Species-dependent methane emissions in a Dutch peatland during paludiculture establishment

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SUMMARY

Paludiculture (crop cultivation on wet peatlands) is an effective means to reduce carbon emissions and nutrient losses from formerly drained peatlands. However, methane (CH_4) emissions and associated pathways may vary substantially between paludicultures, depending on the cultivated paludicrop. Whereas many studies have investigated diffusive CH_4 emissions from paludicrops, large uncertainties exist in relation to the importance of CH_4 ebullition, species-specific differences and seasonal dynamics. In this study we aimed to quantify CH_4 emissions from stands of three paludicrop species, namely *Typha latifolia*, *Typha angustifolia* and *Azolla filiculoides*. CH_4 diffusive fluxes - including plant-mediated emissions - and ebullitive fluxes were studied year-round on a rewetted former agricultural peatland in The Netherlands. Additionally, we tested the effect of irrigation strategy (water table depth) and nutrient loading on CH_4 emissions from stands of both *Typha* species. Diffusive CH_4 emissions were lowest from *A. filiculoides* stands (average 15.4 ± 6.6 mg m^{-2} d^{-1}), followed by open water (36.4 ± 6.0 mg m^{-2} d^{-1}). Substantially higher diffusive emissions were measured for both *Typha* species, with the highest emissions in *T. latifolia* (187.0 ± 29.4 mg m^{-2} d^{-1}). Ebullitive fluxes were generally low (<100 mg m^{-2} d^{-1}), but the highest ebullitive fluxes occurred in the *A. filiculoides* stand, contributing 79 % on average to its total CH_4 emissions. A water table just below the peat surface substantially reduced *Typha* CH_4 emissions, but also led to lower biomass production, compared to a flooded ($+20$ cm) water table. Nutrient loading increased biomass production and did not affect diffusive CH_4 emissions. Our results underline that species choice substantially affects paludiculture carbon dynamics in the first phase after establishment. Quantifying both diffusive and ebullitive fluxes is of paramount importance in adequately assessing paludiculture CH_4 fluxes.

KEY WORDS: *Azolla*, greenhouse gases, nutrient loading, *Typha*, water table depth

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INTRODUCTION

Peat soils form the world's densest terrestrial carbon stock, storing 450 Gt C on only 3 % of the global land area (Joosten 2009, Joosten *et al.* 2016). Peatland drainage for agriculture and peat extraction has resulted in severe degradation of 10 % of peatlands globally, and 50 % of peatlands in the European Union (EU) (Joosten 2009, Tanneberger *et al.* 2017). Drainage boosts aerobic organic matter breakdown and turns peatlands from a weak carbon (C) sink into a strong C source (Frolking *et al.* 2011, Tiemeyer *et al.* 2016). In the EU alone, drained peatlands cause 25 % of agricultural greenhouse gas (GHG) emissions while comprising only 3 % of the agricultural land area (Tanneberger *et al.* 2022). Rewetting drained peatlands is thus essential to mitigate global climate change and attain the goals of the Paris agreement (Günther *et al.* 2020, Tanneberger *et al.* 2021). To smooth the transition from agricultural land to fully restored natural peatland, the cultivation of wetland plants on rewetted peat, i.e. paludiculture, is a promising solution (Wichtmann *et al.* 2016). Wetland plant (i.e. paludicrop) cultivation provides an alternative income for farmers from the production of e.g. horticultural substrate, building and insulation materials or nutrition (Georgiev *et al.* 2014, Gaudig

et al. 2018, Pijlman *et al.* 2019). Simultaneously, the presence of vegetation can speed up the recovery of ecosystem services such as carbon and nutrient sequestration, water retention and habitat provision (Muster *et al.* 2015, Temmink *et al.* 2017, Vroom *et al.* 2022a).

A concern in paludiculture is the potential occurrence of elevated methane (CH_4) emissions after rewetting, which may (partly) offset the newly created CO_2 sink (Hemes *et al.* 2018, Günther *et al.* 2020, Antonijević *et al.* 2023). CH_4 has a 27 times higher global warming potential than CO_2 over a 100-year time horizon (Canadell *et al.* 2021) and is formed when anoxia returns to peat soils after inundation, leading to elevated emissions. Rewetted peatlands will have a lower warming effect than drained peatlands in the long term, as organic matter decomposition rates are substantially lower and CH_4 has a much shorter residence time in the atmosphere than CO_2 (Frolking *et al.* 2001, Günther *et al.* 2020). However, minimising paludiculture CH_4 emissions by making informed management decisions could at the same time boost carbon storage and increase stakeholder support for the implementation of paludiculture.

Paludicrops can affect CH_4 emissions in myriad ways. For a complete overview, see Bodmer *et al.* (2021), Vroom *et al.* (2022b) and Bastviken *et al.* (2023). Briefly, plants can enhance CH_4 production by organic matter provision (root exudates and decaying plants) and by CH_4 transport from sediment to the atmosphere through aerenchyma. In addition, plants can enhance CH_4 production by trapping organic matter, reducing oxygen (O_2) diffusion into the water, and by epiphytic and endophytic CH_4 production. In turn, CH_4 oxidation is promoted by root zone oxygenation (which also reduces CH_4 production), CH_4 ebullition retention by roots or other biomass, and epiphytic and endophytic methanotrophy (Bodmer *et al.* 2021, Bastviken *et al.* 2023). The net vegetation effect depends on plant growth form (emergent/floating/submerged), mode of CH_4 transport (diffusion/pressurised flow), growth stage, and physical condition (e.g. herbivore damage or harvesting), among other factors (Dinsmore *et al.* 2009, van den Berg *et al.* 2020, Vroom *et al.* 2022b).

As a perennial emergent macrophyte that can spread rapidly using rhizomes, *Typha* (cattail) is a paludicrop genus with high production and utilisation potential, particularly in eutrophic systems. *Typha* biomass can be used for a range of purposes such as building material, biodegradable packaging, fodder and biogas (Pijlman *et al.* 2019, Martens *et al.* 2021, Haldan *et al.* 2022). Native species in Europe include *Typha latifolia* (broadleaf cattail) and *Typha*

angustifolia (narrowleaf cattail), which are widespread in wetlands and shallow waters. To aerate their roots, *Typha* spp. have a high proportion of aerenchyma in their tissues, with air spaces composing $>50\%$ of the internal leaf volume (Pazourek 1977). Furthermore, they are capable of pressurised gas transport, resulting in high radial oxygen loss (ROL) but also potentially high CH_4 transport from anoxic sediments to the atmosphere ("chimney effect") (Sebacher *et al.* 1985). Previous research has shown that *Typha* spp. can either increase (McInerney & Helton 2016) or decrease (Jespersen *et al.* 1998, Vroom *et al.* 2018, Antonijević *et al.* 2023) CH_4 emissions compared to an open water situation. Additionally, CH_4 venting through plant biomass could decrease the sediment CH_4 stock, resulting in reduced ebullitive emissions (Van Der Nat *et al.* 1998, van den Berg *et al.* 2020). However, the importance of ebullition as a CH_4 emission pathway in *Typha* and in paludiculture has rarely been studied (van den Berg *et al.* 2024).

Another high-potential candidate paludicrop is *Azolla* (Abel & Kallweit 2022). *Azolla* is a genus of floating ferns that lives symbiotically with N-fixing cyanobacteria *Nostoc / Anabaena azollae* (Peters & Meeks 1989). Due to this symbiosis, *Azolla* has very high clonal growth rates even in N-depleted conditions (Wagner 1997). *Azolla filiculoides* was introduced to western Europe from North America at the end of the 19th century (Pieterse *et al.* 1977, Sheppard *et al.* 2006). The protein-rich biomass can, for instance, be used for human or animal nutrition or as a biofertiliser (Brouwer *et al.* 2014). Dense floating mats of *Azolla* can reduce light and O_2 concentrations in the underlying surface water (Pinero-Rodríguez *et al.* 2021, Aben *et al.* 2022). Studies on CH_4 emissions from *Azolla* have so far mostly focused on rice paddies, where *Azolla* is commonly used as a biofertiliser (Mandal *et al.* 1999, Kollah *et al.* 2016), and there has been one mesocosm study (Aben *et al.* 2022). Our experiment in a Dutch peatland is the first in which *Azolla* has been tested as a paludicrop in a field setting (van den Berg *et al.* 2024).

