



# To Harvest or not to Harvest: Management Intensity did not Affect Greenhouse Gas Balances of *Phalaris Arundinacea* Paludiculture

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## Abstract

The cultivation of flooding-tolerant grasses on wet or rewetted peatlands is a priority in climate change mitigation, balancing the trade-off between atmospheric decarbonisation and biomass production. However, effects of management intensities on greenhouse gas (GHG) emissions and the global warming potential (GWP) are widely unknown. This study assessed whether intensities of two and five annual harvest occurrences at fertilisation rates of 200 kg nitrogen ha<sup>-1</sup> yr<sup>-1</sup> affects GHG exchange dynamics compared to a ‘nature scenario’ with neither harvest nor fertilisation. Fluxes of carbon dioxide (CO<sub>2</sub>), methane (CH<sub>4</sub>), and nitrous oxide (N<sub>2</sub>O), using opaque and transparent chambers, were measured on a wet fen peatland with a mean water table depth of -10 cm below soil surface. Overall, no treatment effect was found on biomass yields and GHG emissions. Annual cumulative CH<sub>4</sub> emissions were low, ranging between 0.3 and 0.5 t CO<sub>2</sub>-C eq ha<sup>-1</sup> yr<sup>-1</sup>. Contrary to this, emissions of N<sub>2</sub>O were high, ranging between 1.1 and 1.5 t CO<sub>2</sub>-C eq ha<sup>-1</sup> yr<sup>-1</sup>. For magnitudes of CH<sub>4</sub> and N<sub>2</sub>O, soil moisture conditions and electrical peat properties were critical proxies. Atmospheric uptake of CO<sub>2</sub> by net ecosystem exchange was higher for the treatments with management. However, this benefit was offset by the export of carbon in biomass compared to the treatment without management. In conclusion, the results highlighted a near-equal GWP in the range of 10.5–11.5 t CO<sub>2</sub>-C eq t ha<sup>-1</sup> yr<sup>-1</sup> for all treatments irrespectively of management. In a climate context, a restoration scenario but also intensive paludiculture practices were equal land-use options.

**Keywords** Paludiculture · Peatland · Greenhouse gas · Harvest · Management intensity · Reed canary grass

## Introduction

Development regarding the cultivation of flooding-tolerant crops on wet or rewetted peatlands, known as ‘paludiculture’ (Tanneberger et al. 2021), was recently highlighted to play a major role as a key priority in mitigating climate change (Evans et al. 2021). This is due to a conflict in global needs in which the first aspect is societal dependence on arable land in times of a growing world population but the simultaneous need for ecological conservation (Mehrabi et

al. 2018). On the other hand, decarbonisation is inevitable in order to limit global warming within the next 30 years to a peak warming of 2 °C (Iyer et al. 2022; Meinshausen et al. 2022). A nature-based solution to decarbonisation is peatland protection and rewetting, known to limit or, in some cases, stop or reverse radiative forcing due to the prevention of high carbon dioxide (CO<sub>2</sub>) emissions by waterlogging (Günther et al. 2020).

Though knowledge on greenhouse gas (GHG) emission factors (EF) for drained, rewetted, and pristine peatlands is fragmented, data exist with an increasing abundance. Contrary to this, there is a lack of disaggregated knowledge regarding emission dynamics for wet or rewetted peatlands under paludiculture practices (Bianchi et al. 2021). The main drivers of variation regarding CO<sub>2</sub> emissions from peatlands are well known, being mainly dependent on the dynamics of water table depth (WTD), biomass growth, and biogeochemical stabilisation of soil organic matter (Moore and Dalva 1993; Skinner 2008; Wang et al. 2021; Kalisz et al. 2021). However, the determination of explicit drivers is

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even more complex for emissions of methane (CH<sub>4</sub>), where pivotal processes still are under debate. Considering the dynamics of anaerobic soil biogeochemistry, the selection of species for paludiculture was highlighted as essential (Noyce and Megonigal 2021). Typical semi-aquatic wetland plants, such as *Typha spp.* or *Phragmites spp.*, are known to potentially contribute to increased CH<sub>4</sub> emissions by allowing CH<sub>4</sub> to bypass possible oxidation processes within the soil by aerenchymatous transport (Minke et al. 2016; Vroom et al. 2022). Contrary to this, bryophyte-dominated (i.e., *Sphagnum spp.*) peatlands are frequently associated with methanotrophic (i.e., methane-consuming) activity (Larmola et al. 2010; Kolton et al. 2022). However, no such direct association between vegetation composition and CH<sub>4</sub> emissions exists for perennial grasses.

*Phalaris arundinacea*, or reed canary grass (RCG), is a bunchgrass species with a wide array of potential habitats. It is cultivated on dry mineral soils but also naturally invades floodplains and wetlands (Ustak et al. 2019). So far, the cultivation and biomass utilisation of RCG has been mainly for forage or as a bioenergy crop, while it recently gained attention as a feedstock in green protein biorefinery (Utama et al. 2018; Nielsen et al. 2021a; Næss et al. 2023). Furthermore, its versatility in various environmental conditions led to unique anatomical features, particularly in their culm and rhizomes, thereby distinguishing RCG from other *Poaceae* species and placing it structurally between mineral-soil adapted and semi-aquatic plants (Zhang et al. 2017). However, while the potential for cultivation of RCG on wet peatlands has been demonstrated, an assessment of associated GHG emissions is still lacking for management options beyond the commonly applied annual harvest in winter months or an experimental two-cut management (Kukk et al. 2010; Heinsoo et al. 2011; Järveoja et al. 2016; Karki et al. 2019). Therefore, the determination of a potential effect of different management intensities on annual GHG balances for RCG cultivation on wet or rewetted peatlands is critical in order to promote the development of knowledge to efficiently balance the needs for atmospheric decarbonisation and agricultural production (Mehrabi et al. 2018; Evans et al. 2021; Bianchi et al. 2021).

Thus, manipulation of the grass stand age is likely to be related to different magnitudes of ecosystem-atmosphere exchanges of GHG. In a previous study, variations in harvest intensities were found to affect root growth significantly and, therewith, the potential for instant belowground accumulation of carbon (C), with lesser harvests leading to increased C storage (Nielsen et al. 2021b). However, more frequent harvest occurrences have the potential to efficiently mitigate N<sub>2</sub>O emissions in summer months, where oxic conditions might occur due to reduced WTD. A dropdown in WTD has the potential to result in increased nitrogen (N)

mineralisation, particularly in nutrient-rich fen peatlands (Minkinen et al. 2020). There, regrowth of aboveground biomass (AGB) following harvest will not be delayed by N scarcity and the optimal partitioning mechanisms of plant growth, prioritising the development of plant organs critical to provide the most limiting resource (Fraser et al. 2015; Yang et al. 2018). Further, regrowth of juvenile plant material is associated with enhanced uptake of atmospheric CO<sub>2</sub>, due to enhanced photosynthetic activity, and N (Walker et al. 2014; Tejera et al. 2022).

Therefore, it was hypothesised that different intensities of harvest and fertilisation frequencies of RCG cultivated on a wet fen peatland will affect annual GHG emissions and the resulting global warming potential (GWP). In this context, the aim was to assess whether intensities of two and five annual harvest occurrences at fertilisation rates of 200 kg N and K per ha<sup>-1</sup> yr<sup>-1</sup> will lead to differing carbon balances and gas exchange dynamics as compared to a 'nature scenario' with neither harvest nor fertilisation.

