Contribution of plant-induced pressurized flow to CH₄ emission from a Phragmites fen

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The widespread wetland species Phragmites australis (Cav.) Trin. ex Steud. has the ability to transport gases through its stems via a pressurized flow. This results in a high oxygen (O₂) transport to the rhizosphere, suppressing methane (CH₄) production and stimulating CH₄ oxidation. Simultaneously CH₄ is transported in the opposite direction to the atmosphere, bypassing the oxic surface layer. This raises the question how this plant-mediated gas transport in Phragmites affects the net CH₄ emission. A field experiment was set-up in a Phragmites-dominated fen in Germany, to determine the contribution of all three gas transport pathways (plant-mediated, diffusive and ebullition) during the growth stage of Phragmites from intact vegetation (control), from clipped stems (CR) to exclude the pressurized flow, and from clipped and sealed stems (CSR) to exclude any plant-transport. Clipping resulted in a 60% reduced diffusive + plant-mediated flux (control: 517, CR: 217, CSR: 279 mg CH₄ m⁻² day⁻¹). Simultaneously, ebullition strongly increased by a factor of 7–13 (control: 10, CR: 71, CSR: 126 mg CH₄ m⁻² day⁻¹). This increase of ebullition did, however, not compensate for the exclusion of pressurized flow. Total CH₄ emission from the control was 2.3 and 1.3 times higher than from CR and CSR respectively, demonstrating the significant role of pressurized gas transport in Phragmites-stands.

Methane (CH₄) produced in soils is to a great extent oxidized to CO₂ before it reaches the atmosphere. The proportion of CH₄ oxidation depends on water table height and the presence or absence of vascular wetland plants. Both factors influence the concentration of oxygen (O₂) in the soil and the pathways by which CH₄ is transported from the soil to the atmosphere. There are three gas transport pathways: diffusion and ebullition from the soil, and plant-mediated transport via aerenchyma of roots and stems of vascular plants.

Diffusion is a relatively slow process, whereby a large part of the produced CH₄ is oxidized when crossing the oxic upper layer of the water column or soil. Ebullition occurs when gas builds up in a submerged soil and bubbles are formed. When bubbles are erupted episodically, the bubbles can rapidly pass through the water-saturated soil and water column above. Because this transport is fast, only a small part of the CH₄ will be oxidized. In general, ebullition is affected by temperature, air pressure and water table height (influencing the pressure in the water column); however, it shows a high spatial and temporal variation and is hard to predict. The contribution of ebullition to the overall CH₄ flux ranges from a few percent to more than 50%.

CH₄ transport mediated by wetland plants occurs through aerenchyma, which has the physiological function to transport O₂ into the roots. Often, more O₂ is transported to the roots than is consumed, leading to O₂ leakage into the rhizosphere. Besides O₂ transport into the soil, other gases (e.g. CO₂ and CH₄) can simultaneously be transported from the soil to the atmosphere. This gas transport via aerenchyma tissue can occur via a diffusion gradient or by a pressure gradient that is built up by the plants. Plants that transport gases via diffusion are, among others, Carex rostrata, Oryza sativa, Scirpus lacustris and Peltandra virginica, whereas gas transport via convective through-flow due to pressure gradient is found in Typha latifolia, Typha angustifolia, Nymphaea odorata, Nuphar luteum, Nelumbo nucifera, Nymphoides peltata and Phragmites australis.