We reported previously that a CO_2 sink was effectively restored in this system after rewetting and planting *T. latifolia*, *T. angustifolia* and *A. filiculoides*. However, annual CH_4 emissions were species-dependent and highest for *T. latifolia* (van den Berg *et al.* 2024). The present study aims to provide additional insights on CH_4 emissions from these paludicrop species in the first phase (2–4 years) after rewetting. Specifically, we aim to answer the following research questions: 1) What is the magnitude of CH_4 emissions from paludiculture stands with *A. filiculoides* (hereafter *Azolla*),

T. latifolia and *T. angustifolia*, and what are the seasonal and diel dynamics?; and 2) how do water table (WT; 0 or +20 cm) and nutrient addition (nitrogen and phosphorous) affect water–atmosphere diffusive CH_4 emissions and plant-mediated emissions (further combined and referred to as 'diffusive emissions') of *Typha*? We hypothesise that the magnitude and pathways of CH_4 emissions depend on plant species and growth form, with higher diffusive emissions and lower ebullitive emissions in *Typha* spp. than in *Azolla* due to plant-mediated CH_4 transport. We expect higher CH_4 emissions during the day in *Typha* spp. as a result of increased pressurised flow. Lastly, we hypothesise that CH_4 emissions will be lowest with 0 cm WT due to increased O_2 availability, and that nutrients will enhance emissions as a result of increased plant productivity.

METHODS

Study site
The study site was situated in the Zuiderveen (North Holland, The Netherlands, 52° 43.78' N, 04° 73.15' E). This is a formerly drained and extensively managed fen grassland which was fertilised with manure and mowed in summer. The site was partially rewetted and paludiculture experiments were established in 2018 (see also van den Berg *et al.* 2024). First, ~ 20 cm of topsoil was removed to create four 23×43 m basins, of which three were included in this study. The fourth basin was not included because a layer of sludge had been added to it, making it incomparable to the others (Figure 1). On the north-east side, the basins were connected to a nearby ditch by small drainage channels, and the water table was regulated with an adjustable weir per individual basin. Each basin also had an overflow outlet which was connected to a second ditch on the south-west side. In one basin (B1) the water table was maintained at the level of the peat surface, whereas in the other two basins (B2, B3) it was set at 20 cm above the peat surface. In B1 and B3 the water table level was logged continuously in dipwells fitted with submerged sensors that measured water pressure (Cera-Diver, Eijkelkamp, Giesbeek, Netherlands), which was recalculated to water table depth by compensating for atmospheric pressure measured with a separate instrument (Baro-Diver, Eijkelkamp, Giesbeek, Netherlands). A wooden boardwalk on poles ran through the centre of each compartment to minimise peat disturbance during sampling. Each basin was split into three compartments (~ 200 m² for *Azolla*, ~ 430 m² for *Typha*) using wooden boards to physically separate the species. One paludicrop

(*Typha latifolia*, *Typha angustifolia* or *Azolla filiculoides*) was introduced to each compartment. Both *Typha* species were introduced in autumn 2018 and spring 2019 by planting shoots (pre-grown in a nursery) at a density of 1 individual m⁻². A floating PVC structure (3×3 m) was placed in each *Azolla* compartment to contain the *Azolla* and minimise plant loss by wind. *Azolla* cultivated in the greenhouse facilities of Radboud University was introduced in March 2020 by placing 950 g m⁻² fresh weight (FW) into the floating structures. *Azolla* covered the water surface at 90–100 % from May to August, after which it declined due to an infestation with the *Azolla* weevil (*Stenopelmus rufinasus*). After infestation, *Lemna* spp. gradually took over, and no *Azolla* remained in December 2020. Bulrush wainscot (*Nonagria typhae*) larvae affected both *Typha* species in spring 2020 and 2021, being more prominent in *T. latifolia*. This herbivorous moth larva feeds endophytically on *Typha* stems from spring until July/August, when it pupates and flies out (Teder & Tammaru 2002).

Experiment 1: CH_4 emissions from three paludicrops
To assess how CH_4 emissions are affected by the three paludicrops, CH_4 emissions (diffusion and ebullition) and pore water concentrations were measured in B2 (average water table in 2020: +17.5 cm, see Figure A1 in the Appendix; for water chemistry, see Table 1) during 14 sampling campaigns (ten with manual and four with automated chambers) throughout 2020. To enable GHG emission measurements in *Typha* without soil disturbance, three wooden frames were installed below the water table in each *Typha* compartment (total n = 6 frames; Figure A2). Manual chamber measurements were carried out on 10 March, 23 April, 14 May, 04 June, 18 July, 04 August, 08 September, 20 October, 09 November and 08 December. For both *Typha* species we used transparent Perspex chambers (diameter 50 cm) that could be stacked to match the height of the plants. Each chamber element was equipped with a fan powered by a power bank. The top part additionally contained a temperature logger and a PAR logger (both HOBO onset, Onset Computer Corporation, Bourne, MA, USA). For *Azolla* we used a floating transparent Perspex chamber (diameter 29 cm, height 26 cm) equipped with a HOBO temperature logger, and PAR was measured with a handheld device (PAR Quantum sensor SKP 215, Skye Instruments, Llandrindod, Wales, UK). Chambers were connected in a closed loop with gastight tubing to either a LI-COR 7810 portable GHG analyser (LI-COR Inc.,
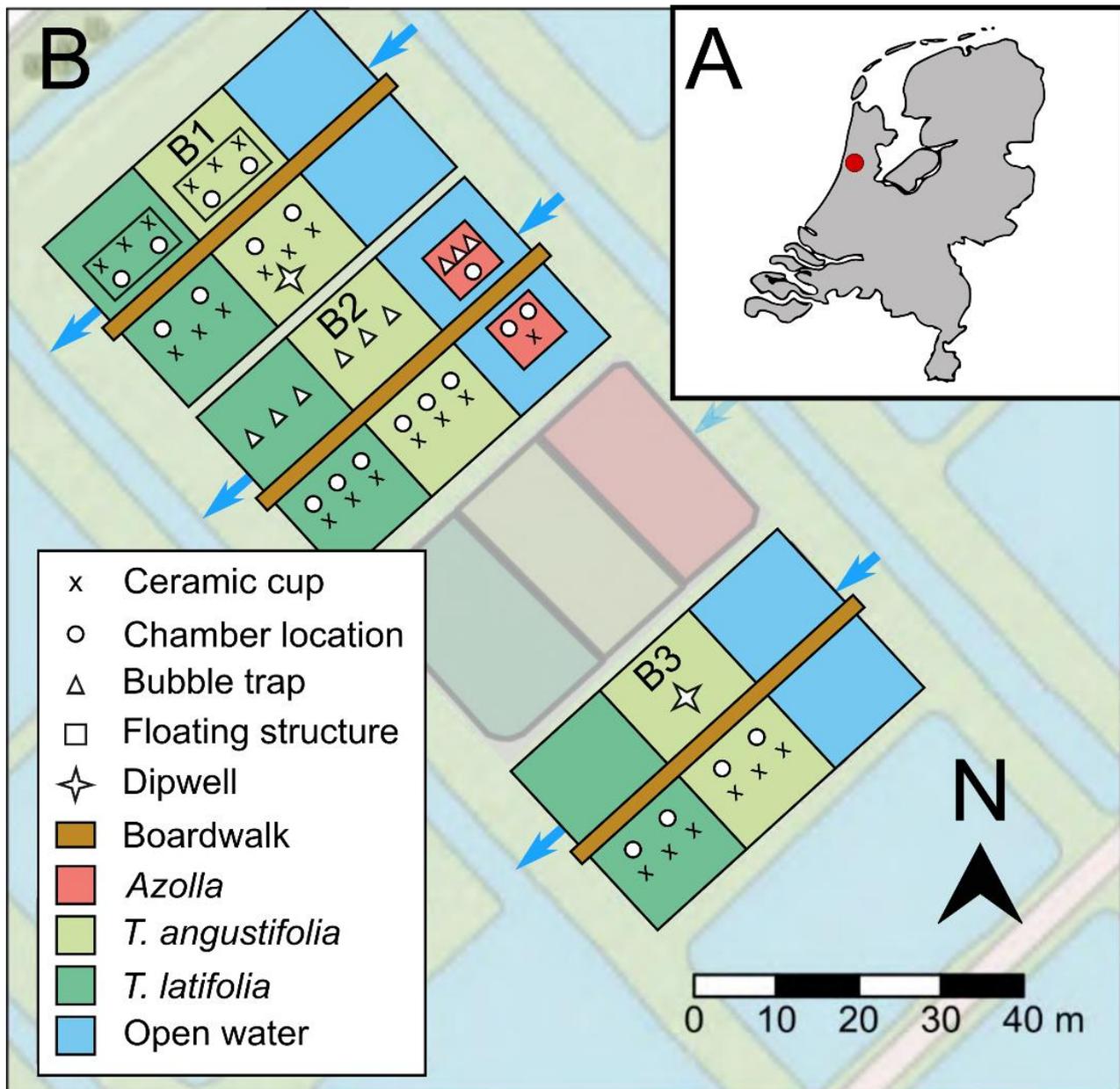
Figure 1. A: Location of the field site in The Netherlands; and B: overview of the experimental paludiculture basins. Measurements were conducted in B1 (Experiment 2), B2 (Experiments 1 and 2), and B3 (Experiment 2). Blue arrows indicate the direction of irrigation water flow. The water table was kept at around the peat surface in B1 and around +20 cm in B2 and B3 throughout the study period. Note that icons are used to indicate the setup per compartment, and are neither to scale nor fully accurate spatially.