## Methods

### Study Site and Biomass Harvest

We determined GHG emissions on four plots, cultivated with *Phalaris arundinacea*, cultivar: Lipaula, on a riparian fen peatland in Vejrumbro, Denmark (56°26'15.3"N, 9°32'44.1"E). The Vejrumbro field site has previously been used in various studies, e.g., regarding biomass productivity (Nielsen et al. 2021a) and the effects of flooding (Malinowski et al. 2015). The climate is temperate, with a long-term annual average temperature of 8.3 °C and an annual average precipitation of 675 mm for 1991–2022 (Aarhus University Viborg, Meteorological Station, Foulum). The Vejrumbro field site has been established in 2018 with various flood-tolerant perennial grass species as part of a biomass study. In the last decades, the site was classified as grassland and mainly used for grazing due to its wetness despite the establishment of drainage systems in the form of ditches and tile drains around the 1950s. Frequent flooding events on the study site and the relative distances of the GHG measurement plots to the Nørre Å river and drainage ditches created spatial heterogeneity of varying organic carbon (OC) contents and other environmental variables across the four plots (Table 1).

The four plots used for GHG measurements were subdivided into six subplots (Fig. 1) with differing management intensities represented by annual frequencies of harvest and fertilisation. Of these, treatments of zero (0-cut), two (2-cut) and five (5-cut) annual cuts were chosen for this study. The treatments with two and five annual cuts received

**Table 1** Annual average environmental variables and biomass properties per plot and treatment, as well as means for treatments across plots, where WTD is the mean water table depth, GH is the grass height,  $T_{soil}$  is the soil temperature in different depths,  $E_h$  is the redox potential at two depths,  $\theta$  is the volumetric water content (in  $cm^3\ cm^{-3}$ ), and NDVI is the normalised difference vegetation index. The standard error is given in brackets, and letters indicate significant differences for means between treatments

Plot	Treatment 0					Treatment 2					Treatment 5				
	12	13	19	22	mean	12	13	19	22	mean	12	13	19	22	mean
WTD (m)	-0.12	-0.12	-0.14	-0.15	-0.1	-0.09	-0.13	-0.16	-0.14	-0.1	-0.13	-0.09	-0.16	-0.13	-0.1
GH (cm)	30.66	35.19	36.75	40.12	35.7	25.42	26	24.82	28.11	26.1	14.84	16.33	15.44	18.31	
$T_{soil}$ -10 cm	8.54	8.71	8.92	8.85	8.8	8.65	8.8	8.85	8.85	8.8	9.13	9.25	9.27	9.16	
$T_{soil}$ -5 cm	8.61	8.72	8.97	8.91	8.8	9.17	8.8	8.74	8.61	8.8	9.03	9.09	9.04	9.01	
$T_{soil}$ -2 cm	9.55	9.75	10.04	9.9	9.8	9.67	10.12	10.18	10.1	10.0	10.66	10.99	10.92	10.45	
$E_h$ -5 cm	284.7	353.2	237.2	235.6	258.3	353.2	237.2	235.6	228.9	270.5	237.2	235.6	228.9	218.9	
$E_h$ -25 cm	130.4	171.7	156	168	139.0	171.7	156	168	186.1	144.1	156	168	186.1	110.8	
$\theta$ (%)	63.98	63.34	62.39	62.52	63.06	59.87	66.21	65.40	61.19	63.17	66.61	66.74	64.02	66.53	
NDVI	0.58	0.57	0.51	0.54	0.6	0.7	0.68	0.65	0.64	0.7	0.69	0.69	0.59	0.64	
Von Post	3	5	5	4	4.3	3	5	5	4	4.3	3	5	5	4	
pH*	4.9	5.7	6.0	6.2	5.7	4.9	5.7	6.0	6.2	5.7	4.9	5.7	6.0	6.2	
Organic C (%)	45.03	25.19	22.17	41.97	33.2	46.07	28.72	24.50	33.61	32.2	43.07	25.47	23.37	34.74	

\*pH was measured solely on plots, not treatment sub-plots

**Fig. 1** Drone picture of the Vejrumbro field site in the Nørre Å river valley. Plots used for the field trial are highlighted in red, with plot numbers indicated within the plots. A close-up shows a schematic illustration of the split-plot design, including infrastructure for greenhouse gas measurements. Drone pictures were taken by Jens Kjeldsen



split-fertiliser applications of in total  $200 \text{ kg N and K ha}^{-1} \text{ yr}^{-1}$ . Details on cultivation and seeding, as well as fertilisation and harvest occurrences, were described by Nielsen et al. (2021a). For this study, the subplots were equipped with permanently installed soil collars ( $55 \text{ cm} \times 55 \text{ cm}$ ), boardwalks, and relevant instrumentation for determining subplot-specific environmental parameters, as described below. The installation was performed in February and March 2020, and first test measurements started in April 2020. Biomass within the collars was harvested with a handheld grass trimmer (Makita, Anjō, Japan) in calendar weeks 24 and 36 (2-cut treatment), as well as 20, 24, 32, 36, and 42 (5-cut treatment). Harvested biomass was weighed, chopped, and oven-dried at  $60 \text{ }^\circ\text{C}$  before further determination of organic carbon (OC) content on a Vario MAX CN (Elementar Analysensysteme GmbH, Hanau, Germany).

### Meteorological and Hydrological Parameters

The meteorological station of Aarhus University Viborg, approximately 7 km from the study site, continuously measured photosynthetically active radiation (PAR), air temperature, and precipitation. In addition, each subplot was equipped with a soil temperature ( $T_{\text{soil}}$ ) logger at  $-5 \text{ cm}$  depth, logging soil temperature every hour. Further, for each GHG sampling occasion and subplot, measurements of  $T_{\text{soil}}$  at  $-2 \text{ cm}$ ,  $-5 \text{ cm}$ , and  $-10 \text{ cm}$  were taken manually with a digital thermometer (Weber Inc., IL, USA). The volumetric water content ( $\theta$ ,  $\text{cm}^3 \text{ cm}^{-3}$ ) was measured during GHG sampling campaigns using a time domain reflectometry (TDR) system (Thomsen 2006). Further, subplots were equipped with electrodes for measurements of redox conditions ( $E_h$ ) at  $-5 \text{ cm}$  and  $-25 \text{ cm}$  depth, as well as one piezometer for manual measurements of water table depth

(WTD), with screens between  $-100 \text{ cm}$  to  $-5 \text{ cm}$  below ground surface. In addition, dipwells (perforated PVC tubes) of  $100 \text{ cm}$  in length were installed within the collar area and equipped with a water depth logger (Levelogger5; Solinst Canada Ltd., Ontario, Canada), measuring automatically every hour. Atmospheric pressure correction was based on barometric loggers of the same manufacturer. For all components, negative signs denote depths below the ground surface and vice versa.