Gas transport in Phragmites australis (Cav.) Trin. ex Steud. (common reed) is highly effective. It builds up a humidity-induced pressure gradient within the leaf sheaths (the part of the leaf that encircles the stems) that...
leads to an airflow from the leaf sheaths towards the rhizomes, which is vented via old and broken stems\(^6,16,18,19\). This transport mechanism is more than five times faster than diffusion\(^1\) and is regulated by the pores (stomata) in the leaf sheaths. These stomata do not transport gas caused by pressure differences, but allow gas transport by diffusion. Due to higher humidity in the internal culm of *Phragmites*, \(\text{O}_2\) and \(\text{N}_2\) concentrations inside the plant are diluted. Therefore, \(\text{O}_2\) and \(\text{N}_2\) are transported along the concentration gradient from the atmosphere into the sheaths, increasing the internal pressure (see Fig. 1). Since this process depends on stomatal conductance, humidity induced convective flow starts after sunrise when the stomata open, reaches an optimum around noon when photosynthetic rates are highest and then decreases till sunset\(^3\). Therefore, this diurnal cycle is also observed in \(\text{CH}_4\) fluxes from *Phragmites* wetlands during the growing season\(^4,17,20,21\). On the one hand, *Phragmites* transports \(\text{O}_2\) into the soil, which leads to higher oxidation rates of \(\text{CH}_4\) and can also be expected to reduce methanogenesis. On the other hand, transport of \(\text{CH}_4\) from the soil to the atmosphere through the plant is facilitated, bypassing the oxic soil and water layer. Grünfeld & Brix\(^4\) showed a 34% decrease in \(\text{CH}_4\) emission after addition of *Phragmites* to a submerged organic soil. In contrast, Hendriks et al.\(^5\) found that vascular plant presence (among others *Phragmites* and *Thypha latifolia*) leads to higher methane emissions, but depends on the water table height.

Since the findings in literature are ambiguous, the following questions remain: (1) how important is plant-mediated gas transport in *Phragmites* compared to the other \(\text{CH}_4\) transport mechanisms (diffusion and ebullition from the soil); (2) how does *Phragmites* influence diffusive and ebullition fluxes; and (3) does the presence of *Phragmites* stands lead to an overall increase or decrease of \(\text{CH}_4\) emission? To study this, a field experiment within a measuring period of three weeks during the growing season was set-up in a large reed area of a minerotrophic peatland. To quantify the importance of plant-mediated \(\text{CH}_4\) transport, we compared fluxes measured with chambers from control *Phragmites* plots with plots where *Phragmites* stems were clipped to exclude pressurized gas transport through the plant. To exclude any gas transport through plants, we also measured \(\text{CH}_4\) fluxes from plots where *Phragmites* stems were clipped and sealed. Ebulition from the soil was determined as well, to assess the relative contribution of all gas transport pathways of \(\text{CH}_4\) from a reed fen.

In addition to the experiment, we were interested to see if the chamber fluxes from the control plots were representative for the total system. Therefore, we made a comparison between \(\text{CH}_4\) fluxes measured with the chamber method and with the eddy covariance method.

**Materials and methods**

**Study site.** The study was conducted in the Federseemoor (48.092°N, 9.636°E), a peatland of 30 km\(^2\) located in the region Upper Swabia in southwest Germany. This peatland has developed via natural terrestrialization from a proglacial lake after the last ice age. As a result, the surface area of the lake declined from 30 to 12 km\(^2\). Between 1787 and 1808, the lake was further reduced to a size of 1.4 km\(^2\) by drainage activities. The newly gained land of 11 km\(^2\) was used as pasture but turned out to be unprofitable due to the recurring high water table. Nowadays it is a nature conservation area, mainly consisting of fen (see van den Berg et al.\(^21\) for a vegetation map).
The lake Federsee is completely surrounded by reed vegetation (*P. australis*), with a total area of 2.2 km² and a density of around 70 living shoots and 75 dead stems per m². During the measurement period (7–10 June) the *Phragmites* plants were 1.2 m high. This is half their maximum height, which is reached at the end of July. The high density of *Phragmites* and lack of other species in the reed belt result from high nutrient concentrations due to wastewater input to the lake since 1951. After 1982, the input of untreated sewage water was stopped, which reduced the nutrient concentrations. Only since 2006 has there been a significant improvement in water quality, and after 2008 the lake water became clear again. The field experiment was installed in the middle of the reed area at around 70 m distance from an eddy covariance (EC) tower, which has been running since March 2013. In a radius of at least 200 m around the EC tower, the vegetation is dominated by *Phragmites* (see van den Berg et al.), meaning only reed dominated the measured EC footprint.