Lincoln, NE, USA) or a Los Gatos Ultra-Portable GHG analyser (ABB - Los Gatos Research, San Jose, CA, USA) that measured CH_4 concentrations every second. Measurements were carried out during daytime and lasted three minutes each. At every flux measurement point (n = 3 for *Azolla*, *T. latifolia*, *T. angustifolia* and open water (unvegetated control, measured in the *Azolla* basins next to the floating structures containing *Azolla*); total n = 12), three transparent measurements and three opaque chamber measurements were carried out during each campaign. To estimate the biomass of *Typha* spp. in the measurement plots, ten living shoots of each species were harvested outside of the measurement plots in September 2020 (n = 1 per basin and species). These shoots were dried at 70 °C for 72 hours, and dry weight per cm shoot length was calculated. This was then multiplied by the number of living shoots and the average shoot height at each measurement time and in each measurement plot.

Table 1. Porewater and surface water characteristics of basin B2, measured monthly in 2020. Values are given as average (min, max). Letters indicate significant differences ($p < 0.05$) between vegetation types. DOC = dissolved organic carbon, NO_3^- = nitrate, NH_4^+ = ammonium, PO_4^{3-} = phosphate, P = phosphorous, Cl = chloride, and H_2S = hydrogen sulphide. For methodological details, see van den Berg *et al.* (2024).

Water type	Variable	Unit	Inflow ditch	*Azolla*	*T. angustifolia*	*T. latifolia*
Surface water	pH	-	7.65 (7.34, 8.35)	7.41 (6.98, 8.36)	7.39 (6.99, 7.72)	7.77 (7.14, 8.6)
	DOC	mmol L^{-1}	3.37 (2.68, 4.85)	3.59 (2.65, 4.83)	3.67 (2.51, 4.95)	4.29 (2.21, 6.35)
	NO_3^-	µmol L^{-1}	8.89 (0.65, 57.62)	4.1 (0.55, 16.72)	4.08 (0.5, 19.58)	2.6 (0.3, 12.93)
	NH_4^+	µmol L^{-1}	43.51 (3.07, 110.49)	28.18 (1.67, 81.63)	10.92 (1.88, 20.25)	10.96 (1.88, 14.55)
	PO_4^{3-}	µmol L^{-1}	5 (0.97, 9.22)	3.34 (0.7, 5.19)[a]	0.97 (0.17, 2.05)[b]	1.41 (0.16, 2.4)[ab]
	Cl	mmol L^{-1}	39.66 (5.68, 59.05)	38.35 (5.76, 49.69)	37.08 (5.73, 47.67)	35.79 (5.69, 44.53)
Pore water	pH	-		6.38 (6.2, 6.66)[a]	4.83 (4.72, 5)[b]	6.42 (6.19, 6.65)[a]
	DOC	mmol L^{-1}		24.14 (7.16, 58.64)	9.88 (6.3, 18.32)	5.38 (4.99, 5.79)
	NH_4^+	µmol L^{-1}		339.07 (128.99, 648.67)[a]	11.89 (0.91, 28.35)[b]	6.97 (2.94, 13.51)[b]
	P	µmol L^{-1}		31.27 (9.29, 55.8)[a]	3.17 (1.14, 6.6)[b]	1.79 (1.07, 2.9)[b]
	Cl	mmol L^{-1}		32.81 (5.69, 52.8)	43.58 (5.8, 75.62)	41.25 (5.7, 55.96)
	H_2S	µmol L^{-1}		0.02 (0, 0.07)	0.05 (0.01, 0.13)	0.02 (0.01, 0.05)

In addition to manual measurements, we carried out automatic chamber measurements at the beginning of March, end of May, end of June and beginning of November 2020. For this, four Perspex chambers (diameter 35 cm) were placed in one vegetation type at a time. The chambers consisted of 0.5 m segments that could be stacked to match vegetation height. Rubber liners and tightening screws prevented leakage between the segments, and guy wires were used to secure the chambers at their maximum height. These chambers were equipped with fans and temperature loggers. They also had hinged lids controlled by a connected Raspberry Pi computer (Raspberry Pi Foundation, Cambridge, UK) and were connected with gastight tubing in four closed loops to a Los Gatos Microportable Greenhouse Gas Analyser. The four chambers were measured in succession, by closing the lid of the requisite chamber for 3 minutes of which the first 30 seconds were excluded from analysis due to flushing time of the tubing. After each measurement the chambers were flushed with atmospheric air for one minute, and then the lid of the next chamber was closed. During the closing time of one chamber, the other chambers remained open. This sequence was continued for three to five days in each vegetation type, providing high resolution data including diel variation in emissions.

To calculate fluxes from both manual (daytime fluxes) and automatic chambers, a plot of CH_4 concentration against time was made for a visual check on linearity. Measurements with significant departures from linearity (due to ebullition or other causes) were rejected. Fluxes were then calculated by linear regression of the gas concentration in the chamber, according to Almeida *et al.* (2016).

To assess CH_4 ebullition from the three paludicrops, we installed three bubble traps in each vegetation type (total n = 9). Each trap consisted of a glass tube connected to a funnel (diameter 20 cm) which was kept floating by a small foam raft (Figure A2). Toothpicks were inserted into the foam rafts to prevent ducks from sitting on them and flipping over the traps. A butyl stopper was fitted at the top of each glass tube to enable gas extraction. Gas volume was determined every 1.5–3 weeks by removing the accumulated gas with a syringe. To determine CH_4 concentrations, gas was sampled once a month (April, May, November, December) or twice a month (June–October): 1 mL of gas was transferred to a 118 mL bottle closed with a butyl stopper. Subsequently, 1 mL of diluted gas was injected into a Los Gatos Ultraportable GHG analyser using an open loop of gastight tubing. The CH_4 concentration was then determined by calculating the area under the curve. As the CH_4 concentrations in the sampled gas were very high (> 3 %), atmospheric CH_4 concentrations (~ 0.0002 %) in the bottles and the greenhouse gas analyser inflow were negligible. Next, ebullitive CH_4 fluxes were calculated by multiplying the gas volume by the CH_4 content and dividing by bubble trap deployment time and area of the funnel.

To assess porewater CH_4 concentrations, ceramic cups were installed in the top 10 cm of soil in each compartment (n = 1 per plant species). Pore water samples were collected in gastight evacuated 12 mL glass exetainers (Labco, Lampeter, UK) containing 1 mL of 0.5 M hydrochloric acid (HCl) for sample preservation. Samples were taken after manual chamber measurements were completed, on 11 March, 24 April, 14 May, 05 June, 23 July, 05 August, 09 September, 21 October, 10 November and 09 December 2020. Pore water CH_4 was measured in the headspace of the exetainers using an HP 5890 gas chromatograph equipped with a Porapak Q column (80/100 mesh) and a flame ionization detector (GC-FID, Hewlett Packard, Palo Alto, CA, USA). The original concentrations in the pore water were calculated using Henry's law (Sander 2015).

Experiment 2: Effect of water table and nutrient loading on *Typha* CH4 emissions

To assess the effect of water table and nutrient loading on CH_4 emissions from both *Typha* species, sampling campaigns were carried out in B1 (average water table -4.5 cm in 2021), B2 and B3 (average water table for both was +19.5 cm in 2021 (Figure 1, Figure A1). To measure emissions, wooden structures similar to those in B2 were installed in B3 (see Experiment 1, n = 3 per species); whereas in B1, two PVC rings (40 cm height, 50 cm diameter) per treatment and species were inserted ± 20 cm into the soil (n = 8 rings in total). In basins B1 and B3, a nutrient treatment was applied using the artificial fertilisers urea $(CO(NH_2)_2)$ and triple superphosphate (45 % P). In 2020, a total of 210 kg ha⁻¹ yr⁻¹ of N and 48 kg ha yr⁻¹ of P was applied, in three applications during the period May–July. In 2021, a total of 140 kg ha⁻¹ yr⁻¹ of N and 32 kg ha yr⁻¹ of P was applied, divided over four occasions in the months April–July. Fertiliser was added directly to the surface water in B3, whereas in B1 the fertiliser was applied in one of the 2 × 4 m enclosures made of wooden boards per species.