### Greenhouse Gas Sampling and Analysis from Opaque Chamber Measurements

White opaque PVC chambers ( $60 \text{ cm} \times 60 \text{ cm} \times 41 \text{ cm}$ ) were used for gas flux measurements of  $\text{CO}_2$ ,  $\text{CH}_4$ , and  $\text{N}_2\text{O}$ . Sampling campaigns were held every second week from the 5th of May 2020 to the 4th of May 2021. The chambers were equipped with a fan to mix headspace air, a vent to ensure pressure equilibrium, and a temperature probe measuring air temperature inside the chamber. During all GHG sampling campaigns, chambers were placed on separate middle pieces ( $60 \text{ cm} \times 60 \text{ cm} \times 41 \text{ cm}$ ), which were pre-positioned 30 min prior to sampling on the permanently installed soil collars. This procedure was in order to avoid methane ebullition as well as to make space for biomass. Five gas samples ( $11 \text{ mL}$ ) were withdrawn at 0, 5, 10, 25, and 50 min after chamber closure, using a syringe ( $20 \text{ mL}$ ) connected to the chamber sampling port by a polypropylene tube of  $1.2 \text{ m}$  and  $4 \text{ mm}$  inner diameter. The sampling port inside the chamber had three air inlets, additionally ensuring the withdrawal of well-mixed air. After discarding  $16 \text{ mL}$  of dead air volume from the tube system, air from the syringe was transferred to pre-evacuated  $6 \text{ mL}$  glass exetainers (Labco Limited, UK). Those were stored dark until analysis on an Agilent 7890 gas chromatograph, equipped with an

automatic injection system (CTC CombiPAL, Agilent A/S, Nærum, Denmark). Gas fluxes were calculated in R (R core team (2021), version 4.1.2 “Bird Hippie”), using the ‘gas-fluxes’ package (Fuss et al., 2020), and a combination of linear and non-linear models for flux estimation with model selection based on the kappa.max technique for reduction of bias and uncertainty as in detail described by Hüppi et al. (2018), considering the GC system-specific precision limit (Petersen et al. 2012) and defining the minimum detectable fluxes to  $3.5 \text{ g CO}_2 \text{ m}^{-2} \text{ h}^{-1}$ ,  $3.2 \text{ } \mu\text{g CH}_4 \text{ m}^{-2} \text{ h}^{-1}$ , and  $1.9 \text{ } \mu\text{g N}_2\text{O m}^{-2} \text{ h}^{-1}$ . From a total of 341 fluxes, 83%, 89%, and 95% of  $\text{CO}_2$ ,  $\text{CH}_4$ , and  $\text{N}_2\text{O}$  fluxes were calculated using the robust linear model. 12 fluxes of  $\text{CO}_2$  and one flux of  $\text{N}_2\text{O}$  were ‘NA’ while the remaining fluxes were determined using the HMR.fit method.

Aggregated annual fluxes of  $\text{CH}_4$  were calculated based on daily dependences with soil temperature using the R package *flux* (Jurasinski et al. 2014; version 0.3-0), using a similar approach as for  $\text{CO}_2$  (Eq. 1), while  $\text{N}_2\text{O}$  emissions were aggregated to annual cumulative values using linear interpolation (Fuß et al., 2020). Fluxes of  $\text{CO}_2$  were only determined and calculated as a quality check (leak test) for  $\text{CH}_4$  and  $\text{N}_2\text{O}$  and were not included in any further calculations.

$$R_{\text{CH}_4} = Q_1 * Q_2 * \text{Temp} \quad (1)$$

In which  $R_{\text{CH}_4}$  is the flux of  $\text{CH}_4$ ,  $Q_1$  is the base respiration rate at  $10^\circ\text{C}$ ,  $Q_2$  is the bias coefficient, and *Temp* is the measured hourly soil temperature at -5 cm.

### Measurements of Carbon Dioxide Using Transparent Chambers

In the same period from the 5th of May 2020 to the 4th of May 20, 2021,  $\text{CO}_2$  fluxes were measured in biweekly intervals using transparent chambers of the same size, including transparent middle pieces, as described for the opaque chambers in the section above. Ideally, measurements were performed the day following the opaque chamber measurements. However, this rhythm was subject to minor alterations of  $\pm$  two days to ensure that measurements were performed in sampling windows (between 10:00 and 15:00) without precipitation.  $\text{CO}_2$  concentrations were measured using a Li-Cor 840 A infrared gas analyser (Li-Cor Inc., Lincoln, USA) connected to an automatic datalogger (Campbell CR850; Campbell Scientific Inc., Logan, USA), over chamber closure times of 120 s. The chambers were equipped with (1) air-temperature sensors, measuring temperature in- and outside the chamber, (2) a temperature control unit, starting automatically when temperature differences exceeded  $1^\circ\text{C}$ , (3) a PAR sensor (Li-Cor 190-SA;

Li-Cor Inc., Lincoln, USA), (4) as well as an  $\text{H}_2\text{O}$  sensor—later used to correct  $\text{CO}_2$  for water vapour concentrations according to Webb et al. (1980). More details on the design for transparent chambers were described by Elsgaard et al. (2012).

To cover different ranges of naturally occurring PAR, artificial PAR blocking was applied by shrouding the chambers with meshes. For each collar placement, first, net ecosystem exchange (NEE) measurements were conducted without shrouding at natural PAR, followed by 50% PAR-blocking and 75% PAR-blocking. Finally, one set of  $R_{\text{eco}}$  measurements was conducted by blocking 100% PAR. Before each PAR-blocked measurement, plants were given 2 min to adapt photosynthesis rates. This procedure resulted in four measurements per collar placement. In addition, the order of transparent chamber measurements was randomised for each sampling campaign.

### Annual Carbon Balances

Based on the flux calculation, hourly fluxes for  $R_{\text{eco}}$  and gross primary production (GPP) were determined by gap-filling for each plot and treatment. Here, a Tier 2 method, as described by Liu et al. (2022), including vegetation height (GH) but using soil temperature (Ft), was applied. This resulted in the following Lloyd and Taylor Arrhenius-type models for  $R_{\text{eco}}$  (Eq. 2) and a Michaelis-Menten-based equation for GPP (Eq. 3).

$$R_{\text{eco}} = R_{\text{ref}} * e^{E_0 * (\frac{1}{T_{\text{ref}} - T_0} - \frac{1}{T - T_0})} + (a * GH) \quad (2)$$

$$GPP = \frac{GPP_{\text{max}} * GH * \alpha * PAR}{(GPP_{\text{max}} * GH) + \alpha * PAR} * Ft \quad (3)$$

Values for GH were interpolated between manual measurement campaigns at each GHG sampling occasion, with a cut set to the stubble height of 7 cm after each harvest occurrence. In those equations,  $T_{\text{ref}}$  is the reference temperature of 283 K, and  $T_0$  is the temperature constant of 227 K, indicating the start of biological processes.  $R_{\text{ref}}$  denotes respiration at  $T_{\text{ref}}$ , while  $E_0$  are estimated values for the activation energy.  $GPP_{\text{max}}$  is the maximum rate of carbon fixation at a PAR of 2000 expressed in  $\text{mg CO}_2\text{-C m}^{-2} \text{ h}^{-1}$ , whereas alpha ( $\alpha$ ) denotes the light use efficiency. Both equations were previously described in detail by Liu et al. (2022) and Oestmann et al. (2022) and are commonly applied. NEE values for each hour were derived by summing  $R_{\text{eco}}$  and GPP.