**Field experiment.** Nine plots of 2 m × 2 m were prepared for three treatments with three replicates: (1) clipped reed (CR), to exclude the pressurized flow in the plants; (2) clipped and sealed reed (CSR), to exclude any exchange via plant stems; and (3) control where reed was not manipulated. In the CR and CSR treatments, living and dead reed stems were clipped to about 10 cm above the water table. In the CSR treatment the clipped reed stems was sealed with an acrylic sealant. Since rhizomes connect plants over longer distances, plots were isolated by cutting rhizomes from the reed plants around each plot to a depth of 50 cm, to avoid gas exchange with the surrounding area. The period between preparation of the plots and measurements was minimized (1–2 days) to reduce possible side effects, such as change in substrate availability for methanogens. One day before the first measurement, the water table rose about 20 cm in the whole field, flooding the prepared sealed stems of one plot already prepared for the CSR treatment. Nevertheless, since no gas exchange is expected from the sealed stems, this plot was still included in the experiment. CH₄ and CO₂ diffusive fluxes from the soil and plant-mediated fluxes were measured with transparent flow through chambers. Pore water was extracted to analyze the effect of the reduced/excluded gas exchange by the plants on soil chemistry. In each plot ebullition was measured as well (see below).

**Diffusive and plant mediated CH₄ flux.** On 7, 9 and 10 June 2016 between 07:00 and 18:00, the gas fluxes of each treatment were alternately measured. Per day, only one of the triplicates per treatment was measured. CH₄ fluxes were measured in the middle of the plots with transparent chambers with a diameter of 50 cm. One chamber was 2 m high and was on the control plots. Two chambers were 1 m high and used on the CR and CSR plots. The 1-m chambers were equipped with a small fan of 8 cm × 8 cm that had a flow capacity of 850 l min⁻¹; two fans were installed in the 2-m chamber. Each day one replicate of every treatment was measured, to be able to capture the diurnal cycle for each plot and to minimize disturbance by translocating the chambers. The chambers were connected with 8 m tubing to a multiport inlet unit attached to a fast greenhouse gas analyzer (GGA) with off-axis integrated cavity output spectroscopy (GGA-24EP, Los Gatos Research, USA) measuring the concentration of CH₄ and CO₂ every second. Every 5 min, the multiport switched between the three chambers, allowing air from each chamber to be alternately pumped through the GGA with a pumping rate of 300 ml min⁻¹ and resulting in four flux measurements per plot per hour (~35 measurements per plot per day). The withdrawn air from the chamber was replaced with ambient air through an opening in the chamber. After 1–2 h of continuous measurements, the chambers were ventilated by lifting the chambers to fully replace inside air with ambient air. After 15 min, the chamber was put back and measurements continued. Since it takes a long time before the chamber CH₄ gets to equilibrium with the water column, 1–2 h of increasing CH₄ concentration in the chamber will have little effect on the measurement accuracy of the CH₄ flux (in contrary to the CO₂ flux). Nevertheless, we used only data from the first 30 min after ventilating to calculate the diffusive flux (five measurements per plot per day), since this is the period where temperature and humidity inside the chamber resemble outside conditions most closely. Only for the comparison between eddy covariance fluxes and chamber fluxes on the control plots we did use data from the whole measurement period.

The concentration for every measurement point was corrected for the change in concentration caused by the inflow of ambient air with known CO₂ and CH₄ concentrations (measured by the EC station) and outflow of chamber air (both with a flow rate of the pump speed of the Los Gatos). The slope of the corrected chamber concentrations over a 4 min period within the 5 min measurement was used to calculate the flux and was checked for non-linear fluctuations due to e.g. ebullition. Fluxes corresponding to an average chamber concentration of >100 ppm CH₄ were discarded, because of the GGA’s detection limit. In total 11% of the fluxes were discarded.