Diffusive CH_4 fluxes were measured eight times using manual chambers in 2021 (07 and 28 April, 18 May, 09 June, 07 and 21 July, 04 and 25 August), according to the above-mentioned methods. We used opaque chambers, since we did not see differences

between transparent and opaque chamber measurements in the previous experiment. Diffusive CH_4 flux (including plant transport) was measured at two sampling points per treatment combination and *Typha* species (n = 16 sampling points in total), and two replicate measurements were taken per sampling point during each sampling campaign.

To compare porewater CH_4 concentrations between treatments, ceramic cups were placed in triplicate in the vicinity of the CH_4 flux measurement points in each treatment combination (total n = 24 cups). Porewater was sampled on 07 and 28 April, 18 May, 09 June, 07 and 21 July, and 04 and 25 August 2021, according to the above-mentioned methods.

In August 2021, biomass was harvested from 1×1 m plots of to determine biomass production. For each treatment, two plots were chosen that were not in the proximity of any GHG measurement setup. In the fertilised 0 cm water table treatment, only one plot per species was harvested due to the small treatment plot size. *Typha* shoots were cut down to ~ 5 cm above the water table or ~ 10 cm above the soil surface (to realistically represent harvesting), weighed, and dried at 70 °C for 72 hours to determine dry weight (mass).

Statistical analyses
All statistical analyses and visualisations were done in RStudio v 4.0.3 using packages ggplot2 (Wickham 2016), nlme (Pinheiro *et al.* 2018) and emmeans (Lenth 2022). Differences between treatments in manually measured diffusive CH_4 fluxes, ebullitive CH_4 fluxes and porewater CH_4 concentrations were assessed using general linear (mixed) models followed by Analysis of Variance (ANOVA). Several models were created for each comparison, including the relevant main effects (vegetation type, date, water table, nutrient addition) and their two-way interactions with, in addition, (i) measurement plot as a random intercept, (ii) a temporal autocorrelation structure, (iii) both (i) and (ii), or (iv) neither. Then, models were compared using ANOVA, and the model with the lowest Aikake Information Criterion (AIC) was chosen. If AICs did not differ significantly based on an ANOVA test, the model with the simplest structure was selected. Residual plots were created for visual assessment of variance normality and homogeneity. To obtain variance normality, CH_4 diffusion and ebullition were log-transformed. Differences between individual treatments were assessed using Tukey post-hoc tests. A significance threshold of p < 0.05 was used. Values are reported in the text as means ± standard error.

RESULTS

Experiment 1: CH_4 emissions from three paludicrop stands
As CH_4 fluxes in manual transparent and opaque chamber measurements did not differ significantly (p = 0.30), diffusive CH_4 flux values were averaged over all six measurements per measurement point (n = 3 points per species) and date. A strong seasonal trend in CH_4 emissions was observed in all treatments, with low emissions during winter months (December, March) and peak emissions during summer months (July–September) (Figure 2A). Overall, diffusive CH_4 emissions differed significantly between plant species (*p* < 0.001). Specifically, emissions were lowest in the *Azolla* stand (9.8 ± 1.6 mg m^{-2} d^{-1}), followed by open water (36.4 ± 6.0 mg m^{-2} d^{-1}) and *T. angustifolia* (64.5 ± 8.4 mg m^{-2} d^{-1}). Emissions were substantially higher in *T. latifolia* (187.0 ± 29.4 mg m^{-2} d^{-1}). There was no difference in estimated biomass production between *T. angustifolia* and *T. latifolia* (p = 0.97).

CH_4 diffusion measured by automated chambers showed distinct diel fluctuations during some months, depending on the species (Figure 3). In March, before the growing season, no patterns could be distinguished. In May, however, CH_4 diffusion in *Azolla* was higher during the night. In June/July, *T. latifolia* had higher emissions during the day, whereas *T. angustifolia* had the highest emissions during the night. In September/October, both *Typha* species again had higher emissions during the day, although the fluctuations were less pronounced. No diel patterns were distinguished in *Azolla* in June/July and September/October. The presence of these diel patterns could lead to a mismatch of daytime (manual) measurements with actual emissions (over 24 h). For instance, measuring *Azolla* at noon in May (6 mg m^{-2} d^{-1}) would under-estimate average emissions over 24 hours (35 mg m^{-2} d^{-1}) by more than fivefold. A similar underestimation, albeit less extreme, would occur for *T. angustifolia* in June/July (300 vs. 325 mg m^{-2} d^{-1} at noon and over 24 hours, respectively), while fluxes from *T. latifolia* would be over-estimated (472 vs. 379 mg m^{-2} d^{-1} at noon and over 24 hours, respectively, in June/July).

CH_4 ebullition was highly variable, with peak emissions in June and August (Figure 2B). Average ebullitive CH_4 emissions were significantly lower in *T. angustifolia* (16.7 ± 5.40 mg m^{-2} d^{-1}) than in *Azolla* (91.5 ± 22.2 mg m^{-2} d^{-1}; p < 0.001) and in *T. latifolia* (46.6 ± 12.2 mg m^{-2} d^{-1}; p = 0.042), with no significant difference between the latter two (p = 0.245). The average contribution of ebullition to total CH_4 fluxes was highest in *Azolla* (69 %), and substantially lower
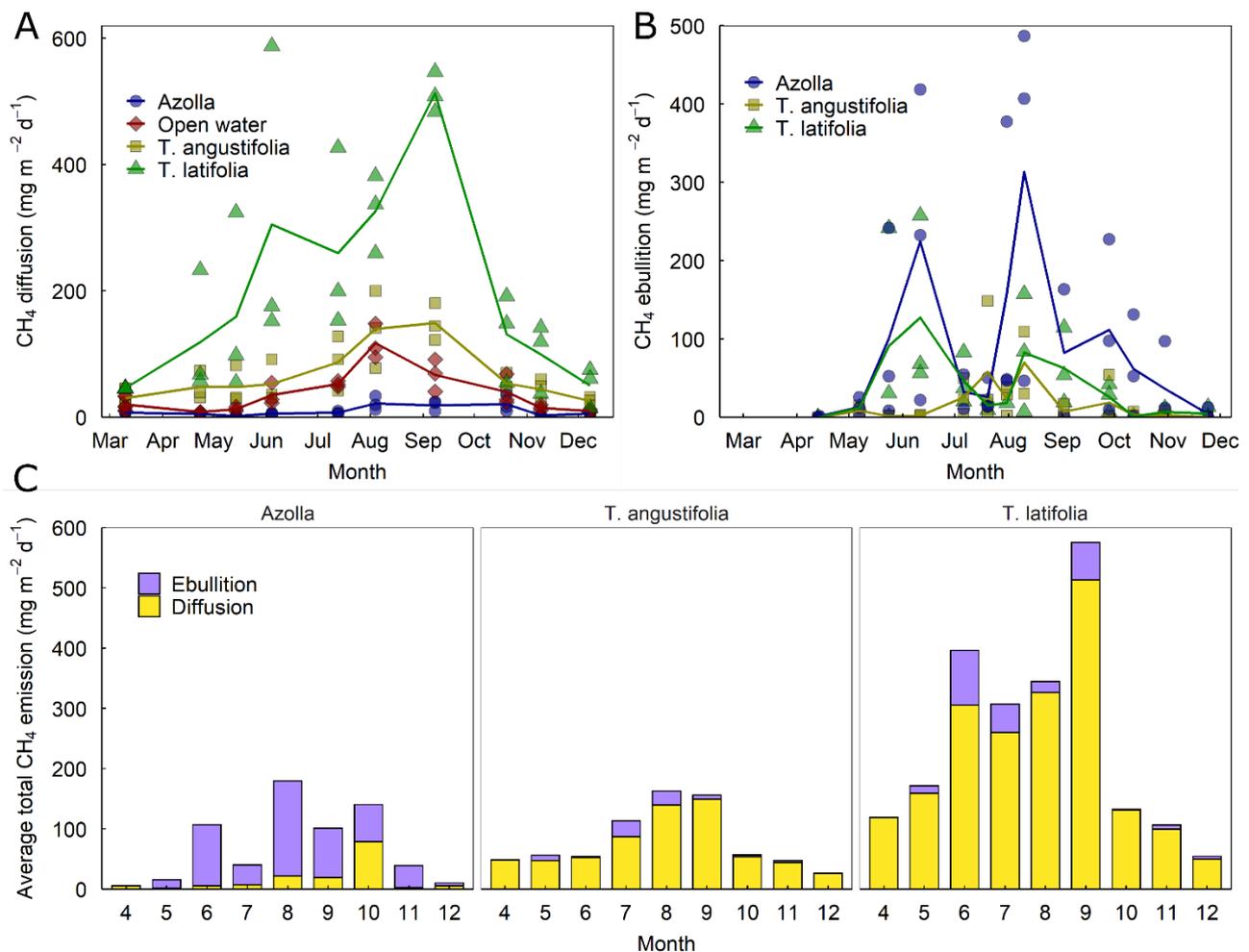
Figure 2. A: Diffusive CH$_4$ fluxes (includes water–atmosphere fluxes and plant-mediated transport) for the three vegetation types and open water; B: Ebullitive CH$_4$ fluxes (not measured in open water); and C: Total CH$_4$ fluxes (diffusion + ebullition) for each vegetation type. Bars show averages for the three subplots. Symbols indicate individual measurements at subplots (for diffusion, averaged over n = 6 repeat measurements over time). Lines indicate average emissions per vegetation type.