Following the approach of Liu et al. (2022) and Hoffmann et al. (2015), the fitted models were controlled for performance using the comparison between modelled and measured values by hands on a variety of performance

indicators: Mean absolute error (MAE), observations standard error (RSR) based on root mean square error, coefficient of determination ( $R^2$ ), modified index of agreement (md), per cent bias (PBIAS), and Nash-Sutcliffe's model efficiency (NSE). Thresholds for performance ratings were adapted from Hoffmann et al. (2015). Models were accepted if 5 out of 6 performance indicators were at least "satisfactory" and two indicators outweighed the "unsatisfactory" performance with a "good" performance rating. Uncertainties for all GHGs and annual balances were determined using a combined bootstrap and Monte Carlo jackknife method with 1000 iterations. Details on this method were previously described by, e.g., Köhler et al. (2012), Beetz et al. (2013), and Günther et al. (2015). To calculate the GWP of  $\text{CH}_4$  and  $\text{N}_2\text{O}$  in terms of  $\text{CO}_2\text{eq}$ , we applied conversion factors of 28 and 265 (Myhre et al. 2013), also adopted by the United Nations Framework Convention on Climate Change (UNFCCC) in 2021. For all GHGs and balances, the atmospheric sign convention was applied where negative values indicate uptake and positive values emissions.

In terms of global warming potential (GWP), annual GHG balances (Eq. 4) for each treatment and plot were calculated considering the export of C by biomass harvest ( $C_{\text{Export}}$ ):

$$GWP = NEE + C_{\text{Export}} + 28 * CH_4 + 265 * N_2O \quad (4)$$

For better comparability, all annual balances were expressed as  $\text{CO}_2\text{-C eq}$ .

## Statistics

For all parameters and values, observations are reported as means with standard errors ( $n=4$ ) to present the data distribution. The significance of differences between means was tested by one-way ANOVA with post-hoc Tukey's HSD at a confidence level of 95%. Effects of co-variables on  $\text{CH}_4$  and  $\text{N}_2\text{O}$  fluxes were assessed using generalised additive models (GAMs) in the package *mgcv* (Wood, Version 1.8–39, 2022) in R (R Core Team (2020) Version 4.1.2– "Bird Hippie"), capable of accounting for linear and non-linear relationships (Marra and Wood 2011; Wood 2011; Wood et al. 2016). Effects of co-variables and categorical treatments on the annual cumulative emissions of NEE,  $\text{CH}_4$ ,  $\text{N}_2\text{O}$  and the resulting GWP were derived using ANOVA-evaluated linear regression models.

## Results

### Meteorological and Hydrological Conditions

The study year 2020/2021 had an annual average of  $8.6\text{ }^\circ\text{C}$  and a total precipitation of 593 mm and was, therefore, warmer ( $+0.2\text{ }^\circ\text{C}$ ) and dryer ( $-82\text{ mm}$ ) than the long-term average over the past 30 years. The annual average WTD for the site was  $-0.10 \pm 0.03\text{ m}$ , with no distinct differences between plots. However, from late May to October, WTD dropped to values below  $-20\text{ cm}$ , dipping down to more than  $-40\text{ cm}$  in August 2020. From November to February, all plots were inundated (Fig. 2a).  $\theta$  varied on an annual basis between  $59.9 \pm 2.2\%$  (plot 12, treatment 2) to  $66.7 \pm 1.8\%$  (plot 13, treatment 5), with a site average of  $64.1 \pm 1.9\%$ . The pH-corrected redox conditions in both depths ( $-5\text{ cm}$  and  $-25\text{ cm}$ ) differed among plots and subplots without a link to a potential treatment effect. For instance, redox conditions at  $-5\text{ cm}$  ranged between  $227.8 \pm 11.2$  (plot 13) to  $291.7 \pm 17.7$  (plot 12)  $E_h$ , with no distinct treatment-specific differences (Table 1).

### Biomass Yields and Carbon Content

Mean harvested biomass yields were similar for the treatments with two ( $10.6 \pm 1.1\text{ t DM ha}^{-1}\text{ yr}^{-1}$ ) and five annual cuts ( $9.4 \pm 1.0\text{ t DM ha}^{-1}\text{ yr}^{-1}$ ), resulting in the export of  $4.7 \pm 0.5$  and  $4.2 \pm 0.5\text{ t of C}$ , and  $0.25 \pm 0.03$  and  $0.29 \pm 0.03\text{ t of N}$  by biomass harvest (Table 2).

