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Plant roots but not hydrology control microbiome composition and methane flux in temperate fen mesocosms

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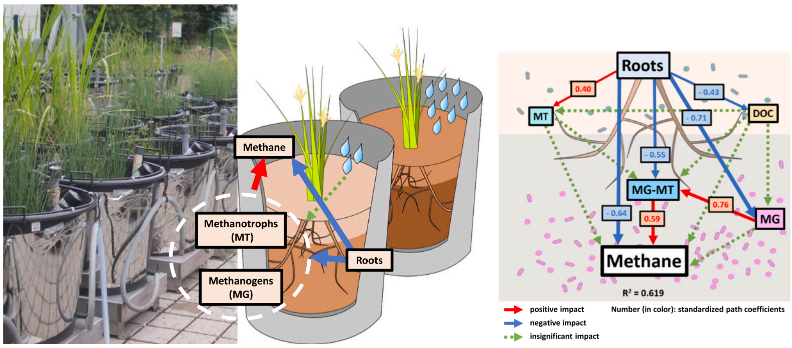
HIGHLIGHTS

- Peat microbiomes, plant root biomass and CH_4 fluxes were linked in fen mesocosms.
- Microbiome composition was mostly determined by plant presence and depth.
- Root biomass was inversely linked to methanogen relative abundances and CH_4 fluxes.
- Methanogens and methanotrophs together, in interaction, determined the CH_4 fluxes.
- Water level regimes showed limited effects on microbiomes and CH_4 fluxes.

GRAPHICAL ABSTRACT



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ABSTRACT

The rewetting of formerly drained peatlands can help to counteract climate change through the reduction of CO_2 emissions. However, this can lead to resuming CH_4 emissions due to changes in the microbiome, favoring CH_4-producing archaea. How plants, hydrology and microbiomes interact as ultimate determinants of CH_4 dynamics is still poorly understood. Using a mesocosm approach, we studied peat microbiomes, below-ground root biomass and CH_4 fluxes with three different water level regimes (stable high, stable low and fluctuating) and four different plant communities (bare peat, *Carex rostrata*, *Juncus inflexus* and their mixture) over the course of one growing season. A significant difference in microbiome composition was found between mesocosms with and without plants, while the difference between plant species identity or water regimes was rather weak. A significant difference was also found between the upper and lower peat, with the difference increasing as plants grew. By the end of the growing season, the methanogen relative abundance was higher in the sub-soil layer, as

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well as in the bare peat and *C. rostrata* pots, as compared to *J. inflexus* or mixture pots. This was inversely linked to the larger root area of *J. inflexus*. The root area also negatively correlated with CH_4 fluxes which positively correlated with the relative abundance of methanogens. Despite the absence or low abundance of methanotrophs in many samples, the integration of methanotroph abundance improved the quality of the correlation with CH_4 fluxes, and methanogens and methanotrophs together determined CH_4 fluxes in a structural equation model. However, water regime showed no significant impact on plant roots and methanogens, and consequently, on CH_4 fluxes. This study showed that plant roots determined the microbiome composition and, in particular, the relative abundance of methanogens and methanotrophs, which, in interaction, drove the CH_4 fluxes.

1. Introduction

Peatlands cover an area of approximately 488 million ha, which only amounts for 3.8 % of Earth's surface, yet store up to 33 % of Earth's total terrestrial organic carbon (600,000 Mt. C) (UNEP, 2022). However, the sequestering of carbon and the resulting climate cooling through peatlands is disturbed by draining for horticultural or agricultural means. Drainage of peatlands oxygenates the normally anoxic peat that causes degradation and increasing aerobic carbon mineralization processes by the peat microbiome and thereof leading to a massive release of the greenhouse gas (GHG) carbon dioxide (CO_2) to the atmosphere. Currently, drained peatlands worldwide emit about 2000 Mt. CO_2 equivalents (CO_2e) amounting up to 4 % of anthropogenic caused CO_2 release, not counting peatland fires that can substantially increase the CO_2 emission (UNEP, 2022). Rewetting peatlands stops CO_2 emissions effectively (Evans et al., 2021; Nugent et al., 2018; Purre et al., 2019; Wilson et al., 2022) and can reinstate them as a carbon sink. The return of the peatland to a more natural state, however, can be accompanied by increasing emissions of the potent GHG methane (CH_4). As the lifetime of this GHG in the atmosphere (~12 yrs.) is much lower than that of CO_2 (over thousands of yrs.), reduced CO_2 emissions are more relevant in the long term, causing rewetted peatlands to have a long-term cooling effect on the global temperature (Frolking et al., 2006).

The legacy of the pre-rewetting disturbance, however, can change local plant communities that differ from original peatlands by more fluctuating water tables and higher bulk density of the remaining peat, thus forming novel ecosystems (Emsens et al., 2020; Kreyling et al., 2021). However, the legacy impacts on the peat microbiome in these novel ecosystems are not well understood yet. Since water content is a major driving factor for changes in pro- and eukaryotic communities, rewetting does also change the GHG fluxes (Weil et al., 2020). For peatlands, where rewetting leads to large-scale inundation or frequent water table fluctuations, the increase in CH_4 emissions due to higher activity of methane-producing archaea (methanogens) might be a potential problem (Putkinen et al., 2018; Reumer et al., 2018; Wen et al., 2018; Urbanová and Bárta, 2020).

The interplay between plants, peat, water, and the peat microbiomes as ultimate determinants of CH_4 dynamics is still poorly understood, despite recent progress in field studies. Investigating all the environmental effects on the microbiome, especially on the methane producing archaea, and the resulting changes in the GHG (e.g., CH_4) emissions, is in fact challenging. For instance, water table fluctuations and nutrient dynamics as well as parameters such as salinity, nitrogen and phosphorus concentrations are influenced by seasonal dynamics (Kieckbusch and Schrautzer, 2007; Feng et al., 2020; Wang et al., 2021). Additionally, plants and their roots are in close interaction with the peat soil microbiome, hence changes in vegetation also affect the microbiome composition (Elliott et al., 2015; Ward et al., 2015; Ritson et al., 2020). The aforementioned studies have shown that plants have an impact on the soil microbiome and that the microbiome itself is the major source of methane. The relationship between plants and the microbiome has been broadly studied (Bieniada et al., 2023; Elliott et al., 2015; Ritson et al., 2020; Ward et al., 2015), but only a few studies have linked this relationship to GHG fluxes.