in both *Typha* species (9 % in *T. latifolia*, 8 % in *T. angustifolia*) (Figure 2C). Average annual total CH$_4$ fluxes were highest in *T. latifolia* (245.4 ± 57.1 mg m^{-2} d^{-1}), followed by *T. angustifolia* (80.2 ± 16.9 mg m^{-2} d^{-1}, p = 0.020) and *Azolla* (71.1 ± 21.0 mg m^{-2} d^{-1}, p = 0.012), with no significant differences between the latter two (p = 0.938).

Porewater CH$_4$ concentrations (top 10 cm of sediment) remained rather stable (~ 80 µmol L^{-1}) over time in both *Typha* species whereas a clear accumulation was observed in *Azolla*, rising from 47 µmol L^{-1} in March to 1305 µmol L^{-1} in November (Figure 4). Overall, mean porewater CH$_4$ concentrations were significantly higher in *Azolla* (481 ± 129 µmol L^{-1}) than in *T. angustifolia* (50 ± 13 µmol L^{-1}; p < 0.001) and *T. latifolia* (111 ± 31 µmol L^{-1}, p < 0.001), and did not differ between *T. latifolia* and *T. angustifolia* (p = 0.056).

Experiment 2: Effect of water table and nutrient loading on *Typha* CH$_4$ emissions

Biomass yield for both *Typha* species was higher in the +20 cm WT conditions, with the more marked increase in *T. latifolia* (Figure 5A). Similarly, nutrient addition increased the biomass yield of both species, but more prominently of *T. latifolia*. *T. latifolia* dry biomass was 0.41 (0 cm WT) and 1.96 (+20 cm WT) kg m^{-2} in the unfertilised treatment and 0.53 (0 cm WT) and 3.81 (+20 cm WT) kg m^{-2} in the fertilised treatment. *T. angustifolia* dry biomass was 0.87 (0 cm WT) and 1.35 (+20 cm WT) kg m^{-2} in the unfertilised treatment and 0.62 (0 cm WT) and 2.23 (+20 cm WT) kg m^{-2} in the fertilised treatment.

As in Experiment 1, diffusive CH$_4$ emissions were substantially lower (65 % on average) in *T. angustifolia* than in *T. latifolia* at the high WT
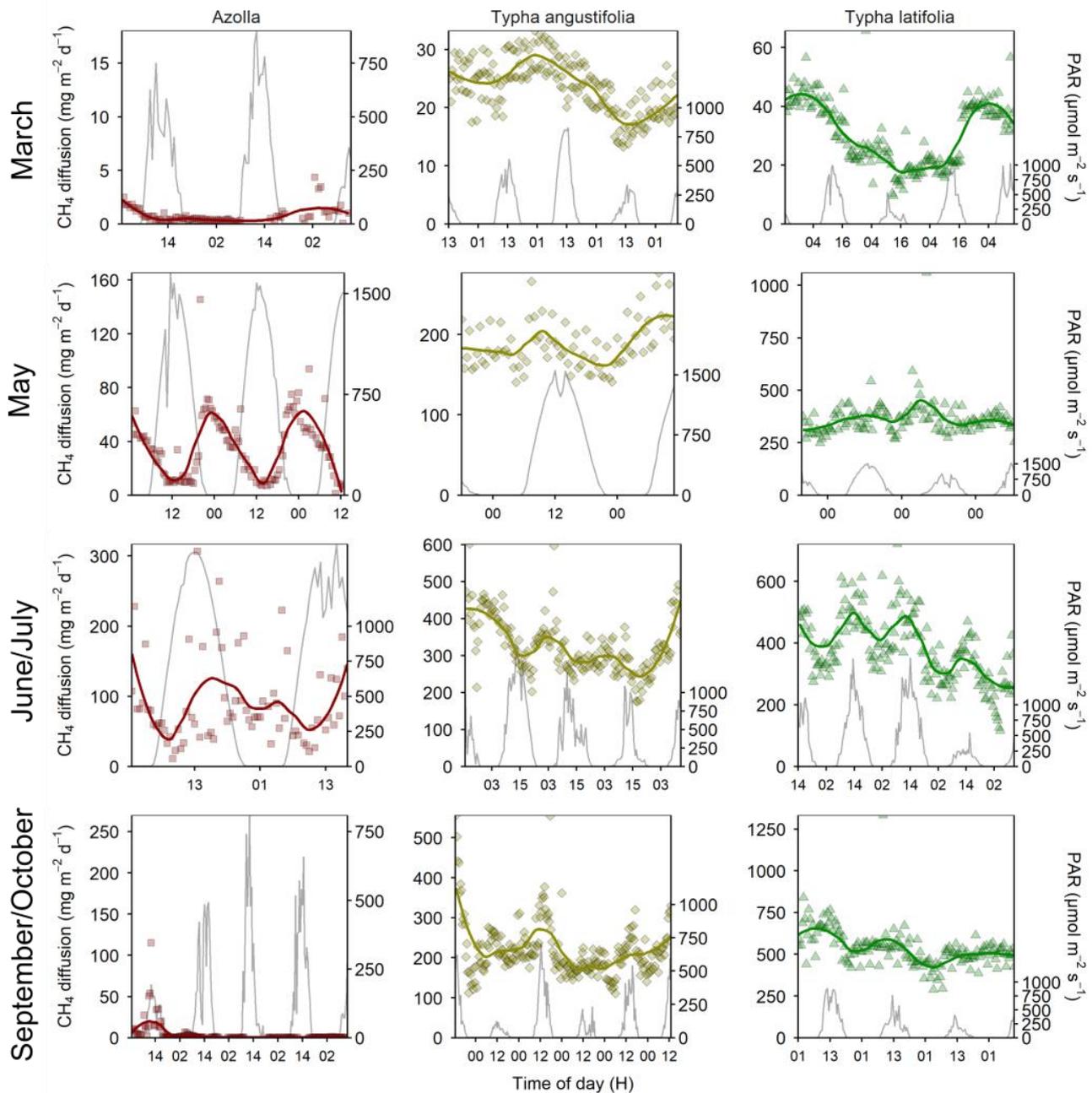
Figure 3. Diel patterns of CH_4 fluxes in the three vegetation types measured using automated chambers. Locally estimated scatterplot smoothing (loess) models are shown by the coloured lines. Grey lines represent PAR values at the times of measurements. NB: y-axis scales vary. *Azolla* was not yet present in March.

(p < 0.001) (Figure 5B). At the low WT, however, emissions did not differ significantly between the two species (p = 1.000). Overall, emissions were substantially lower at the low WT (p < 0.001). This difference was more prominent in *T. latifolia* (87 % lower, 466.7 ± 57.5 and 53.0 ± 10.6 mg m^{-2} d^{-1} at the high and low WT, respectively), but also substantial in *T. angustifolia* (71 % lower, 123.8 ± 11.9 and 35.4 ± 2.2 mg m^{-2} d^{-1} at the high and low WT,

respectively). Despite its effect on biomass production, nutrient addition had no effect on CH_4 emissions (p = 0.342).