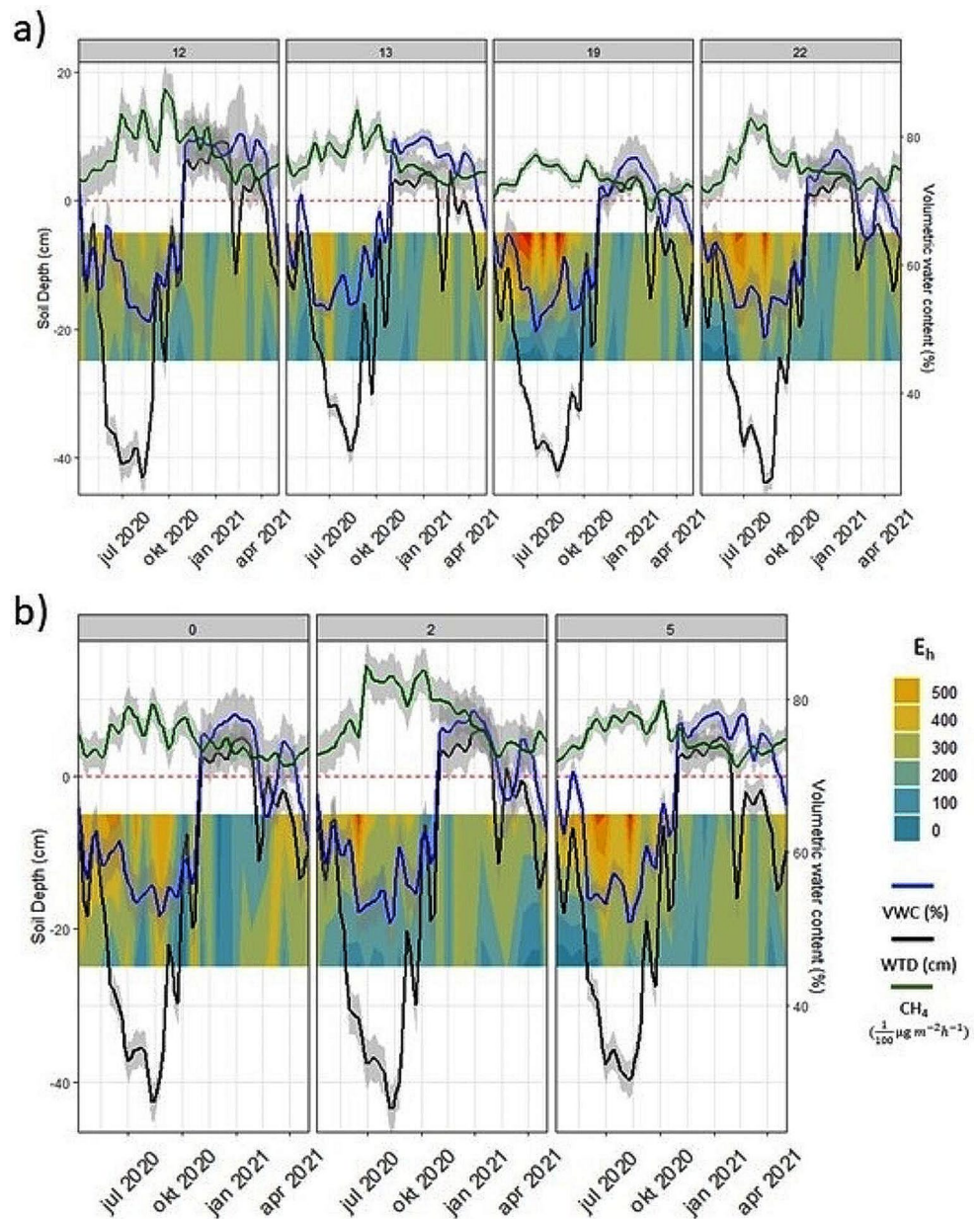
### Carbon Dioxide

NEE for all treatments was positive on an annual basis, ranging between  $3.8 \pm 3.2$  (2-cut) to  $9.6 \pm 2.2$  (0-cut)  $\text{t CO}_2\text{-C ha}^{-1}\text{ yr}^{-1}$  with no statistical difference between the annual values (Table 3). However, the treatments with two and five annual cuts were both characterised by negative (net uptake) NEE during the early growing season in April–June (Fig. 3). This observation was absent for the 0-cut treatment. A similar pattern was observed for GPP, which was higher for the treatments subject to harvest. Here the 2-cut treatment showed the highest GPP, with peaks prior to harvest seen on a plot basis (Figure S1).  $R_{\text{eco}}$ , however, was similar for all treatments, averaging  $21.5 \pm 2.7\text{ t CO}_2\text{-C ha}^{-1}\text{ yr}^{-1}$ .

### Methane Emissions

Methane emissions were low for all treatments (Table 3), with any significant difference. However, the highest value of cumulative emissions was found for the 2-cut treatment ( $0.5 \pm 0.13\text{ t CO}_2\text{-C eq ha}^{-1}\text{ yr}^{-1}$ ) compared to the 0- and 5-cut treatments ( $0.3 \pm 0.09$  and  $0.3 \pm 0.05\text{ t CO}_2\text{-C eq ha}^{-1}$

**Fig. 2** Redox conditions, expressed as the for pH corrected redox potential ( $E_h$ ), in the soil layer of -5 cm to -25 cm depth (left y axis) over the course of the study period for (a) each plot, and (b) each treatment. Blue lines indicate the volumetric water content ( $\theta$ ) in % (right y axis), while black lines show the fluctuation of water table depth (WTD) at cm soil depth (left y axis). For illustration, the green line shows interpolated emissions of  $CH_4$  (in  $1/100 \mu g m^{-2} h^{-1}$ , left y axis). Red dashed lines indicate zero



$yr^{-1}$ , respectively). Across plots and treatments, the highest  $CH_4$  emissions were observed at low  $E_h$  values, typically following increases in  $\theta$  (Figure S2). In addition, while  $CH_4$  fluxes per measurement campaign correlated with soil temperature and GH, no effects on annual cumulative values were found (Tables S1, S2).

### Nitrous Oxide

Both fluxes and annual cumulative emissions of  $N_2O$  were high for all treatments, with no significant difference, despite 0.4 less  $N_2O$  (in  $t CO_2-C eq ha^{-1} yr^{-1}$ ) emitted from the treatment with five annual cuts (Table 3). In this context, no effect of harvest and fertilisation frequency was

detected on neither  $N_2O$  fluxes per measurement campaign nor annual cumulative emissions (Tables S1, S2). Inconsistent directions of treatment effects of harvest and fertilisation frequency also depicted this lack of correlation. Here, for instance,  $N_2O$  emissions increased with management intensification in plot 19, while the opposite was observed in plot 12 (Fig. 4).

### Annual Greenhouse Gas Balances

The annual GHG balances of all treatments were dominated by NEE and the export of harvested C in biomass, together accounting for 81% (2-cut) to 88% (5-cut) of the GWP. NEE alone accounted for 84% of the GWP for the 0-cut treatment.

**Table 2** Mean dry matter (DM) biomass yields, as well as carbon (C) and nitrogen (N) exports with SE ( $n=4$ ) in brackets per harvest occurrence as indicated by calendar week and as total annual sum per hectare. For the 0-cut treatment, no biomass was harvested. For the treatment with two annual cuts, yields from week 24 in 2020 are shown for comparison but not included in any calculations

Treatment	Year	Week	t DM ha <sup>-1</sup>	t C ha <sup>-1</sup>	t N ha <sup>-1</sup>
0 cut			NA	NA	NA
	(2020	24	4.90 ( $\pm 0.40$ )	2.21 ( $\pm 0.18$ )	0.11 ( $\pm 0.01$ )
2 cut	2020	36	6.28 ( $\pm 0.59$ )	2.79 ( $\pm 0.27$ )	0.16 ( $\pm 0.02$ )
	2021	24	4.28 ( $\pm 0.52$ )	1.95 ( $\pm 0.23$ )	0.09 ( $\pm 0.01$ )
Sum	2020–		10.55	4.74 ( $\pm 0.49$ )	0.25
	2021		( $\pm 1.09$ )		( $\pm 0.03$ )
5 cut	2020	24	1.91 ( $\pm 0.27$ )	0.86 ( $\pm 0.12$ )	0.06 ( $\pm 0.01$ )
	2020	32	3.55 ( $\pm 0.66$ )	1.60 ( $\pm 0.31$ )	0.08 ( $\pm 0.01$ )
	2020	36	1.25 ( $\pm 0.20$ )	0.56 ( $\pm 0.09$ )	0.05 ( $\pm 0.01$ )
	2020	42	1.67 ( $\pm 0.07$ )	0.74 ( $\pm 0.03$ )	0.07 ( $< 0.01$ )
	2021	20	0.99 ( $\pm 0.24$ )	0.45 ( $\pm 0.11$ )	0.03 ( $\pm 0.01$ )
Sum	2020–		9.37 ( $\pm 1.02$ )	4.21 ( $\pm 0.46$ )	0.29 ( $\pm 0.03$ )
	2021				

**Table 3** Annual average greenhouse gas balances per treatment with SE given in brackets. Letters indicate the significance between means

	Treatment 0	Treatment 2	Treatment 5
Biomass C export (t C ha <sup>-1</sup> yr <sup>-1</sup> )	NA	4.7 ( $\pm 0.49$ )a	4.2 ( $\pm 0.46$ )a
Reco (t CO <sub>2</sub> -C ha <sup>-1</sup> yr <sup>-1</sup> )	21.4 ( $\pm 2.46$ )a	21.4 ( $\pm 3.11$ )a	21.8 ( $\pm 2.46$ )a
GPP (t CO <sub>2</sub> -C ha <sup>-1</sup> yr <sup>-1</sup> )	-11.8 ( $\pm 0.46$ )a	-17.6 ( $\pm 1.35$ )b	-15.8 ( $\pm 0.46$ )ab
NEE (t CO <sub>2</sub> -C ha <sup>-1</sup> yr <sup>-1</sup> )	9.6 ( $\pm 2.15$ )a	3.8 ( $\pm 3.17$ )a	5.9 ( $\pm 2.24$ )a
CH <sub>4</sub> (t CO <sub>2</sub> -C eq ha <sup>-1</sup> yr <sup>-1</sup> )	0.3 ( $\pm 0.09$ )a	0.5 ( $\pm 0.13$ )a	0.3 ( $\pm 0.05$ )a
N <sub>2</sub> O (t CO <sub>2</sub> -C eq ha <sup>-1</sup> yr <sup>-1</sup> )	1.5 ( $\pm 0.31$ )a	1.5 ( $\pm 0.13$ )a	1.1 ( $\pm 0.25$ )a
GWP (t CO <sub>2</sub> -C eq ha <sup>-1</sup> yr <sup>-1</sup> )	11.4 ( $\pm 2.11$ )a	10.5 ( $\pm 2.69$ )a	11.5 ( $\pm 2.61$ )a