**Ebullition.** In each plot ebullition was measured by catching bubbles from a fixed surface with an ebullition trap, composed of a 20 cm diameter funnel, to which a glass bottle of 300 ml was attached. The bottles were filled with water from the site and the ebullition trap was installed under the water table on 8 June and carefully anchored between reed stems (no open endings of stems were below the trap) on the soil surface around 0.55 m below the water surface. Bubbles were captured in the glass bottle for 18 days, after which the bottles were removed and gas samples were taken in the field. The total volume of ebullition gas was determined and the concentration of CH₄, CO₂ and N₂O were measured by gas chromatography (7890B GC, Agilent Technologies, USA) in the lab.

**Environmental variables.** In each chamber, temperature and radiation were measured with a temperature/light sensor (HMP155, Vaisala Inc., Finland) logging at an interval of 30 s. Every minute soil temperature was measured in each plot in the upper 0–0.05 m with a Soil Water Content Reflectometer (CS655, Campbell Scientific Inc., USA) around 0.56 m below the water table. Air temperature, air relative humidity (HMP155, Vaisala Inc., Finland) and incoming and outgoing shortwave and longwave radia-
tion (CNR4, Kipp & Zonen Inc., The Netherlands) were measured at a height of 6 m close to or at the EC station. Groundwater table was continuously measured with a water level pressure sensor (Mini-Diver datalogger, Eijkelkamp Agrisearch Equipment Inc., The Netherlands) placed at 1.45 m depth in a 2-m long filter pipe that was placed 1.60 m into the soil. Data were recorded at a 30 min interval.

**Pore water sampling and analysis.** To see if the treatments had any effect on the methane production, pore water samples were analyzed. At two locations in each plot, pore water was extracted anaerobically (Eijkelkamp Agrisearch Equipment Inc., The Netherlands). Pore water from 10, 20, 30 and 50 cm depth was collected by vacuum suction in syringes and transported to the lab. In the lab, pore water was diluted with a ratio of 1:3. Dissolved organic carbon (DOC) concentration was measured with a Dimatoc 100 DOC/TN-analyzer (Dimatec, Germany). A second pore water sample was taken in vacuumed 13 ml exetainers with 3 g of NaCl. The concentration of CH4 in the headspace of these exetainers, representing the CH4 concentration in pore water, was determined on a HP gas chromatograph (Hewlett Packard, USA). A third pore water sample was fixed with 0.2% 2.2-bipyridin in 10% CH3COOH buffer in the field to determine Fe(II) measuring photo-metrical absorption at 546 nm in the lab.

**Eddy covariance.** The EC tower was located at a distance of around 70 m from the prepared plots. The tower was 6 m high and consisted of a LI-7700 open path CH4 gas analyser (LI-COR Inc., USA), a LI-7200 enclosed path CO2/H2O gas analyser (LI-COR Inc., USA) and a WindMaster Pro sonic anemometer (GILL Instruments Limited Inc., UK). Molar mixing ratio/mass density of the gases and wind speed in three directions were measured at a frequency of 10 Hz. Fluxes were calculated for an averaging interval of 15 min with the software EddyPro version 6.1.0. For more detailed information about the set up and calculations of the fluxes, see van den Berg et al.21.

**δ13C measurements.** CH4 oxidation and transport lead to isotopic fractionation of δ13C of CH4.23 The difference between δ13C of the CH4 present in the soil and the CH4 emitted to the atmosphere may therefore reveal the importance of both methane oxidation and the different emission pathways.

The δ13C of CH4 tends to be much lower than the natural abundance in organic compounds, because methanotrophic prokaryotes prefer the lighter 12CH4 to 13CH4 thereby increasing the δ13C of CH4. Diffusion rates for 12CH4 are higher than for 13CH4 decreasing the δ13C of the emitted CH4.23 Although 13C enrichment (compared to produced CH4) has been found in internal spaces of plants due to CH4 oxidation14, the fractionation at the plant-atmosphere surface reduces the δ13C by about 12–18‰ due to the faster transport rate of 12CH4, which makes that emitted CH4 can have a lower fraction of δ13C than the produced CH4. Differences in δ13C between sediment and overall emission are larger for plants with diffusive internal gas transport than for plants with convective gas transport23.