In situ studies struggle to disentangle explicit functional linkages between plants, peat, water and the microbiome composition and activity due to the complexity of the system. Robust explanatory models for CH_4 fluxes are consequently missing so far. Mesocosm studies are well suited to resolve some of these linkages by reducing complexity and increasing experimental control as compared to in situ studies.

Using such a mesocosm approach, we aimed to obtain a mechanistic understanding of the rewetting impact on a temperate fen ecosystem. We set up three different water level regimes (stable high, stable low and fluctuating) as well as colonization with two different native plants, *Carex rostrata* Stokes and *Juncus inflexus* L., and their mixture during one growing season. Experimental manipulations of water table regimes and repeated measurement of plant root area gave us the opportunity to dissect the relative importance of those factors on the microbiome dynamics and ultimately CH_4 fluxes. We hypothesized that water table manipulations will have a larger effect on the microbiome composition and resulting CH_4 fluxes than plant identity and composition, and that microbiome composition, especially methanogen abundance, is tightly linked to CH_4 fluxes.

2. Material and methods

2.1. Experimental design

The mesocosm experiment was conducted at the mesocosm facility of the University of Greifswald (54,091878 N, 13,3644833E) as part of the WETSCAPES project (Jurasinski et al., 2020) (wetscapes.de) to study the effects of rewetting on peat ecosystems. For this purpose, peat was excavated out of a percolation fen near the village Tribsees (northern Germany, 54,0770 N, 12,7414E) from a depth of at least 2 m in August 2018. The peat was mixed and filled into 36 pots of 125 L each (3 water regimes × 4 plant communities × 3 replicates). Each pot has a height of 54 cm and a diameter on the top of 64 cm and narrow down to a diameter of 60 cm at the bottom. All pots were inoculated on 5th of December 2018 with ditch water from a fen peatland (54°05′59.1″N 12°44′14.2″E) rewetted about 20 years ago (Fig. 1). Three water regimes were assigned to represent different peatland conditions: stable high, i.e. water level constantly at the peat surface, resembling successful rewetting; stable low, i.e. 20 cm below the peat surface, resembling insufficient rewetting; and fluctuating, i.e. changing between low and high every second week, mimicking the increased water table fluctuations commonly found after rewetting. The water regimes were maintained by a weight-based automated irrigation system using rainwater from a local cistern and local tap water in case the cistern ran empty.

The plants used in the mesocosms were sampled from a semi-natural fen peatland (53°55′21.7″N 13°25′41.4″E) on 29th of September 2018 and larger bunches or sods were divided into single, evenly sized tillers in the following day. The tillers were individually planted into 10x10x20 cm^3 pots filled with the target peat substrate until planting them into the mesocosms. Eight plants were planted into each mesocosm as equidistant as possible on Oct. 29th. The plant community treatment consisted of four levels, i.e., monocultures of *Carex rostrata* and *Juncus inflexus*, their mixture (four individuals per species), and a bare peat control. The whole mesocosm experiment was conducted outside. All pots were subject to the ambient temperature and natural day and night cycles that were the same as for the place where the original peat soil was taken from.

2.2. Belowground plant root area

Root area was monitored using minirhizotrons as described in Schwieger et al. (2021). Briefly, one transparent tube was installed in each pot at an angle of 45° in the soil to insert a root image scanner (CI-600 In-Situ Root Imager; CID Bio-science Inc.), taking *c.* 350° scans (image size: 21.6 × 19.6 cm) of the tube-soil interface and thus roots at two depths (0–15 and 15–30 cm). Light was excluded from the tubes with a cap and the tubes were wrapped with mirror foil to reduce thermal differences. The minirhizotrons were scanned biweekly, and root area was measured as the percentage of root-covered area to the total area of the minirhizotron scan quantified with the AI-based algorithm "RootDetector" (Peters et al., 2023). Root area is used as a proxy of the belowground production in this study.

2.3. Methane flux and dissolved organic carbon

The CH_4 flux was measured similarly to Wang et al. (2023). During the measurement, an opaque circular chamber (volume = 0.22 m^3) made from high-density polyethylene (HDPE) was fixed on each pot for 3 min. The chamber was equipped with fans to ensure the consistent mixing of the air inside during measurements. CH_4 concentrations were measured biweekly using a G2508 Gas Concentration Analyzer (Picarro, Santa Clara). Calculation of CH_4 fluxes follows Wang et al. (2023). For each flux measurement, the porewater samples were taken with Rhizon samplers from a depth of 10 cm below surface. The concentration of the dissolved organic carbon (DOC) was measured using a DIMATEC analyzer. The root area, DOC and methane flux data matching the microbiome samplings (Fig. 1) were used for downstream analyses.

2.4. Peat sampling and DNA extraction

We took triplicate soil samples from each mesocosm at four different time points in the year 2019 (April, June, August, and October) from two different depths, upper peat layer (0–5 cm) and lower peat layer (25–30 cm) using a Mini ViscoSampler (Bürkle, Bad Bellingen, Germany). Triplicates were mixed on a sterile petri-dish and stored in a sterile 1.5 mL-tube which was put on dry ice and later stored at −70 °C until further processing. About 250 mg of soil material was subjected to DNA extraction using the DNeasy Power Soil™ kit (QIAGEN, Hilden, Germany). The bead beating step was performed in a FastPrep® machine with the following parameters: intensity of 5 m/s for 45 s. The extracted DNA was quantified using Qubit™ 4 Fluorometer and stored at −80 °C.