For the treatment with five annual cuts, this resulted in the highest GWP of all treatments ( $11.5 \pm 2.61$  t CO<sub>2</sub>-C eq ha<sup>-1</sup> yr<sup>-1</sup>), despite the lowest contribution of N<sub>2</sub>O and CH<sub>4</sub>. However, there was no significant difference regarding the

GWP of the three treatments, all of which ranged around approximately 11 t CO<sub>2</sub>-C eq ha<sup>-1</sup> yr<sup>-1</sup> (Fig. 4).

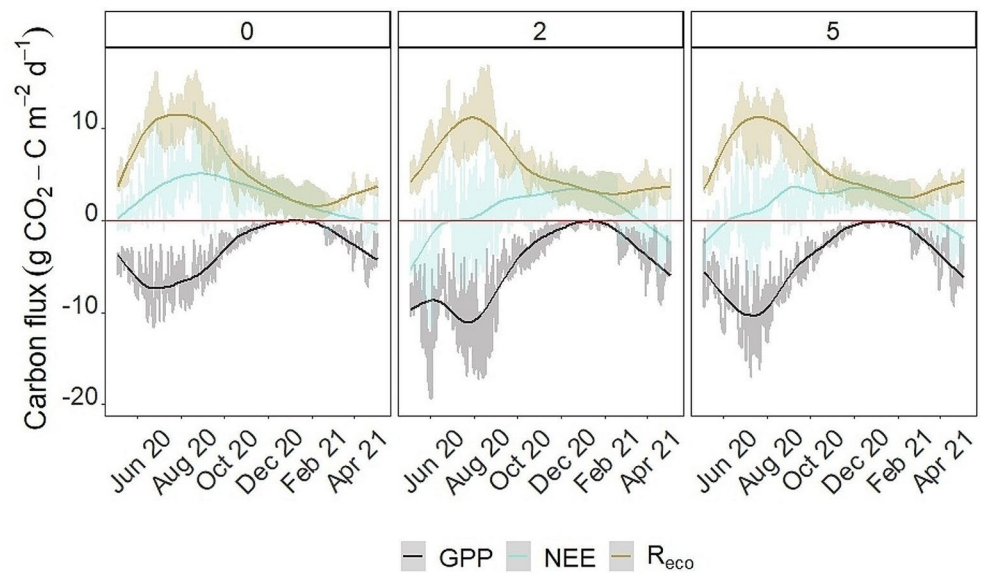
## Discussion

### Management Intensity did not Affect the Global Warming Potential

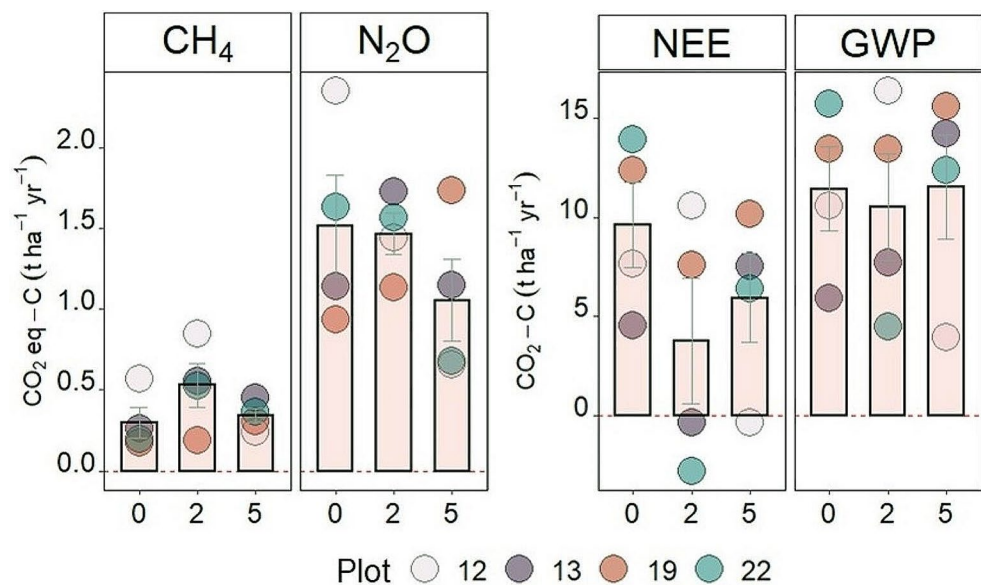
In this study, the magnitudes of annual GHG emissions for RCG cultivation on a wet fen peatland under different intensities of harvest and fertilisation, with either zero, two, or five annual cuts, were assessed. Annual biomass production for the treatments including harvests was similar and within the range of previously reported yields for RCG in a temperate climate (Tilvikiene et al. 2016) but lower compared to a study in the same area and similar WTD (Karki et al. 2019). The similarity in biomass yields was also depicted by near-equal R<sub>eco</sub> emissions for all treatments, both aspects previously being correlated by Liu (2022). Given that gap-filled annual R<sub>eco</sub> on both the 0-cut and 2-cut treatment were equal (both 21.4 t CO<sub>2</sub>-C ha<sup>-1</sup> yr<sup>-1</sup>), we estimated that above-ground biomass production on the 0-cut treatment also must have been similar. This assumption is supported by the previously reported yield of 8.3 t DM ha<sup>-1</sup> yr<sup>-1</sup> for RCG in the same study area, with one annual summer cut (Nielsen et al. 2021a). However, despite similar biomass growth and R<sub>eco</sub>, we found differing CO<sub>2</sub> dynamics for partitioned fluxes of GPP and, subsequently, NEE. Here, the treatment with two annual cuts resulted in the smallest annual CO<sub>2</sub> emissions of 3.8 t CO<sub>2</sub>-C ha<sup>-1</sup> yr<sup>-1</sup>, which is in line with the IPCC 2013 EF for shallow-drained temperate grassland on nutrient-rich peat (IPCC 2014), but high in comparison to other studies. For instance, Schrier-Uijl et al. (2014) found an annual cumulative NEE of 1.1 t CO<sub>2</sub>-C ha<sup>-1</sup> yr<sup>-1</sup> for intensively and extensively managed sites in the Netherlands with similar drops in WTD over summer months as on our study site. However, a point of discussion in this context is the applicability of the annual mean WTD for comparisons between sites. For instance, according to Wilson et al. (2016a) and the IPCC (2014), our study site would be classified as rewetted due to the annual average WTD of -10 cm. Nevertheless, it is a site with an existing shallow drain system and reduced WTD during summer months. Another point of discussion is whether emissions or net carbon balances are reported. For instance, we found NEE emissions in the range of 3.8–9.6 t CO<sub>2</sub>-C ha<sup>-1</sup> yr<sup>-1</sup>, depending on management, therewith being similar to the range of EFs for drained cropland or deeply-drained intensively used grassland (Wilson et al. 2016a; Weideveld et al. 2021). If also accounting for the export of biomass, known as the net ecosystem carbon balance (NECB), nowadays commonly applied following