Since fractionation of CH4 emitted through ebullition in shallow waters is negligible, these gas bubbles can be used to know the isotopic composition of CH4 produced in sediment23. We therefore compared the δ13CH4 signature of ebullition gas with the signatures of CH4 from the chambers. Gas samples from the chamber were taken when the CH4 concentration was at least 10 times the ambient concentration, from each plot in the afternoon. The δ13CH4 signature was measured with an isotope-ratio mass spectrometer Delta plus XP (Thermo Finnigan, Germany).

**Statistics.** Chamber fluxes were measured at different times of the day, which means that environmental variables like temperature and radiation were varying. To be able to compare the different treatments without the variation resulting from environmental conditions, an analysis of covariance (ANCOVA) was conducted with the environmental variables as covariables. For the analysis, the data of the different measurement days were pooled together per treatment. The residuals of the model were normally distributed. With the parameters of the ANCOVA model, average fluxes were calculated with average environmental variables for the period ebullition was measured (8–27 June), to be able to compare the chamber fluxes with ebullition.

To test if the means of the ebullition measurements or pore water concentrations were different between the treatments, an analysis of variance (ANOVA) test was performed with Fishers Least Significant Difference (LSD) post hoc test to find the specific differences between the treatments.

**Results**

**Environmental conditions.** May and the first week of June were very wet with extreme rain events (up to 45 mm day). This caused the water table to rise to 55 cm above the surface, while normal fluctuations of the water table are between ~2 and 40 cm with an average of 8 cm above surface. During the measurement days rainfall was little to zero (see Table 1). Only on 9 June the weather was more cloudy with some rain in the morning. Ambient daily average temperature was close to the monthly average of 18.1 °C.

**Pore water.** Most roots are concentrated at 30 cm depth (personal observation). From this depth and lower, Fe is reduced to Fe(II) and CH4 production is enhanced and/or CH4 oxidation reduced, given the increase in CH4 concentration at this depth (see Fig. 2). Concentrations of elements in pore water show small (not significant) differences in CH4 and Fe(II) concentration between the treatments. A significant increase is found in DOC concentrations for the CSR treatment compared to the control (p<0.05) at 30 cm depth.
Comparison eddy covariance versus chamber fluxes. During the experiment, the CH$_4$ fluxes measured by the EC and the chamber method on the control plots show a similar data range and show the same diurnal pattern (Fig. 3). The later increase in CH$_4$ flux in both EC and chamber data on 9 June compared to the other days is most probably due to the low radiation by cloud cover and rain in the morning. From 9 June, there are no EC data available between 7:00 and 11:00 due to a rain event, which disturbed the functioning of the open-path CH$_4$ sensor. Chamber fluxes data that did not match the EC flux pattern well appeared to originate from the first measurement after ventilating the chamber (see Fig. 3). Therefore, all first measurements after ventilating were discarded in further analyses.

These results show that side effects of the chamber like temperature increase or high relative humidity did not affect the CH$_4$ flux (and thus gas transport through the plant) much, which makes the comparison between the control and other treatments reliable.

Diffusive and plant-mediated fluxes. All treatments show a diurnal cycle that correlates well with the inside air temperature of the chamber. However, the stems of the CSR plot measured on 7 June were flooded. This clearly affected the CH$_4$ flux and the relation with chamber air temperature (Fig. 4), compared to the other measurements in the CSR treatment. Flooding of the sealed stems resulted in a further decrease of the gas flux. Due to the different conditions, these data were excluded from further data analysis. The ANCOVA analysis gives a significant result for the interaction chamber temperature * treatment ($p<0.05$, F = 84.2), which means that temperature has a significantly different effect on CH$_4$ flux between the treatments. This interaction is used
in the model, with the results given in Table 2. The regression models for each treatment (the results of the ANCOVA analysis) are plotted together with the measured fluxes in Fig. 4.