2.5. Sequencing and data processing

Bacterial and archaeal 16S rRNA genes were amplified from the extracted DNA using a nested PCR protocol with the 16SF_515YF_lib and 16SR_B806R_lib primer pair (Caporaso et al., 2011, 2018; Apprill et al., 2015), targeting the V4 region of the 16S rRNA gene plus a sequence which was also used as the primer for the second round PCRs. The primers were also indexed for the second round PCRs. The amplicons were cleaned using the Nucleofast® 96 PCR-Clean-up kit (Macherey-Nagel), pooled and then sequenced on a MiSeq instrument (Illumina) (2 × 250 bp). After demultiplexing, the data was processed using the *dada2* protocol in R v4.0.3. The raw sequences were truncated at 250 and 200 bps for forward and reverse reads, respectively. Sequences failing to meet the filter criteria (maxEE = 2, truncQ = 2, maxN = 0) were discarded. Those filtered sequences were de-replicated, the amplicon-sequencing-variants (ASVs) were deduced, and the paired-end sequences were merged. Afterwards the chimeric sequences were removed. The sequence of each ASV was assigned to taxonomy using the SILVA SSUref_NR_138 database. Furthermore, ASVs with only one sequence and ASVs that were assigned to mitochondria and chloroplasts were removed. Additionally, samples with sequence numbers below 3000 were removed, leaving 256 samples for downstream analysis, with a total of 25,329 ASVs.

2.6. Downstream analysis

The complete downstream analysis was done using R v4.0.3 (R Core Team, 2021). Principal Coordinate Analysis (PCoA) based on the Bray-Curtis-Dissimilarity was performed to reveal the microbial community compositions. Furthermore, correlations between relative abundance of methanogens (MG) and methanotrophs (MT), methane fluxes and root area were investigated with scatter plots, and Pearson's method was used to test these correlations. The significance of the impact of factors on community compositions was determined by permutational multivariate analysis of variance (PERMANOVA) using *vegan* package v2.5.7. Since some data were not normally distributed, Kruskal−Wallis post hoc Dunn's tests were performed to compare the means of methanogen

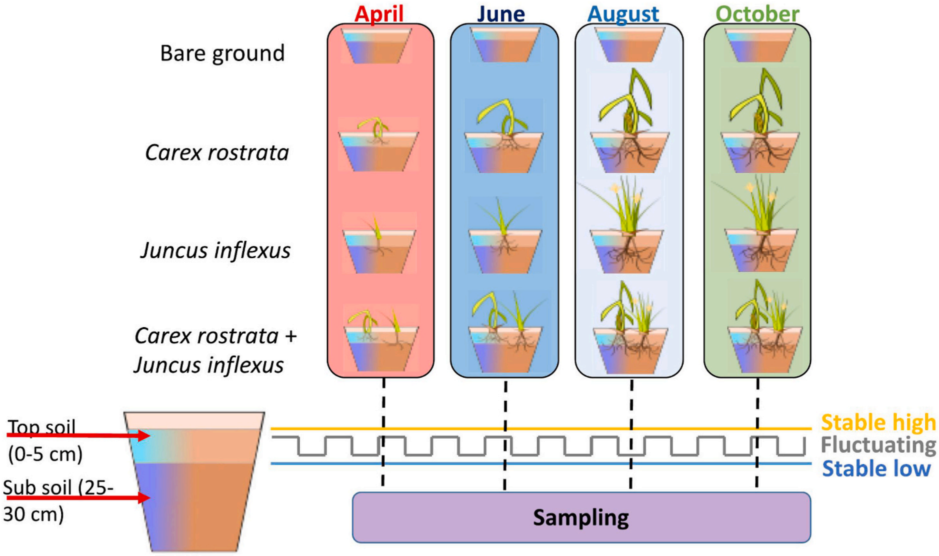

Fig. 1. Scheme of the mesocosm experiment showing the pot setup with the corresponding water regimes, plant communities, sampling depth and sampling time points. The periods of fluctuating water level changed every two weeks.

relative abundance, methane fluxes and root area between different plant communities, water regimes, soil depths, and time points using *vegan* package v2.5.7. The *p*-values were adjusted using the “false discovery rate” (fdr) method (Benjamini and Hochberg, 1995). In all cases, the statistical significance level $\alpha = 0.05$ was used.

Structural equation modelling (SEM) was performed to explore the causes and consequences of microbiota changes by assuming that plants could directly influence CH_4 fluxes and indirectly through their effect on the methanogen and methanotroph abundances which further determine CH_4 fluxes. Water table was initially included in the model. However, water table showed no significant impact on any variables in the model and was therefore excluded from the model. SEM was constructed using covariance-based method with *lavaan* package (Rosseel, 2012). All variables were checked for normality, and the non-normally distributed ones were $\log_{10}$ transformed. A minimum value (0.0001) was added to variables with negative values to generate positive values before $\log_{10}$ transformation, including methanogen and methanotroph relative abundances and their subtraction. Since all values were smaller than 1, the values turned negative. The multivariate normality of the final dataset showed insignificant multivariate Kurtosis.

2.7. Accession numbers

All sequencing data were deposited in the European Nucleotide Archive of European Molecular Biology Laboratory with the study accession number PRJEB61714 (secondary Accession: ERP146796).

3. Results

3.1. Spatial and temporal dynamics of peat microbiome composition

Depth was the strongest factor impacting the microbiomes (PCoA-Fig. 2A, PERMANOVA $R^2 = 0.15$, $p = 0.001$), in which the upper peat layer microbiomes differed strongly from the ones in the lower layers.

