil: Modelling and Measurement. In *Denitrification in Soil and Sediment*; Revsboech, N.P., Sørensen, J., Eds.; Plenum Press: New York, NY, USA, 1990; pp. 229–244.
32. Malhi, Y.; Baldocchi, D.D.; Jarvis, P.G. The carbon balance of tropical, temperate and boreal forests. *Plant Cell Environ.* **1999**, *22*, 715–740. [[CrossRef](#)]
33. Yang, W.H.; McNicol, G.; Teh, Y.A.; Estera-Molina, K.; Wood, T.E.; Silver, W.L. Evaluating the Classical Versus an Emerging Conceptual Model of Peatland Methane Dynamics. *Glob. Biogeochem. Cycles* **2017**, *31*, 1435–1453. [[CrossRef](#)]
34. Magonigal, J.P.; Whalen, S.C.; Tissue, D.T.; Bovard, B.D.; Allen, A.S.; Albert, D.B. A Plant-Soil-Atmosphere Microcosm for Tracing Radiocarbon from Photosynthesis through Methanogenesis. *Soil Sci. Soc. Am. J.* **1999**, *63*, 665–671. [[CrossRef](#)]
35. King, J.Y.; Reeburgh, W.S.; Thieler, K.K.; Kling, G.W.; Loya, W.M.; Johnson, L.C.; Nadelhoffer, K.J. Pulse-labeling studies of carbon cycling in Arctic tundra ecosystems: The contribution of photosynthates to methane emission. *Glob. Biogeochem. Cycles* **2002**, *16*, 10–11. [[CrossRef](#)]
36. Guenet, B.; Danger, M.; Abbadie, L.; Lacroix, G. Priming effect: Bridging the gap between terrestrial and aquatic ecology. *Ecology* **2010**, *91*, 2850–2861. [[CrossRef](#)] [[PubMed](#)]

37. Hedin, L.O.; Von Fischer, J.C.; Ostrom, N.E.; Kennedy, B.P.; Brown, M.G.; Robertson, G.P. Thermodynamic Constraints on Nitrogen Transformations and Other Biogeochemical Processes at Soil-Stream Interfaces. *Ecology* **1998**, *79*, 684–703. [[CrossRef](#)]
38. Belyea, L.R.; Baird, A.J. Beyond “The limits to peat bog growth”: Cross-scale feedback in peatland development. *Ecol. Monogr.* **2006**, *76*, 299–322. [[CrossRef](#)]
39. Lohila, A.; Aalto, T.; Aurela, M.; Hatakka, J.; Tuovinen, J.-P.; Kilkki, J.; Penttilä, T.; Vuorenmaa, J.; Hänninen, P.; Sutinen, R.; et al. Large contribution of boreal upland forest soils to a catchment-scale CH₄ balance in a wet year. *Geophys. Res. Lett.* **2016**, *43*, 2946–2953. [[CrossRef](#)]
40. Simona, C.; Ariangelo, D.P.R.; John, G.; Nina, N.; Ruben, M.; José, S.J. Nitrous oxide and methane fluxes from soils of the Orinoco savanna under different land uses. *Glob. Chang. Biol.* **2004**, *10*, 1947–1960. [[CrossRef](#)]
41. Lindau, C.W.; Patrick, W.H., Jr.; DeLaune, R.D. Factors affecting methane production in flooded rice soils. In *Agricultural Ecosystem Effects on Trace Gases and Global Climate Change*; Karl, D.M., Ed.; American Society for Agronomy: Madison, WI, USA, 1993; pp. 157–165.
42. Yagi, K.; Minami, K. *Spatial and Temporal Variations of Methane Flux from a Rice Paddy Field*; Oremland, R.S., Ed.; Biogeochemistry of Global Change; Chapman and Hall: New York, NY, USA, 1993; pp. 353–368.
43. Pangala, S.R.; Enrich-Prast, A.; Basso, L.S.; Peixoto, R.B.; Bastviken, D.; Hornibrook, E.R.C.; Gatti, L.V.; Marotta, H.; Calazans, L.S.B.; Sakuragui, C.M.; et al. Large emissions from floodplain trees close the Amazon methane budget. *Nature* **2017**, *552*, 230–234. [[CrossRef](#)] [[PubMed](#)]
44. Carmo, J.B.D.; Keller, M.; Dias, J.D.; De Camargo, P.B.; Crill, P. A source of methane from upland forests in the Brazilian Amazon. *Geophys. Res. Lett.* **2006**, *33*. [[CrossRef](#)]
45. Miller, J.B.; Gatti, L.V.; D’Amelio, M.T.S.; Crotwell, A.M.; Dlugokencky, E.J.; Bakwin, P.; Artaxo, P.; Tans, P.P. Airborne measurements indicate large methane emissions from the eastern Amazon basin. *Geophys. Res. Lett.* **2007**, *34*, 1–5. [[CrossRef](#)]
46. Querino, C.A.S.; Smeets, C.J.P.P.; Vigano, I.; Holzinger, R.; Moura, V.; Gatti, L.; Martinewski, A.; Manzi, A.O.; De Araújo, A.C.; Röckmann, T. Methane flux, vertical gradient and mixing ratio measurements in a tropical forest. *Atmos. Chem. Phys.* **2011**, *11*, 7943–7953. [[CrossRef](#)]

Publisher’s Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



© 2020 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<http://creativecommons.org/licenses/by/4.0/>).