All regression lines in the model are forced through an intercept of 5 °C, assuming that there is no significant microbial activity below this temperature. This was done by subtracting 5 degrees from the measured temperature and excluding an intercept in the model. With this model, including the three regressions, the F value is 84.2 \( (p < 0.001) \) and the effect size \( (\eta^2) \) 0.869. The control treatment showed the highest flux and CR treatment the lowest (Table 2). The slope of the control treatment is about twice as high as that of the other treatments \( (p < 0.001) \), but the slopes of CR and CSR are not significantly different from each other \( (p = 0.359) \).

**Ebulition.** Total volumes of trapped ebulition gas from the soil differed between the treatments and control: CR and CSR have a 2–3 times higher release of ebulition gas than the control (76 ml m\(^{-2}\) day\(^{-1}\)). The difference between CSR and control is significant \( (p < 0.05) \). For the CH\(_4\) volume both treatments are significantly different from the control \( (p < 0.05) \), but not significantly from each other. In both CR and CSR treatments, CH\(_4\) is clearly dominating ebulition: the percentage of CH\(_4\) in the ebulition gas was around 4 to 5 times higher in the CR (51%) and CSR (71%) treatments compared to the control (13%). Zero (CR and CSR plots) or very low amounts (0.6 ml m\(^{-2}\) day\(^{-1}\)) (control plots) of N\(_2\)O were found in the ebulition traps. Besides CH\(_4\) and CO\(_2\), ebulition gas could consist of nitrogen gas and water vapor, which were not measured.

**Relative contribution different flux pathways.** To compare the ebulition flux with the diffusive/plant mediated flux, we calculated the average daily chamber flux based on the relation of CH\(_4\) flux with temperature from the ANCOVA model (Table 2). The fluxes were calculated for a temperature of 14.7 °C, which is the daily average ambient temperature from 8 to 27 June (the period in which ebulition gas was captured), and resulted in an average flux of 517 (control), 217 (CR) and 279 mg m\(^{-2}\) day\(^{-1}\) (CSR). The fraction of ebulition from the total flux is 13 to 16 times higher in the CR and CSR treatments than in the control (Fig. 5).

**δ\(^{13}\)C change from soil CH\(_4\) to emitted CH\(_4\).** In all treatments the CH\(_4\) sampled in the chamber show lower δ\(^{13}\)C than CH\(_4\) from ebulition (Table 3). The largest depletion was found for CR, followed by CSR and control.

![Figure 4](https://example.com/figure4.png)

**Figure 4.** CH\(_4\) flux in relation to chamber temperature. (A) The treatment clipped and sealed reed (CSR), in black the measurements in the plot with the stems below water (7 June) and in grey the plots with stems above water (9 June, 10 June). (B) All treatments (clipped reed (CR), clipped and sealed reed (CSR) and control) excluding the measurements from CSR with stems below water. The linear regression models are all significant \( (p < 0.001) \).

| Treatment | N   | Mean | SD     | Beta interaction | Sig beta |
|-----------|-----|------|--------|------------------|----------|
| CR        | 17  | 300.5| 289.6  | 14.7             | 0.000    |
| CSR       | 9   | 370.7| 649.4  | 19.0             | 0.000    |
| Control   | 15  | 792.2| 508.7  | 35.1             | 0.000    |

**Table 2.** Descriptive statistics and slopes (beta) with significant levels for the different treatments in the ANCOVA model. The interaction is (chamber temperature − 5) * treatment. N is amount of data points.
Discussion