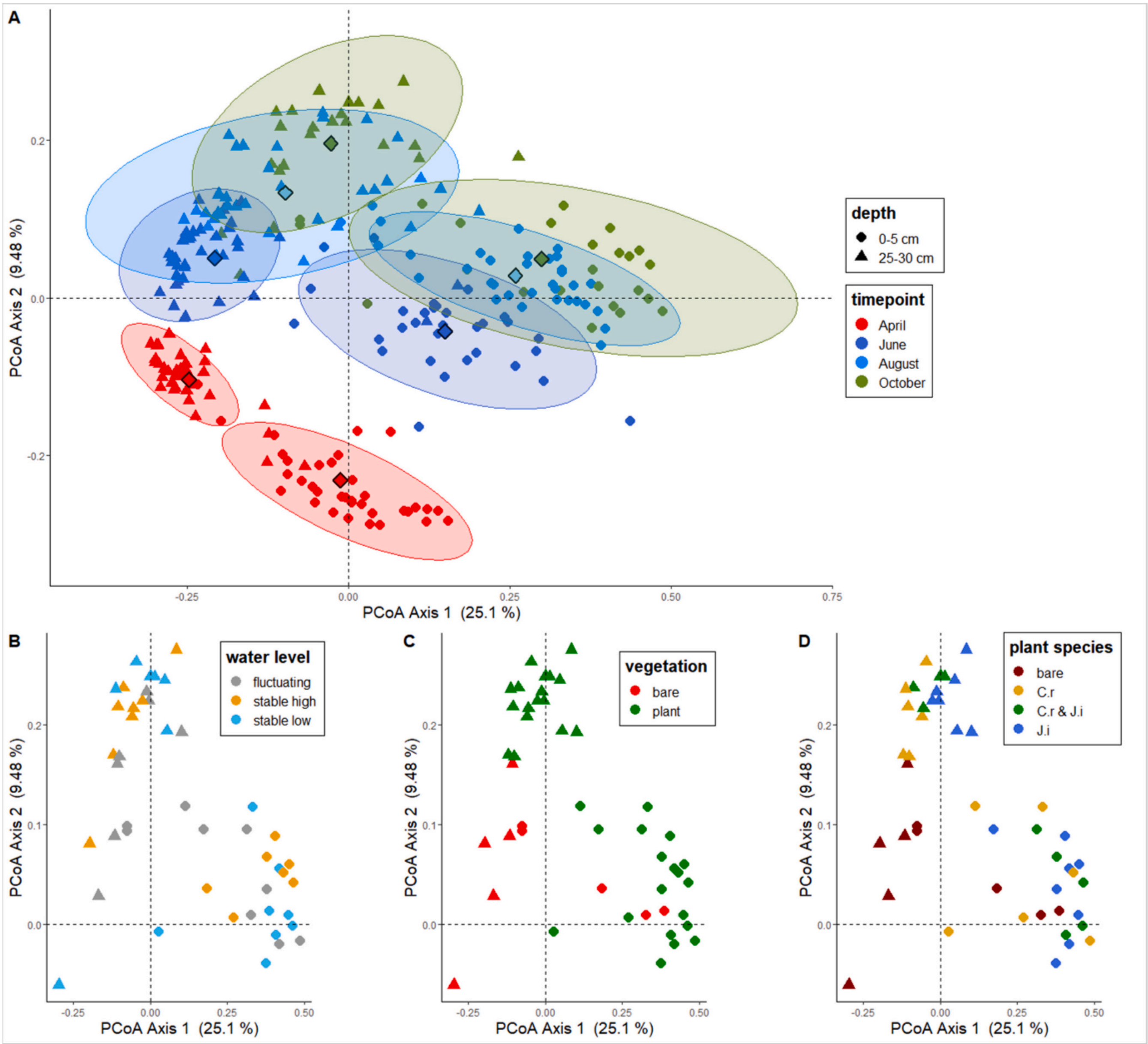

Fig. 2. Spatial and temporal changes in beta-diversity of fen microbiota. A: PCoA plots of the peat microbiomes labelled by sampling time and depth. B: Microbiomes labelled by water level and depth (October). C: Microbiomes labelled by presence of plants and depth (October). D: Microbiomes labelled by plant species and depth (October).

This, however, changed with time as the microbiomes developed a larger alpha-diversity in the upper layers (data not shown), indicated by the variance. Especially the microbiome composition had a larger variability in August and October when compared with the microbiome at earlier time points. The difference in microbiome composition between different water regimes was rather weak but still significant (Fig. 2B, $R^2 = 0.01, p = 0.003$). Microbiomes from mesocosms with plant presence differed clearly from the bare peat pots (Fig. 2C, $R^2 = 0.02, p = 0.001$). This distinction was clearer in the lower than in the upper layer. A detailed analysis of the plant species effect showed that there was a significant difference between the lower peat microbiomes in *C. rostrata* and *J. inflexus* pots (Fig. 2D, $R^2 = 0.31, p = 0.001$). Furthermore, in the lower peat, the microbiome composition of mixed plant species was in the middle between microbiome compositions of the two respective single species in the ordination space (Fig. 2D).

3.2. Methanogen and methanotroph compositions and abundances

To get insights into the functional guilds responsible for CH_4 production and oxidation, we analyzed the composition and abundance of methanogenic archaea (Fig. 3) and methanotrophic bacteria (Fig. 4), respectively. Hereafter, the (relative) abundance refers to the proportion of methanogens or methanotrophs to the total prokaryotes. Five orders of methanogenic archaea were abundant: *Methanosarciniales*, *Methanomicrobiales*, *Methanomassiliicoccales*, *Methanobacteriales* and *Methanocellales* (Fig. 3), involving methanogenic groups conducting all three main methanogenic pathways, i.e. hydrogenotrophic, acetoclastic and methylotrophic methanogenesis. At every time point, consistently higher relative abundances of methanogens (0.7 %) were observed in the lower peat as compared to the upper peat (0.4 %) microbiomes (Fig. 3). In both peat layers, a decline of their relative abundance was observed with time in the planted pots, while the relative abundance was rather stable in the bare peat (Fig. 3). The constant decline of the methanogen abundance over time with plants resulted in a much lower abundance of methanogens in the plant pots compared to the bare peat in October (Fig. 3).

The relative abundance of methanotrophs was much lower, accounting for only up to 0.15 % of the total microbial community (Fig. 4). The methanotroph abundance showed a similar change over time as observed with methanogens in the plant pots, while the abundance in the bare peat declined in June and August, followed by a sharp increase in October (Fig. 4). The methanotroph abundance was much higher in the upper peat layer and in the bare peat (Fig. 4).