Porewater CH_4 concentrations were more than ten times higher at the + 20 cm WT than at the 0 cm WT (283 ± 30 µmol L^{-1} and 23 ± 5 µmol L^{-1}, respectively, p < 0.001) (Figure 5C). Porewater CH_4 concentrations did not differ between plant species (p = 0.086) or fertilisation treatments (p = 0.606).
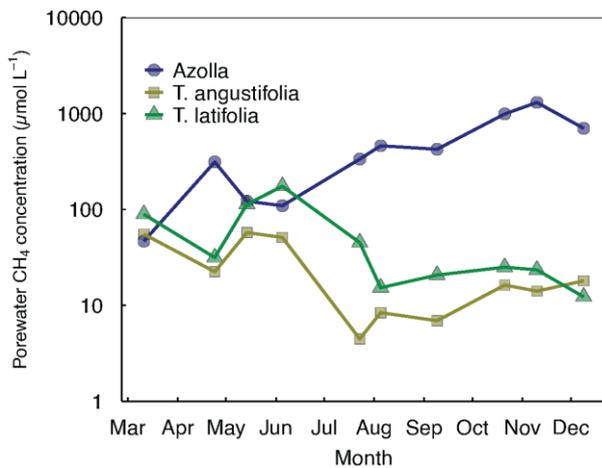
Figure 4. Porewater CH_4 concentrations (note the logarithmic y-axis scale) for the three vegetation types through time.

DISCUSSION

Paludiculture can halt intense CO_2 emissions from formerly drained peatlands and substantially decrease negative climate effects compared to drainage, despite coinciding increases in CH_4 emissions (Günther *et al.* 2020). Moreover, CH_4 emissions, particularly occurring in flooded conditions with high carbon and nutrient concentrations, could be minimised by informed management choices, including the choice of paludicrop species (Antonijević *et al.* 2023, van den Berg *et al.* 2024). In our field study conducted in the third and fourth year after rewetting, we found that the magnitude and pathways of CH_4 emissions were paludicrop-dependent in stands that had not yet been harvested. CH_4 emissions were diffusion-dominated in the emergent *Typha* spp., but ebullition-dominated in the free-floating *Azolla*. *T. latifolia* stands had substantially higher total emissions than stands of its congener *T. angustifolia* or of *Azolla*. CH_4 emissions in *Typha* were substantially lower at a water table of 0 cm than of +20 cm. N and P addition led to higher biomass production in *Typha* at both water tables, but did not affect CH_4 emissions.

CH_4 emissions in free-floating *Azolla* were ebullition-dominated

This experiment was the first to quantify in-situ CH_4 emissions from *Azolla* outside of rice paddy systems, and the first to quantify CH_4 ebullition in an outdoor water body covered by *Azolla*. Previous studies in rice paddies showed that *Azolla* dual cropping generally led to lower diffusive CH_4 emissions than growing rice alone, potentially as a result of higher dissolved O_2 concentrations in surface water and, thus, a higher sediment redox potential (Xu *et al.* 2017, Bharati *et al.* 2000). In our study *Azolla* appeared to be a good candidate paludicrop in terms of CH_4 emissions, with lower diffusive emissions than the open water control (although open water ebullitive emissions were not quantified here). Lower diffusive emissions may be explained by reduced CH_4 diffusion through the floating *Azolla* mat, ROL from *Azolla* roots to the surface water and/or presence of methanogens on *Azolla* roots, leading to enhanced CH_4 oxidation (Kosten *et al.* 2016). ROL may also explain the diel CH_4 patterns in May, with lower fluxes during the day (O_2 production and release through photosynthesis) than the night. Alternatively, higher night-time CH_4 fluxes may be explained by heat loss from the warmer water to the air, resulting in convective mixing and increased CH_4 diffusion (Anthony & Macintyre 2016). In June/July, automated chamber measurements took place in a part of the *Azolla* stand that was particularly affected by *S. rufinasus*, explaining the absence of this pattern and the discrepancy between automated and manual measurements (undertaken in a healthy part of the stand). Additionally, shading by the *Azolla* mat may have led to lower sediment temperatures and, therefore, lower rates of methanogenesis (Segers 1998). However, porewater CH_4 concentrations in *Azolla* stands did show a steady increase during our experiment. When *Azolla* is not rooted in the sediment, the lack of a plant-transport pathway can result in sediment CH_4 accumulation and bubble formation (Van Der Nat & Middelburg 1998, van den Berg *et al.* 2020). The observation that ebullitive CH_4 emissions exceeded diffusive CH_4 emissions highlights the importance of quantifying this pathway in systems with *Azolla* or other floating plants. In the longer term, release of organic material from the *Azolla* mat may lead to enhanced CH_4 production and ebullition, as was observed in a two-year mesocosm experiment (Vroom *et al.* unpublished results). However, our bubble trap funnels were positioned below the floating vegetation which is likely to lead to over-estimation of the ebullitive flux that reaches the atmosphere. Bubbles rising up from the sediment may be retained naturally by the floating plants and, from there, part of the trapped CH_4 may diffuse into the water or be oxidised in the rhizosphere. Bubble retention has been found to be around 10 % at an *Azolla* cover of 100 % (Kosten *et al.* 2016). Additionally, ebullitive fluxes are likely to be under-estimated for all species, since 'trapped' bubbles may be oxidised or dissolve in between bubble trap sampling times.
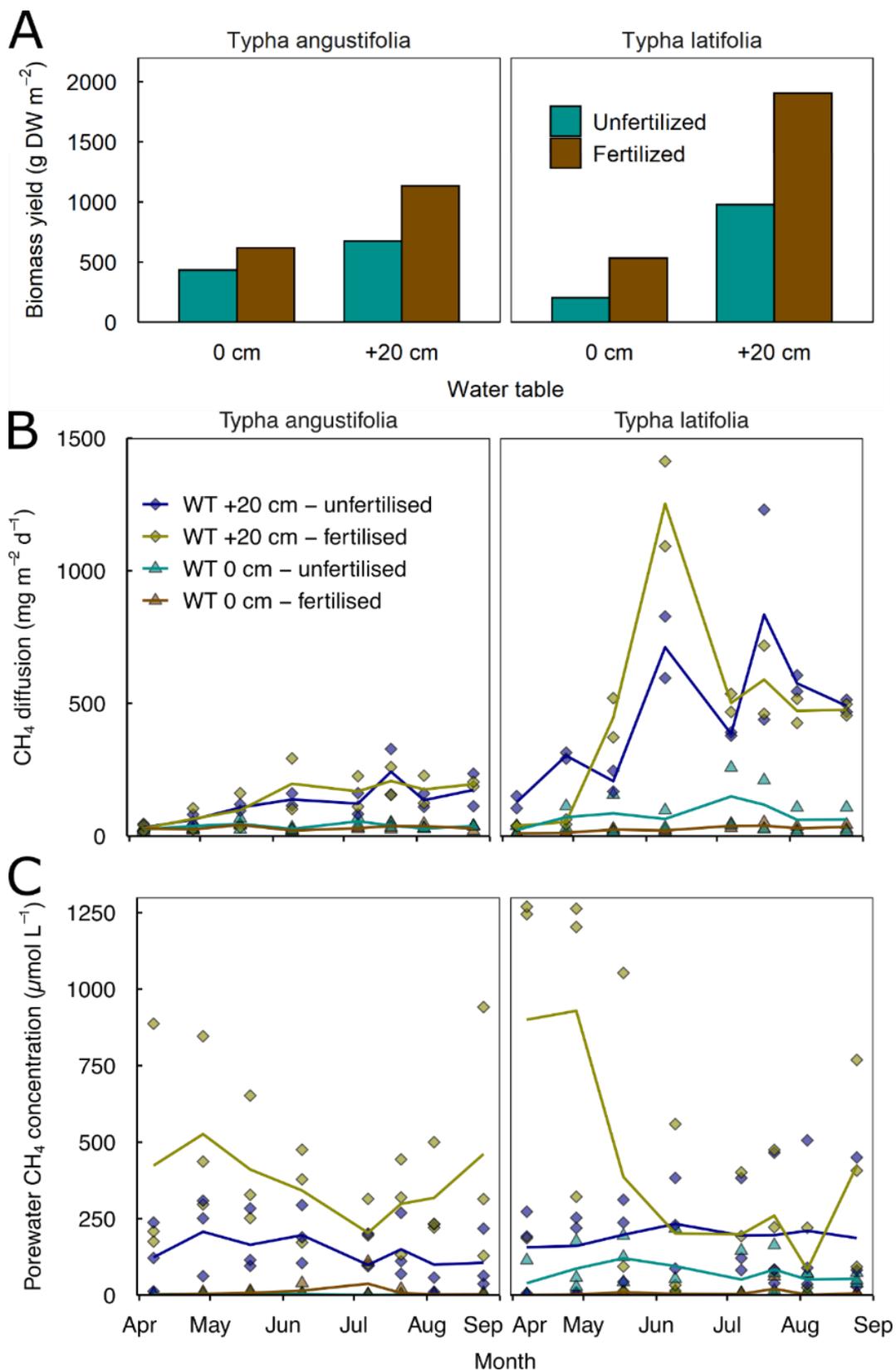
Figure 5. A: Average biomass yield measured at the end of August 2021 (n = 2 per treatment, n = 1 for the 0 cm WT, fertilised treatment). B: diffusive CH$_4$ fluxes measured in 2021 at 0 cm and +20 cm WT, with and without fertiliser application, for both *Typha* species. Symbols indicate the average of three measurements per plot (n = 2 plots per treatment for CH$_4$ diffusive flux, n = 3 samples per treatment for CH$_4$ concentration). Lines indicate average emissions per WT and fertilisation treatment.