The total CH₄ flux (chamber + ebullition) decreased by 45% and 23% when we clipped the reed (CR) and clipped and sealed the reed (CSR), respectively, compared to intact reed (control) (see Fig. 5). The contribution of ebullition to the total flux increased much by clipping: 2% in the control plots compared to 24% in CR and 37% in CSR (see Fig. 5). van der Nat et al.¹⁷ found a lower CH₄ flux from water saturated bare soil compared to Phragmites-vegetated soil, with a difference of 75%. They also found that in bare soil (compared to Phragmites vegetated soil) ebullition was the main gas transport pathway, while in a Phragmites-vegetated soil more than 98% of the CH₄ was transported through the reed. Our findings, however, contradict the findings of Grünfeld & Brix⁴. They showed in an experiment a 34% lower CH₄ emission with Phragmites in a submerged organic soil compared to the same soil without Phragmites and argued that methanogenesis is reduced and CH₄ oxidation increased because of the transport of oxygen by Phragmites into the rhizosphere. In the soil without reed, the gas transport would be dominated by ebullition. The reason for the different findings of Grünfeld & Brix⁴ compared to our data, could be due to differences in experimental set up. Their experiment was conducted with single Phragmites seedlings (6 months old) and CH₄ flux was measured 9 weeks after planting. This means that there was no rhizome network present as in a developed reed bed. Because CH₄ is taken up by the rhizomes and transported upwards, the amount of CH₄ transport in Phragmites seedlings would be lower than at our site. Seemingly, the oxygen transport to the soil was not much limited by the undeveloped rhizome network of the seedlings. In our study CR and CSR reduced or excluded plant-mediated gas transport, but fluxes are not directly comparable to those from bare soil.

In CR, roots and clipped stems still allowed gas transport via diffusion, but not via pressurized flow as in the control plots. The clipped stems act as chimneys that connect deeper soil layers to the atmosphere. In line with this, Greenup et al.²⁵ found that CH₄ flux from Sphagnum vegetation increased after inserting glass tubes into the soil. CR only showed slightly lower chamber flux than CSR (217 vs 279 mg m⁻² day⁻¹) (see Fig. 5), indicating that sealing the stems hardly affected the chamber CH₄ flux (disregarding ebullition). Only the CSR plots with stems below water (see “Material and methods” section) showed a clear reduced CH₄ flux compared to plots with stems above water. This suggests that our sealing method was not fully effective. Possibly gas could still escape from, for example, cracks in the stem. Despite this chimney function, we found that diffusive fluxes from clipped reed were two times lower compared to the control (see Fig. 4). We found that despite the higher ebullition from CR and CSR treatment, the increase does not compensate for the excluded pathway via the pressurized air flow. This could indicate a significant role of pressurized flow in intact reed beds.

The total CH₄ flux could be overestimated in our data, and should therefore be interpreted with care. Ebullition could exist of episodical outbursts and/or a steady flow (small bubbles continuously released from the soil)¹¹.

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Table 3. δ¹³C measured in ebullition and chamber flux. The difference is a measure of the fractionation due to oxidation or gas transport pathways.

| Treatment | Chamber flux  δ¹³C (°) | SE | Ebullition  δ¹³C (°) | SE |
|-----------|------------------------|----|----------------------|----|
| CR        | −62.21                 | 1.44 | −50.93               | 1.59 |
| CSR       | −63.05                 | 1.53 | −55.95               | 0.87 |
| Control   | −60.35                 | 1.29 | −55.44               | 1.46 |

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Figure 5. Total CH₄ flux, consisting of ebullition and diffusive flux corresponding to a temperature of 14.7 °C (average ambient temperature between 7 and 27 June) for the treatments clipped reed (CR), clipped and sealed reed (CSR), and control. The contribution of the diffusive flux to the total flux is given in percentages.
Steady ebullition cannot be separated from diffusion in chamber flux measurements since the flow is constant, but is at the same time captured in bubble traps. So it could be that we double counted for this steady ebullition. It is hard to say if steady ebullition occurred or how much it contributes to the total flux. There is little scientific literature available that describes the prediction or characteristics of this type of gas flow. The conclusions about the increase in ebullition by clipping would, however, not change. And since ebullition is higher in the CR and CSR treatment, the double counting would be higher in the treatments as well, meaning that the difference in the total CH4 flux of the control vs treatments would only increase.