3.3. Links between microbiomes, plants, and methane fluxes

In October, the highest methanogen abundance was found with bare peat (~0.72 %), followed by *C. rostrata* pots (~0.14 %); abundance of methanogens in pots with *J. inflexus* and with mixed species was significantly lower (Fig. 5A). However, no significant difference in

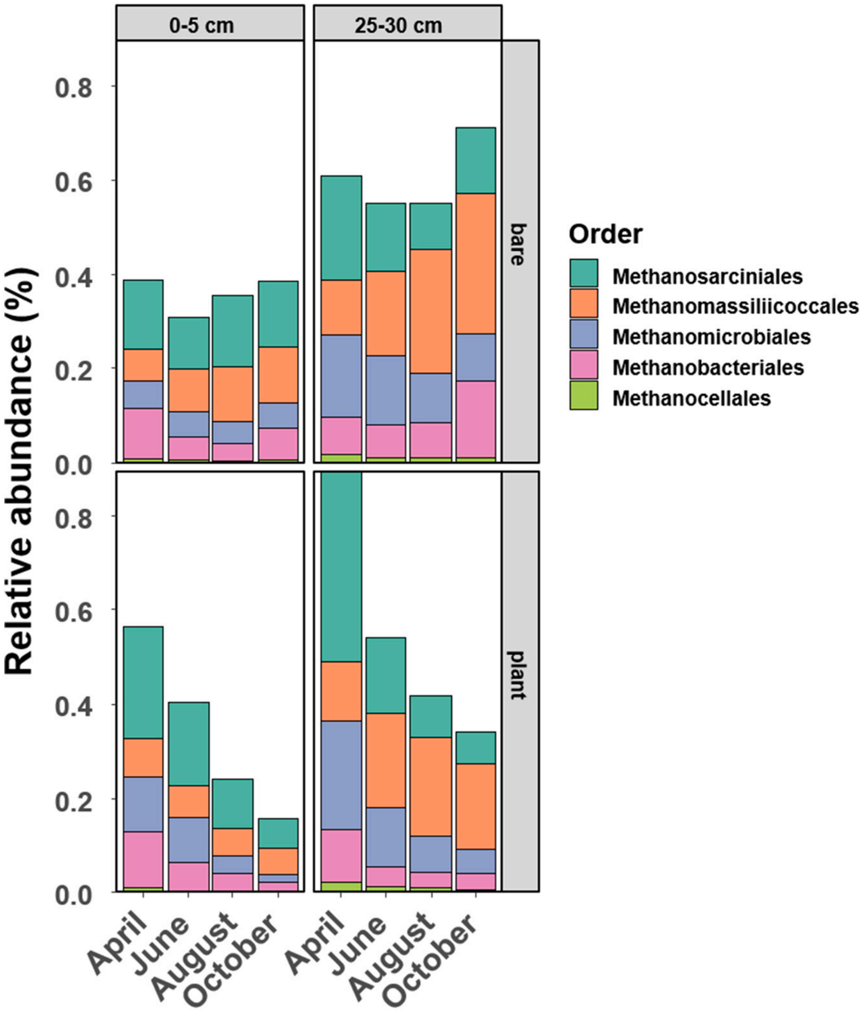

Fig. 3. Barplots depicting the relative abundance of different methanogen orders for all time points at two depths. Bare, pots without any plants; plant, all pots with plants.

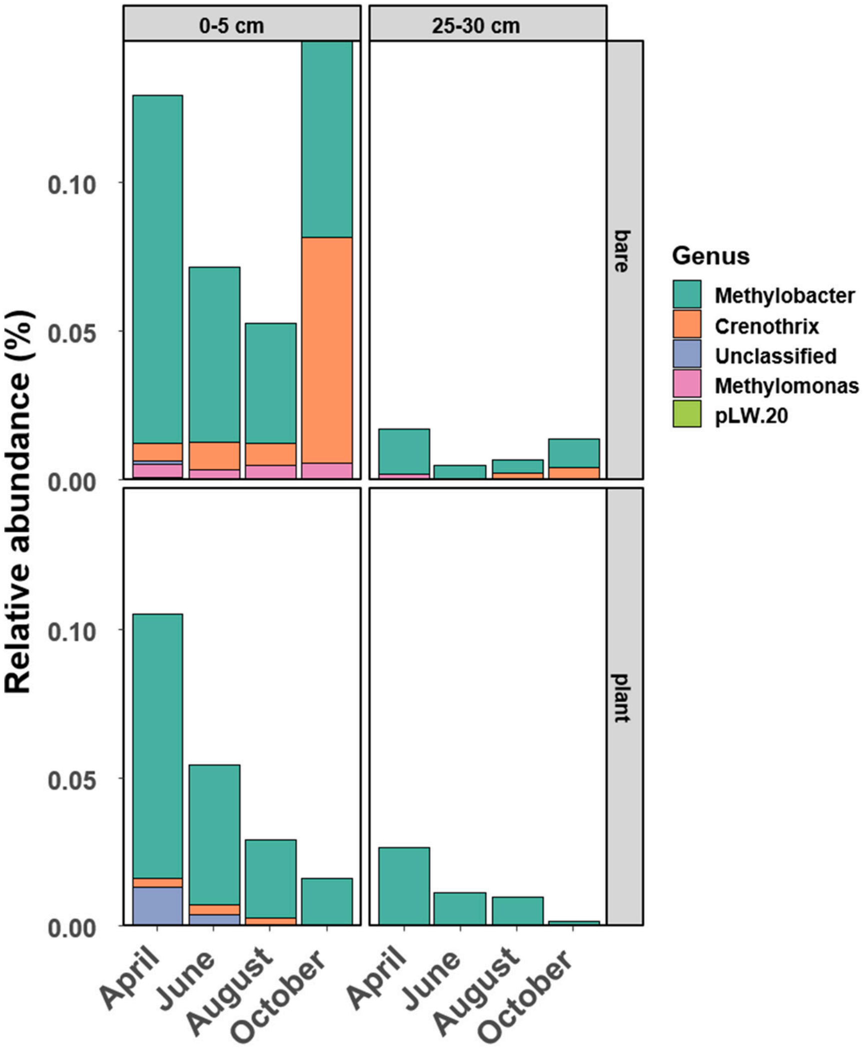

Fig. 4. Barplots depicting the relative abundance of different methanotroph orders for all time points at two depths. Bare, pots without any plants; plant, all pots with plants.

methanogen abundance was observed between different water regimes (Kruskal-Wallis test $p > 0.05$). Similarly, methane fluxes were significantly higher in bare peat and *C. rostrata* pots than in *J. inflexus* and mixed species pots (Fig. 5B). The root area mirrored that pattern with larger root areas in pots with *J. inflexus* and the mixed pots compared with bare and *C. rostrata* pots (Fig. 5C). The concentration of DOC was not significantly different between pots with different plant communities (Fig. 5D).