CH$_4$ emissions from *Typha* stands were species-dependent

Typha productivity in our field site was relatively low (< 1.5 kg m^{-2} dry mass), which can be explained by high Cl concentrations, low nutrient availability and *N. typhae* invasion. Still, CH$_4$ fluxes from *Typha* were generally high (> 100 mg m^{-2} d^{-1}), especially during the summer months. This magnitude is in line with previous field studies of *T. latifolia* stands in a boreal lake (Desrosiers *et al.* 2022), a subtropical swamp (Whiting & Chanton 1996) and a temperate wetland (Yavitt 1997), but higher than *T. latifolia* in a temperate rewetted fen (Günther *et al.* 2014) and higher than expected after the substantial topsoil removal that took place (Quadra *et al.* 2023). Fewer studies exist on *T. angustifolia*: much higher CH$_4$ emissions (> 1000 mg m^{-2} d^{-1} during the warm season) were found in a temperate marsh (Strachan *et al.* 2015), while slightly lower emissions were found in one EC study in a temperate *T. angustifolia* stand (annual average 73 mg m^{-2} d^{-1}) (Fritz & van Giersbergen 2023) and two mesocosm studies (~80 mg m^{-2} d^{-1}) (Kao-Kniffin *et al.* 2010, 2011).

Generally, high CH$_4$ emissions in our site (despite top soil removal) may be explained by high dissolved organic carbon (DOC) concentrations (> 2.7 mmol L^{-1}) in the incoming surface water, supplying a carbon source for methanogenesis (Chanton *et al.* 1995, Liu *et al.* 2012). Especially in summer months, when evapotranspiration rates and water demand were high, DOC inflow may have been substantial. On the other hand, DOC concentrations in surface water and pore water were equally high in all vegetation types, ruling out the explanation that differences between vegetation types were caused by spatial differences in DOC concentrations. Our outcomes warrant further research into the effect of DOC loading and composition, and rewetting methods (e.g. flooding or ditch irrigation), on paludiculture CH$_4$ emissions.

Diffusion (including plant mediated transport) was the dominant CH$_4$ emission pathway in both *Typha* species. Porewater CH$_4$ concentrations became very low during the growing season, and ebullitive CH$_4$ emissions were low in both *Typha* species compared to *Azolla*. This indicates that plant transport substantially reduced the sediment dissolved CH$_4$ pool (Chanton 2005, Green & Baird 2012). CH$_4$ emissions were substantially higher in *T. latifolia* than in *T. angustifolia*, even though both species transport CH$_4$ through pressurised gas flow (Tornbjerg *et al.* 1994). However, *T. angustifolia* generally grows in deeper water and has higher pressurised flow rates and ROL rates than *T. latifolia* (Bendix *et al.* 1994, Inoue & Tsuchiya 2008). This could result in higher root zone CH$_4$ oxidation rates in *T. angustifolia* and subsequently lower CH$_4$ emissions, which is supported by the observed lower sediment pH in *T. angustifolia* (on average 7.39 compared to 7.77 in *T. latifolia*). A low pH could also directly affect CH$_4$ dynamics, e.g. by reducing methanogenesis (Segers 1998). Additionally, although aboveground biomass did not differ, the *T. angustifolia* stand may have been healthier compared to *T. latifolia*, as it was less affected by *N. typhae* and *T. angustifolia* has a higher salt tolerance (McMillan 1959). This may have concurred with a higher root density and, therefore, increased ROL.

Observed dips in *T. angustifolia* CH$_4$ emissions during daytime in June could be explained by decreased pressurisation and gas flow rates under illumination in this species (Bendix *et al.* 1994). The opening of stomata at sunrise increases the effective pore size above the optimum diameter (~ 0.2 µm) and could thereby reduce pressurisation efficiency (Inoue & Tsuchiya 2008, Vroom *et al.* 2022b) and CH$_4$ transport. However, ROL is not affected by illumination in this species, because the lower pressurised flow is compensated by higher photosynthetic rates and, thus, O$_2$ concentrations (Inoue & Tsuchiya 2008). Higher emissions during daytime than during night-time in *T. latifolia*, on the other hand, correspond with earlier findings of increased pressurised flow rates during daytime in this species (Chanton *et al.* 1993). It is likely that *T. latifolia* has longer and/or more tortuous sub-stomatal air pathways, increasing resistance to mass flow and explaining why illumination does not decrease pressurisation in this species (Sorrell & Brix 2003, Inoue & Tsuchiya 2008). However, increased temperatures during the day do increase internal pressurisation, and, therefore, gas flow rates (Vroom *et al.* 2022b). The presence of these diel dynamics in paludicrops emphasises the importance of capturing both day and night emissions, especially during the summer months, when creating carbon balances in paludiculture. In our case, daytime measurements may have led to an emission over-estimation for both *Typha* species during summer months, with the exception of *T. angustifolia* in June/July which had potential emission under-estimation.

Our biogeochemical data show no substantial differences between compartments, and we found no structural differences in CH$_4$ emissions between measurement spots within compartments. However, due to the experimental design, we cannot fully exclude potential spatial effects on CH$_4$ emissions (e.g. effects of the basin margins). We therefore recommend future studies to further unravel

differences between both *Typha* species in different environmental conditions (e.g. healthier stands, different water tables). Additionally, the effect of a closed chamber on gas flow through *Typha* plants (by altering e.g. temperature and humidity) warrants future investigation.

Typha CH$_4$ emissions were driven by water table, but not by nutrient addition

As expected, a water table just below the soil surface led to decreased CH$_4$ emissions, but also to lower *Typha* biomass production compared to a flooded (WT +20 cm) situation. As the water table increases from (far) below the surface to above the surface (flooded), microbial breakdown shifts from respiration and CH$_4$ oxidation (CO$_2$-dominated fluxes) to methanogenesis (CH$_4$-dominated fluxes), due to a decrease in O$_2$ availability (Blain *et al.* 2014, Minke *et al.* 2016). Assessing the balance by quantifying CO$_2$ emissions, plant carbon storage, site hydrology and economic value of the produced biomass are necessary when choosing an optimal water table. Water tables close to the surface may be more realistic for paludiculture in temperate climates, given a water surplus of 200–300 mm during winter and spring that is consumed by evapotranspiration in May through July. Water tables below the surface from June onwards would, therefore, be more common when *Typha* stands approach peak biomass (Günther *et al.* 2014).

Although nutrient addition led to a biomass increase in both *Typha* species, it did not affect CH$_4$ diffusion. This corroborates previous findings from mesocosm experiments (e.g. Bhullar *et al.* 2013, Boonman *et al.* 2023) and suggests that increasing biomass leads not only to an increase in plant-mediated CH$_4$ transport and labile carbon release, but also to an increase in ROL and subsequent CH$_4$ oxidation. Additionally, added nutrients could have affected methanogenesis (Bodelier *et al.* 2000, Veraart *et al.* 2015). Our findings imply that the admission of surface water that is rich in nutrients, particularly NH$_4^+$ and PO$_4^{3-}$ at moderate concentrations, can boost biomass production without exacerbating CH$_4$ emissions in the establishment phase of *Typha* paludiculture.