Another effect that we cannot quantify is the change in root exudates by cutting the reed. Root exudates are a substrate for methanogens and are expected to decrease by clipping due to the exclusion of photosynthesis. The change in photosynthates can occur within several hours, although a peak response of CH4 emission can be expected after several days. The possible decrease in photosynthates did not noticeably increase DOC or CH4 concentrations in the pore water (see Fig. 2) and we therefore expect it had little influence on the fluxes.

The δ13C signature change we found shows the difference in the gas transport mechanism between clipped and unclipped Phragmites (see Table 3). Without considering δ13C signature change due to gas transport, emitted CH4 is expected to be enriched with 13C compared to produced CH4, since part of the CH4 is oxidized. This would result in a positive δ13C signature change, that we expected to be highest in unclipped Phragmites. We found the opposite: the δ13C depletion was larger in the CR and CSR treatments (~11‰ and ~7‰ respectively) than in the control (~5‰). This change in δ13C signature in CR and CSR is in the same range as in CH4 emission from plants with diffusive gas transport. That clipped vegetation can be considered as plants with diffusive gas transport, is in line with the findings of Greenup et al. They did not find a significant difference between CH4 flux from clipped stems of E. vaginatum above the water table and from unclipped vegetation. Gas transport in E. vaginatum is known to be due to diffusion. The lower fractionation of CSR compared to CR treatment can be explained by the fact that gas transport through the stems is partly limited due to the sealing in the CSR treatment. Chanton compared δ13C change from soil to atmosphere from different wetland plants and found, on average, a smaller change for plants with convective transport than for those with diffusive transport. That corresponds to our results. Assuming gas transport in clipped reed to be diffusive, we can summarize that pressurized CH4 transport in intact reed leads to a CH4 emission two times higher than clipped reed with diffusive transport through stems.

Our chamber measurements from the control plots show diurnal patterns very similar to the EC measurements, with low fluxes in the morning and simultaneous increases when light intensity and temperature increased. Such a similarity in fluxes is not often found in comparisons between the two methods. Our results can be explained by the very homogeneous EC footprint of our site in regard to vegetation and landscape development (see Study site). Thereby, ebullition contributes for a small amount to the total flux, this leads to a more constant flux without abrupt and random emission peaks of CH4. The highest discrepancy between chamber and EC fluxes were found within 10 min after ventilating. Lifting the chambers and placing them back has likely caused disturbances. Apparently, it takes several minutes before CH4 production and emission are eliminated.

In summary, pressurized flow in Phragmites does seem to increase the total CH4 emission, even though ebullition is much reduced. It means that the effect of CH4 bypassing the oxic water layer due to plant transport is much larger than the effect of O2 transport by the plants on CH4 oxidation and production in the rhizosphere. This research was only covering a period within the growing stage of Phragmites with a high water table, giving a first indication of the contribution of the different flux pathways. To know the overall effect of the pressurized flow in Phragmites on CH4 emission, this experiment should be repeated to cover the whole growing season and variations in water table. Overall, this research emphasizes that plants with pressurized gas transport mechanism can be an important contributor to CH4 emission from wetlands.

Data availability
All data generated or analyzed during this study are included in this published article.

Received: 6 September 2018; Accepted: 22 June 2020
Published online: 23 July 2020

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Acknowledgements

We would like to thank the Federal State of Baden-Württemberg (BWPLUS program) for their financial support. We would like to thank Nils van Rooijen, Jelle Abma and Giani Gangloff for their assistance in the field. And our chamber flux calculations were made much easier thanks to the work of Ralf Aben.

Author contributions

M.v.d.B. was responsible for the literature review. M.v.d.B. and E.v.d.E. contributed equally in the study design, conducting the field and lab work, and analyzing the results. M.v.d.B., E.v.d.E., J.I., L.M.P.L. and S.K. evaluated the results. All authors contributed to the writing of the manuscript.

Competing interests

The authors declare no competing interests.

Additional information

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