**Fig. 3** Averaged gap filled daily carbon (C) fluxes, partitioned into gross primary productivity (GPP), net ecosystem exchange (NEE), and ecosystem respiration ( $R_{eco}$ ), per treatment as indicated by the number of annual biomass cuts (0, 2, 5) throughout the study period from May 2020–May 2021



**Fig. 4** Cumulative annual values for methane ( $CH_4$ ), nitrous oxide ( $N_2O$ ), net ecosystem exchange (NEE) and the global warming potential (GWP). Values are given as means per treatment (bars) and individual values per plot (coloured dots), including the standard error ( $n=4$ ) of the mean. Red dashed lines indicate zero



the IPCC guidelines (IPCC 2014), all our treatments were similar with regard to  $CO_2$  emissions, having a NECB of 8.5 (2-cut) to 10.1 (5-cut)  $t CO_2-C ha^{-1} yr^{-1}$ . However, the results were far above the recently defined EF for German peatlands, which were within the range of 0–4  $t CO_2-C ha^{-1} yr^{-1}$  for the same average WTD as on our study site (Tiemeyer et al. 2020). Thus, defining a universal range of  $CO_2$  emissions for wet peatlands under the land use of permanent grass or paludiculture is not a straightforward task due to the dependence on biomass yields as a response to soil nutrient and mineral status.

The observation of near-equal emission magnitudes for all management intensities did not change when also including annual emissions of  $CH_4$  and  $N_2O$ .  $CH_4$  emissions were very low, while  $N_2O$  emissions were unexpectedly high. However, annual emissions for both gases were

close to equal among different management intensities. For instance, annual  $CH_4$  emissions were 0.3  $t CO_2-C eq ha^{-1} yr^{-1}$  for both extremes of assessed treatments: no harvest and fertilisation, as well as intensive management with five annual cuts. In the case of the 0-cut treatment, this might be explained by a combination of enhanced root growth (Nielsen et al. 2021b) in combination with potential  $CH_4$  oxidation by aerenchymatous oxygen transport to the root zone. However, an explanation the other way around is more likely: that dry soil in periods with enhanced soil temperature led to increased  $CH_4$  oxidation over the entire study area, disregarding of treatment. In addition, in cases of high captured  $CH_4$  fluxes, mainly on the 2-cut treatment, a lag-time effect has been observed— meaning that higher  $CH_4$  fluxes were detected delayed with regard to the occurrence of optimal conditions of  $\theta$  and soil temperature. This

lag-time effect has also been observed in a study by Yuan et al. (2021), who found that methanogenic bacteria are unable to utilise C substrate availability immediately after optimal soil moisture conditions are reached. According to a review by Abdalla et al. (2016) on CH<sub>4</sub> emission in northern peatlands of different stages of naturalness, our observed CH<sub>4</sub> emissions were way below values reported for natural fens (1.77 t CH<sub>4</sub> in CO<sub>2</sub>-C eq t ha<sup>-1</sup> yr<sup>-1</sup>), but above CH<sub>4</sub> dynamics from drained fen peatlands (0.05 t CH<sub>4</sub> in CO<sub>2</sub>-C eq t ha<sup>-1</sup> yr<sup>-1</sup>). Thus, for all treatments, it was found that annual cumulative emissions of CH<sub>4</sub> that were more within the range of dynamics for natural bog peatlands (0.1–1.0 t CH<sub>4</sub> in CO<sub>2</sub>-C eq t ha<sup>-1</sup> yr<sup>-1</sup>), usually associated with high rates of methanotrophy (Kolton et al. 2022). However, compared to a compilation of annual CH<sub>4</sub> emissions for German grasslands (Tiemeyer et al. 2020), our observed values from a wet fen peatland with an annual WTD of -10 cm were higher than values reported for a similar mean WTD (0.05 t CH<sub>4</sub> in CO<sub>2</sub>-C eq t ha<sup>-1</sup> yr<sup>-1</sup>), as well as across all WTDs and under grassland-use (0.007 t CH<sub>4</sub> in CO<sub>2</sub>-C eq t ha<sup>-1</sup> yr<sup>-1</sup>).

Even regarding emissions of N<sub>2</sub>O, we did not find significant differences between treatments. This, however, is curious since the 0-cut treatment did not receive any N fertiliser application— and yet, emissions were equal to those from the 2-cut treatment, having received 200 kg of N h<sup>-1</sup> yr<sup>-1</sup>. In other words, fertilisation did not result in higher N<sub>2</sub>O emissions for the 2-cut and 5-cut treatments. Overall, N<sub>2</sub>O emissions were high and with 1.1 (5-cut) and 1.5 (0-cut, 2-cut) t CO<sub>2</sub>-C eq t ha<sup>-1</sup> yr<sup>-1</sup> within the range of emissions for drained peatlands under the land-use of fertilised cropland (Wilson et al. 2016a). Considering the IPCC default EF (IPCC 2019) for direct N<sub>2</sub>O emissions from agricultural soil, a fixed percentage of approximately 1% of fertiliser-N applied, as well as priors of approximately 7.9 and 4.6 kg N<sub>2</sub>O-N ha<sup>-1</sup> yr<sup>-1</sup> for fertilised and unfertilised organic soils, respectively (Tiemeyer et al. 2020; Mathivanan et al. 2021), our results were exceeding average EFs. In detail, it was observed that the treatments with two and five annual cuts emitted 7.5% and 5.5% of the N applied. In the case of the 0-cut treatment without fertiliser application, the observed high emissions of N<sub>2</sub>O exceeded expectable prior emissions by 30% and were likely caused by N mineralisation during summer months with low WTD (Minkinen et al. 2020). Considering the large observed N<sub>2</sub>O emissions from the prior on the unfertilised plots, the harvest of biomass is likely to have mitigated potentially higher N<sub>2</sub>O emissions for the fertilisation treatments due to plant rejuvenation (Walker et al. 2014; Tejera et al. 2022).

Although our methods effectively determined treatment effects, the sampling procedure may not represent actual GHG budgets due to its temporal constraints. For instance,

Dooling et al. (2018) found that annual cumulative emissions for CH<sub>4</sub> are underestimated if only based on daytime measurements, even if this might be an artefact resulting from nocturnal stratification (Stieger et al. 2015). Further, due to the bi-weekly measurement campaigns, it is without much doubt that we have not adequately captured hot moments of CH<sub>4</sub> and N<sub>2</sub>O emissions, which contributed to 140 and 45% of mean annual fluxes in a study by Anthony and Silver (2021). In addition, also modelled daily values of CO<sub>2</sub> fluxes, including their partitioning, are likely to not be flawless due to artificially derived PAR reduction by shrouding. Even though plants were given time to acclimate their photosynthesis rates to the reduced light levels before measurements were started, we could not capture fluxes under different temperature levels. Instead, a diurnal sampling campaign approach over naturally occurring levels of PAR and temperature would have been of benefit.