Recommendations and implications for paludiculture

Limiting CH$_4$ emissions in paludiculture could accelerate carbon sink restoration and increase stakeholder support for implementation. Examples of CH$_4$ emission drivers that can be actively managed include water table, nutrient input and paludicrop choice (Karki *et al.* 2014, Günther *et al.* 2015, Vroom

et al. 2018). We found similar CH$_4$ emissions in *Azolla* and *T. angustifolia* stands during the first period after rewetting, whereas emissions from *T. latifolia* were substantially higher. Although these results arise from only one paludiculture site with high water table and limited biomass production, and only in the third and fourth year after rewetting, this implies that even congeners with very similar morphology may have drastically different effects on carbon cycling in paludiculture. In our study, growth of *Azolla* and *Typha* was hampered by low nutrient availability as a result of substantial topsoil removal. Despite initial concerns expressed by scientific advisors, this occurred during site construction for various (practical and bureaucratic) reasons. If high biomass production is desired, we would recommend a lower amount (~10 cm) of topsoil removal, to still limit CH$_4$ emissions and nutrient mobilisation while allowing substantial biomass production (Quadra *et al.* 2023). Growth of our paludicrops was further limited by *S. rufinasus* and *N. typhae*. Further research on insect herbivory in paludiculture and possible ways to prevent infestation is recommended before scaling up to larger paludicrop 'monocultures' when high biomass production is an (economic) incentive. Meanwhile, the tradeoff between lower CH$_4$ emissions and lower biomass production at a (summer) water table around the peat surface needs to be addressed, for instance using life cycle analysis. Future research could add insights by focusing on CH$_4$ production at different soil depths and relating this to plant rooting depths at different WTs. If CH$_4$ is produced in deeper layers, emissions may be substantial even at WTs below the peat surface. Bubble release may be impacted, in turn, by plant roots. Assessing carbon dynamics in simple pilot field trials may aid in paludicrop species choice prior to scaling up; however, the effect of the incoming water (e.g. carbon input) needs critical evaluation. Lastly, to fully assess carbon dynamics in paludiculture, the effect of harvesting (including the use of machinery) on CH$_4$ emissions should be assessed, for instance by the use of eddy covariance towers during a harvest.

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AUTHOR CONTRIBUTIONS

RV planned and carried out fieldwork, analysed data, and is the lead author. TG planned and carried out fieldwork, analysed data. JvH and CvH carried out automated chamber measurements and analysed data. AS, SK and BvdR conceptualised and supervised the study and acquired funding. MvdB conceptualised and supervised the study and the manuscript, analysed data, and validated outcomes. All versions of the manuscript were written by RV and revised by TG, JvH, CvH, AS, SK, CF and MvdB. All authors approved the final manuscript.

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Appendix

Pictures of the *Typha* stands during the 2020 field season can be found at: https://doi.org/10.5281/zenodo.13348040
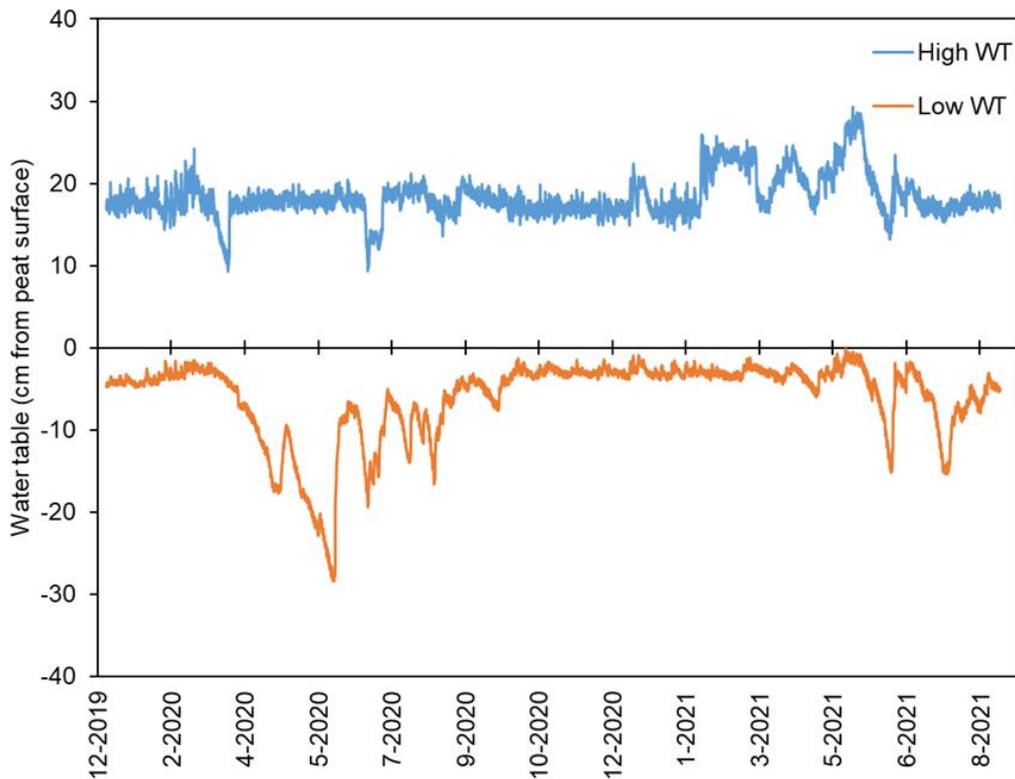

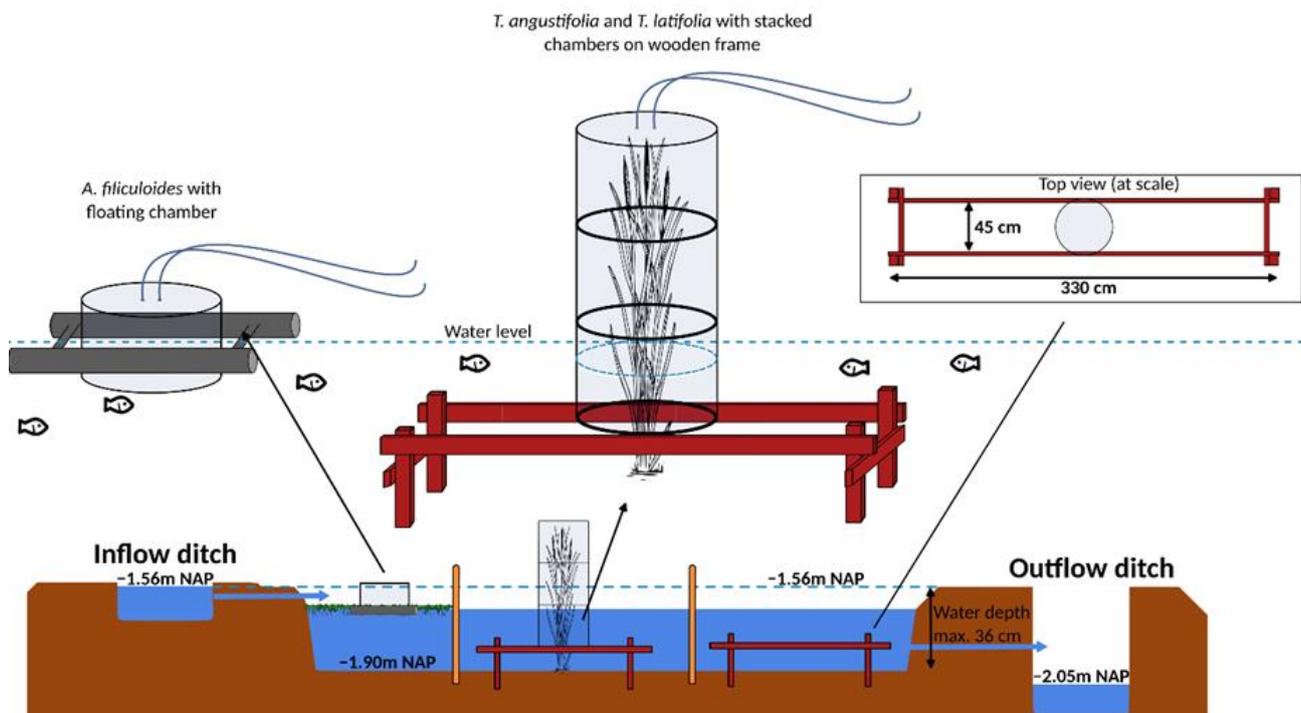Figure A1. Water table height in the +20 cm WT (B2, B4) and 0 cm WT (B1) basins.

Figure A2. Visual representation of the diffusive flux measurement setup (see also van den Berg *et al*. 2024). Water tables are relative to Amsterdam Ordnance Datum (NAP).