Linear correlation analysis revealed a significant, positive correlation between methanogen abundances and methane fluxes across all mesocosms (Fig. 6A). Root area was significantly and negatively correlated with both methanogen abundance and CH_4 flux (Fig. 6B and C), suggesting a negative plant impact on methanogens and thus CH_4 fluxes in peat. Due to the low abundance and absence of methanotrophs in most samples, the correlations between methanotrophs and CH_4 fluxes could not be statistically verified. However, since methanogens and methanotrophs both impact CH_4 dynamics, the subtraction between total methanogen and methanotroph abundances (MG-MT) was applied. Interestingly, an improved correlation with CH_4 fluxes was observed compared with methanogen abundance alone (Fig. 6A and D).

A structural equation model was constructed to show the causes and consequences of microbiome changes in our mesocosm system (Fig. 7). The root area was assumed as the initial factor impacting the DOC and they together impacted the methane-cycling microbiomes which further determined the CH_4 fluxes. The CH_4 fluxes might also be impacted directly by roots and DOC. The root area showed significant influences including a positive impact on methanotroph abundance and a negative impact on DOC, methanogen abundance and MG-MT. DOC showed no significant influence on the microbiomes. Neither methanotroph nor methanogen abundance significantly impacted the CH_4 flux, while the difference of their abundances showed a positive and strong impact on the CH_4 flux. Despite that MG-MT was largely determined by methanogen abundance (Fig. 7), these results suggested that the interplay of methanogens and methanotrophs, together with plants, determined CH_4 fluxes in the experimental setup. The abiotic and biotic factors in total explained 61.9 % of the variance of methane fluxes.

4. Discussion

Plant roots had a strong impact on the microbiome composition and methanogen abundance, which further influenced CH_4 fluxes, while the water regime showed a weak impact in our peatland mesocosm experiment. This contrasted with our hypothesis that water level regimes would have a large effect on the microbiome composition and resulting CH_4 fluxes. Although methanogen abundance correlated significantly with CH_4 fluxes, the difference between methanogen and methanotroph abundances was determining the fluxes in the structural equation model.

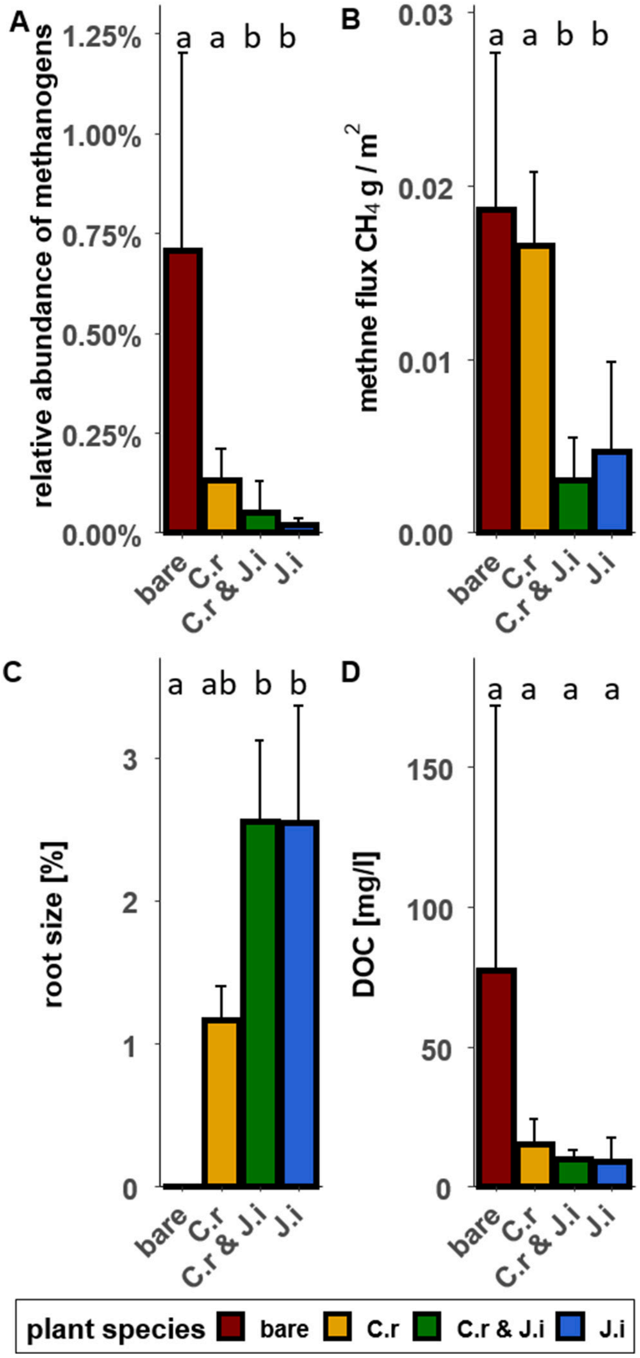

Fig. 5. Barplots showing relative abundance of methanogens (A), methane flux (g m^{-2} h^{-1} CH_4) (B), root area (% scanned area) (C) and amount of dissolved organic carbon (DOC [mg/L]) (D) in bare peat and the corresponding plant community mesocosms in October. Difference is significant when no same letter exists between groups (adjusted $P < 0.05$), $n = 3$.

While plant species identity had only a mild impact, the difference between plant-presence and bare peat microbiome compositions was strong, especially in the lower layer. Thus, plant presence and plant identity played an important role in the structuring of the microbiome, especially in the later time points of the growing season, which is in line with the view that plants are major architects and controllers of the below-ground ecosystem.