### Water Table Depth is Only One Potential Proxy

For both main aspects related to this study, (1) biomass yields, including their accumulation of C and N, as well as (2) GHG emissions in dependence on management intensity, we did not find a treatment effect. However, we found intra-site variations that were distinct on a plot basis. First, in dependence on the distance to the adjacent Nørre Å river, organic carbon contents between plots with differences of up to 20% OC. This plot-specific difference was also found for pH and redox conditions. The most pronounced inter- and intra-plot specific differences were observed when relating the hydrological conditions of WTD,  $\theta$ , and  $E_h$  to GHG fluxes. Typically, mean WTD levels are used as proxies for annual emission rates and are proposed to be utilised in national inventory reports (Tiemeyer et al. 2020). However, WTD is only one proxy, not necessarily being strictly correlated to two other critical hydrological aspects: the capillary fringe intensity (Gnatowski et al. 2002; Macrae et al. 2013) and the soil's water holding capacity, which are also affected by precipitation rates (Irfan et al. 2020; Dai et al. 2022). As also observed in a study by Estop-Aragonés et al. (2012), soil moisture contents for our plots were not continuously developing parallel to changes in WTD. With both  $\theta$  and  $E_h$  being related to suitable conditions for fluxes of CH<sub>4</sub> and N<sub>2</sub>O (van den Pol-van Dasselaar et al. 1998; Wang et al., 2018b; Zhao et al. 2019; Korkiakoski et al. 2022), we found that soil-moisture related differences between plots explained the variation of observed emission magnitudes, irrespectively of treatment. Säurich et al. (2019) found the highest N<sub>2</sub>O production rates from denitrification at a water-filled pore space of 80–95%, which is similar to the average measured volumetric water contents of 60–67% during summer months for our site, but with distinct differences

between sub-plots. Nonetheless, Jungkunst et al. (2008) and Tiemeyer et al. (2016) also found high  $N_2O$  emissions of up to  $12 \text{ kg } N_2O\text{-N ha}^{-1} \text{ yr}^{-1}$  on sites with a similarly intermediate WTD of around  $-20 \text{ cm}$  to  $-10 \text{ cm}$ . Furthermore, Berendt et al. (2022) concluded their study on the influence of peatland rewetting on  $N_2O$  fluxes by stating that emissions were highly variable across states of soil wetness and related to hot moments and hot spots. In addition, the assessment of peat Eh revealed how quickly the border between aerated ( $E_h > +400 \text{ mV}$ , pH 7) and anaerobic ( $E_h < +350 \text{ mV}$ , pH 7) was fluctuating in the top 25 cm of soil depth, even in periods with low WTD. In our study, measurements of peat Eh supported the findings of both dynamics: high  $N_2O$  flux rates and low  $CH_4$  emissions due to the lack of extended anaerobic conditions of  $E_h < -150 \text{ mV}$  (pH 7). Thus, in accordance with previous studies (e.g. Masscheleyn et al. 1993; Pezeshki and DeLaune 2012; Wang et al. 2018 a,b; Zhang and Furman 2021), we found that peat  $E_h$  conditions were a better proxy explaining the variation in GHG dynamics than WTD.

### To Harvest or Not to Harvest?

Our results showed that irrespective of management: (1) with and without fertilisation and (2) under different intensities of harvest, the GWP for all scenarios was similar. In the first instance, this implies that both extremes, a restoration scenario and intensive paludiculture practices were similarly climate-affecting land-use options. In areas where arable land is not a restricted resource, rewetting and restoration is likely to be the most cost-efficient option for atmospheric decarbonisation and ecological restoration (Mehrabi et al. 2018; Evans et al. 2021; Bianchi et al. 2021). In addition, previous research on belowground biomass (BGB) development under different harvest intensities showed significantly higher root biomass development for non-harvested RCG stands (Nielsen et al. 2021b). Therewith, direct C inputs from BGB, and long-term storage under water-logged conditions, are likely to be higher in non-harvested areas in the long run. However, in areas under pressure regarding issues of concurrent production and sustainable peatland management, intensive paludiculture practices might offer a solution to socioeconomically and environmentally responsible peatland agriculture (Wijedasa et al. 2016). Various options exist for paludiculture, ranging from the production of bio-energy plants to forage and protein concentrates for livestock feed (Damborg et al., 2019; Nielsen et al. 2021a; Martens et al. 2021). In this context, a frequent harvest of paludiculture biomass might add the benefit of nutrient removal and thus to the long-term biological restoration of peat environments (Hinze et al. 2021; Vroom et al. 2022; Zak and McInnes 2022). However, our results are only based on one year of

data and under hydrological conditions with a clear potential for improvements in WTD and its stability. Considering that peatlands have shown the potential for sudden developments regarding their C sink function (Roulet et al. 2007; Wilson et al. 2016b), our results only represent a snapshot in time regarding the effect of management intensities on the GWP for wet or rewetted fen peatlands.

### Conclusion

This study aimed to assess whether intensities of two and five annual harvest occurrences at fertilisation rates of  $200 \text{ kg N and K per ha}^{-1} \text{ yr}^{-1}$  will lead to differing carbon balances and gas exchange dynamics compared to a 'nature scenario' with neither harvest nor fertilisation.

First,  $CH_4$  emissions were low for a wet fen with a mean WTD of  $-10 \text{ cm}$  but characterised by a drop in WTD in August of down to  $-40 \text{ cm}$ . This was found for all plots and treatments due to soil moisture conditions and the associated redox potential, providing pathways for  $CH_4$  oxidation in the upper 25 cm of the peat layer when WTD was low and temperatures optimal. Contrary to the low emissions of  $CH_4$ , we found substantially high flux rates of  $N_2O$ , translating to EFs in the range of agricultural cropland. Here also, the unfertilised treatment was characterised by unforeseen high fluxes, which was interpreted as prior emissions from N mineralisation and denitrification during summer months, characterised by low WTD and an  $E_h$  of between  $+300$  and  $+500 \text{ mV}$ . Overall, it was found that peat  $E_h$  and  $\theta$  conditions were better proxies explaining the variation in GHG dynamics than WTD.

Second, fertilisation and harvest did in no case of management intensity result in higher cumulative emissions of  $N_2O$  due to the rejuvenescence of biomass. In addition, biomass yields, and their composition, were equal for both harvest intensities. Thus, while GPP was higher and NEE lower for the treatments with harvest occurrences, these C benefits were offset by the export of C in biomass as compared to the treatment without management. Based on these observations, our results highlighted a near-equal GWP in the range of  $10.5\text{--}11.5 \text{ t CO}_2\text{-C eq t ha}^{-1} \text{ yr}^{-1}$  for all scenarios irrespectively of management. In a climate context, our findings supported that both extremes, a restoration scenario but also intensive paludiculture practices, were similar land-use options regarding their climate impact. Finally, this indicates that site-specific hydrological and electrical peat properties are more critical regarding their climate impact than paludiculture management practices.

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**Author Contributions** All authors contributed to the study design, reading and revision of the manuscript, and approved the final version. CN developed and performed the study design and experimental work, analysis of the data, and writing of the manuscript. WL developed the script for modelling. MK contributed significantly to data collection in the field. PL was involved in gathering of funds.

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**Data Availability** The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

## Declarations

**Conflict of Interest** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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