Field data suggests that rewetting changes the peat biomes (bacteria, archaea, fungi, protists) as compared to drained fens (Emsens et al., 2020; Jurasinski et al., 2020; McNicol et al., 2020; Weil et al., 2020; Wang et al., 2021, 2022, 2023). These shifts indicate drastically changed functions in the below-ground microbiome. For instance, the functional guild of methanogens within the complex peat microbiome is the most important determinant of the potential flux of CH_4 from wetlands to the atmosphere (Wang et al., 2023). Unexpectedly, the water regime affected the microbiome, albeit to a much lesser extent than expected. Such a minor effect was also found in another mesocosm experiment (Potter et al., 2017). This is in contrast to in situ studies, where the microbiomes of drained and rewetted sites differed strongly (Emsens et al., 2020; Weil et al., 2020). This discrepancy can have several causes. For one, the microorganisms are resilient to short term changes in the water table and effects of drought and flooding (Andersen et al., 2013; Basiliko et al., 2013; Kitson and Bell, 2020; Emsens et al., 2020; Reumer et al., 2018; Ritson et al., 2020). Another explanation is that the lower peat layers were always saturated with water during our experiment, thereof creating stable anoxic niches for microorganisms (Kitson and Bell, 2020). Another reason might be related to the rather short time frame of our experiment, in which the microbiomes might not have had time to adapt (Kitson and Bell, 2020), as compared to the decade-long rewetting in some of the in situ studies (Weil et al., 2020). We found a strong temporal dynamic during the time of the mesocosm experiment. This dynamic development of the original microbiome stemming from deep peat was also evident in the compositional difference between the microbiomes in the upper and lower peat layers at the onset of the sampling in April 2019. At that time the microbiomes that originally stemmed from the same deep peat sample had developed in the pots for six months. The microbiome as CH_4 drivers at that time was “mature” and showed the most distinct differentiation.

Moreover, it is even more difficult to disentangle plant and water level effects in field studies, as plant species identity or root biomass are important parts of the ecosystem response to the water level. The scale of water level is also different from our mesocosms, as the water level of −20 cm used in our study would be considered almost wet in the field while deeply drained fens are considered with a water level < -50 cm. Lastly, the peat used for mesocosms was from deep layer (< -2 m) of the field site whereas the depth effect might play a role in the monitoring in situ.

The water regime also showed no strong influence on the methanogen relative abundance. The effect of plant presence on methanogens and the resulting CH_4 fluxes, however, was particularly evident. Methanogens were most abundant in the pots without plants, both in lower as well as upper peat microbiomes, while their relative abundance was lower in the presence of plants. Also, the relative abundance of methanogens kept declining over time as the plants grew and their root systems expanded. One likely explanation for this effect was the development of a root density that facilitated the diffusion of oxygen into the peat, creating oxic and suboxic zones in an otherwise anoxic environment (Armstrong, 1971; Flessa and Fischer, 1992; Brune et al., 2000; Galand et al., 2005). Additionally, root exudates distributed through the roots to the surrounding rhizosphere might have influenced the development of the microbiome as well (Galand et al., 2005; Badri et al., 2009; Chaparro et al., 2012).

It was remarkable to observe a differential effect of the plant species onto the methanogens and the general microbiome: mesocosms with *J. inflexus* had a lower relative abundance of methanogens as the ones with *C. rostrata*. The possible explanation of this effect is the belowground root area, which was much larger for *J. inflexus* (Henneberg et al., 2016). More roots likely distribute more oxygen in the surrounding area, which is detrimental for the obligatory anaerobic methanogens (Murray et al., 2019). This was evident when the methane emissions correlated positively with the relative abundance of methanogens but correlated negatively with the root area. A previous study confirmed that wetland plant species significantly impacted the methanogen abundance and thus methane emissions through changing dissolved oxygen levels in their rhizospheres, and this effect was even stronger than temperature changes (Zhang et al., 2018).

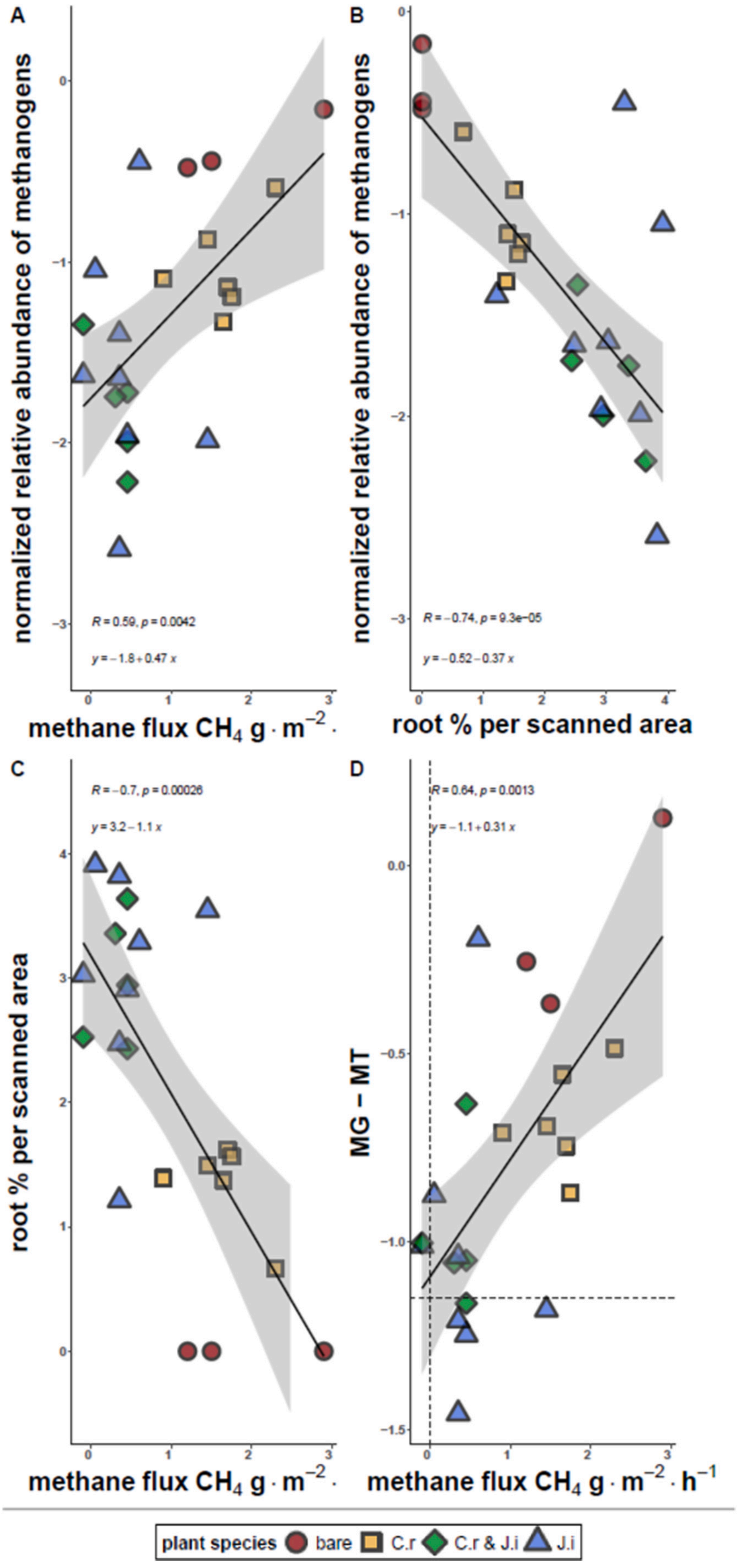

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Fig. 6. Correlation analyses between relative abundance of methanogens and methane flux (A) or root area (B), between methane flux and root area (C) or MG-MT (D). The dotted lines in the scatter plots indicate the 0 values in the original data. MG, methanogen abundance; MT, methanotroph abundance; MG-MT, subtraction between methanogen and methanotroph abundances. A minimum value (0.0001) was added to relative abundance data to avoid zeros before $\log_{10}$ transformation (normalization).

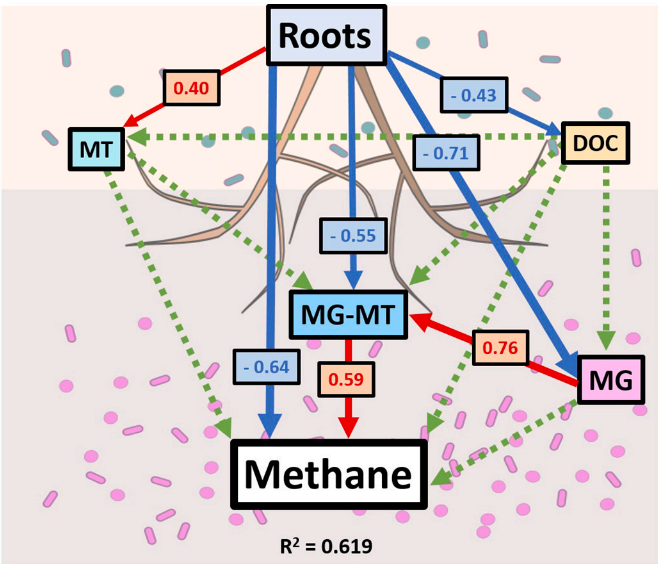

Fig. 7. Structural equation model showing causes and consequences between different factors. The dotted arrows indicate insignificant impacts, while the red and blue solid arrows indicate significantly positive and negative impacts, respectively. The numbers along with arrows are standardized path coefficients. The width of the arrows is scaled with coefficient values. Model statistics: df = 1; $p = 0.088$; CFI = 0.974; $\text{RMSEA}_{\text{lower 90\% CI}} = 0$; SRMR = 0.048; $\chi^2 = 2.907$. MG, methanogen abundance; MT, methanotroph abundance; MG-MT, subtraction between methanogen and methanotroph abundances.

While the methanogen abundance correlated significantly and positively with CH_4 fluxes, the integration of methanotroph abundance improved the quality of the correlation with CH_4 fluxes. It was surprising that the subtraction between methanogen and methanotroph relative abundances, but not the methanogen or methanotroph relative abundance alone, significantly determined the CH_4 flux in the structural equation model. This suggested this relationship as a potential promising microbial proxy in predicting CH_4 flux in peat. Supporting this, one study showed that the interaction between functional gene markers of methanogens and methanotrophs better correlated with CH_4 fluxes in response to summer drought in rewetted peatland soils, when compared to either marker alone (Wang et al., 2023). However, integrating the environmental characteristics (e.g., temperature, redox, substrate level) and other associated biotic factors (e.g., microbial predators) is needed to establish a full predicting model.

In conclusion, this interdisciplinary study sheds light on how the complex interplay between plants, hydrology and the fen microbiome affect CH_4 emissions. Remarkably, water regime showed a limited impact on the microbiome composition and no significant effect on methanogen relative abundances, which is in contrast to our hypothesis and field studies. This discrepancy indicates the necessity to disentangle plant, water, and other effects in field studies. Importantly, it also indicated that the presence of plants as well as the plant species identity determined the relative abundance of methanogens and methanotrophs, microbiome composition and thereby the resulting CH_4 fluxes accordingly. Rooting intensity appeared to be the major link from the plants to the microbiome. Thus, future restoration and rewetting projects including paludiculture, should take belowground plant production into account, if CH_4 emissions are an important factor for consideration.

CRediT authorship contribution statement

Marc Piecha: Writing – original draft, Visualization, Investigation, Formal analysis, Conceptualization. **Juergen Kreyling:** Writing – review & editing, Methodology. **John Couwenberg:** Writing – review & editing. **Michael Pester:** Writing – review & editing, Resources. **Anke Guenther:** Writing – review & editing, Investigation. **Levke Henningsen:** Investigation. **Micha Weil:** Investigation. **Gerald Jurasinski:** Writing – review & editing, Resources. **Gesche Blume-Werry:** Writing – review & editing, Investigation. **Tim Urich:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization. **Haitao Wang:** Writing – review & editing, Supervision, Methodology, Formal analysis, Data curation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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