Degradation legacy and current water levels as predictors of carbon emissions from two fen sites

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SUMMARY

Drainage-induced shifts in physicochemical peat properties are irreversible on a decadal time span. We investigated whether carbon emissions from fen peat can be estimated using two proxies: current water levels and peat properties (as affected by drainage history, i.e. degradation legacy). We collected bare peat monoliths from a long-term drained and an undrained fen. In a crossed design, half of the monoliths was kept wet and the other half was drained. Highest carbon dioxide (CO_2) emissions came from the originally undrained peat under low water levels (607 mmol m^{-2} d^{-1}). Overall, CO_2 emissions were primarily determined by drainage history, with 141 % higher emissions from the originally undrained peat. In addition, low current water levels correlated with 42 % higher emissions. Highest methane (CH_4) emissions were measured in the originally undrained peat under high water levels (123 mmol m^{-2} d^{-1}). Overall, CH_4 emissions were primarily determined by current water levels, with 430 % higher emissions under high water levels. In addition, the originally undrained peat had 180 % higher emissions. The lower C efflux from originally drained peat correlated with lower concentrations of methanogens and of easily-degradable carbon substrate (cellulose). We conclude that substrate limitation in long-term drained fens ensures low baseline carbon emissions, which provides opportunities for renewed carbon sequestration by rewetting.

KEY WORDS: carbon dioxide, climate change, methane, peatlands, rewetting

INTRODUCTION

Peatlands are home to vast carbon (C) stocks: estimates suggest that approximately 455–500 Gt C is stored in northern peatlands alone (Gorham 1991, Yu 2012). This fragile C stock is increasingly destabilised by anthropogenic drainage and drought-induced water table drawdown, which triggers increased peat decomposition rates (Knorr *et al*. 2008, Fenner & Freeman 2011, Brouns *et al*. 2014). Following the concomitant spike in carbon dioxide (CO_2) emissions, peatlands shift from net sink to source of atmospheric carbon. It is estimated that an annual ~2 Gt of CO_2 is currently emitted from degraded peatlands alone, with a more than 25 % increase in emissions since 1990 (Joosten *et al*. 2016).

Ample studies have focused on the quantification of carbon fluxes from acidic ombrotrophic peatlands ("bogs") or permafrost peatlands (e.g. Alm *et al*. 1999, Turetsky *et al*. 2002, Creevy *et al*. 2020). During the last few decades, the investigation of carbon emissions from minerotrophic peatlands ("fens") has gained more interest (see e.g. Moore *et al*. 1990, Euskirchen *et al*. 2019). Fens are the dominant peatland type in the temperate zone and are characterised by a higher base saturation and pH, a higher availability of electron acceptors and nutrients, and they are typically covered by plant communities of brown mosses (mainly from the Amblystegiaceae family) and sedges (e.g. *Carex* spp.) rather than peat mosses (*Sphagnum* spp.) (Succow & Joosten 2001). Since fens predominantly occur in the more densely populated regions of Europe and North America, most have already been drained many decades to centuries ago (Lamers *et al*. 2015).

Besides shifting the net C balance, long-term drainage of peat also results in altered physicochemical properties of the remnant peat matrix (Zeitz & Velty 2002). Alterations of the peat matrix include shifts in peat soil carbon quality and quantity (Leifeld *et al*. 2012), the mobilisation of nutrients and other inorganic compounds (Zeitz & Velty 2002, Zak & Gelbrecht 2007, Zak *et al*. 2010), and peat soil subsidence (Leifeld *et al*. 2011). Such shifts are irreversible within a decadal time span, and we refer to them as a "degradation legacy". This

degradation legacy, in turn, drives shifts in the composition of the vegetation and of soil microbial communities (Malson *et al*. 2008, Emsens *et al*. 2020).

Rewetting is a first crucial step to restore drained fens and may contribute to the global mitigation of climate change (Günther *et al*. 2020). Although there is consensus that this is a prerequisite to conserve the remnant peat C stock, which manifests itself as a significant reduction in CO_2 emissions to the atmosphere after rewetting (Strack & Zuback 2013, Günther *et al*. 2020), it is much less clear how rewetting impacts total carbon fluxes. This uncertainty largely stems from the recovery of methanogen communities (Wen *et al*. 2018, Emsens *et al*. 2020), resulting in an increase in methane (CH_4) emissions (Estop-Aragones & Blodau 2012, Hahn *et al*. 2015). Since CH_4 is a more potent greenhouse gas than CO_2, increased CH_4 emissions cannot be neglected in fen rewetting projects.

To gain a better understanding of how fen restoration by rewetting will affect fluxes of CO_2 and CH_4, especially in relation to climate change mitigation, it is key to experimentally isolate the effects of potentially explanatory factors. In this study, we conducted a mesocosm experiment to investigate the interactive effects of degradation legacy and water table on carbon emissions stemming from peat soil respiration, i.e. the C efflux from peat and root decomposition. We hypothesise that if effects related to differences in peat volume and vegetation composition are excluded, then the emission of both CH_4 and CO_2 from fen soils will correlate with two simple proxies: (i) the current physicochemical properties of the remaining peat matrix, i.e. soil organic matter quantity and quality, and (ii) current water levels.

METHODS

Study area and peat monolith collection

We selected two adjacent sites in the valley of the Zwarte Beek, the largest remaining fen complex in the Campine area of Flanders, Belgium. Site selection was based on historical (oral) information provided by the land owner (nature organisation "Natuurpunt"), as well as on past research in the fen complex by our research team (Emsens *et al*. 2016). The first site was located in a well-preserved and relatively undrained, near-natural part of the fen complex (N51° 04' 48.4" N; 5° 17' 10.6" E) with a deep peat layer (> 1.2 m). The vegetation consisted of sedges (mainly *Carex rostrata* Stokes), horsetails (mainly *Equisetum fluviatile* L.), rushes (mainly *Juncus acutiflorus* Ehrh. ex Hoffm.), *Menyanthes trifoliata* L., and brown mosses (dominance of *Calliergonella cuspidata* (Hedw.) Loeske and *Calliergon cordifolium* (Hedw.) Kindb.). The second site was located 950 m farther up the same valley in a highly similar but long-term drained part of the fen (N51° 04' 51.5"; E5° 17' 58.6") with a (currently) more shallow peat layer (approximately 25–40 cm). Although the exact drainage history and intensity is unknown for the site, most of the surrounding area was already drained from the 18th century onwards (visible as drainage structures on the map of Ferraris, made in 1777).

The vegetation was characterised by a high presence of fen meadow and moist grassland species such as *Potentilla erecta* (L.) Raeusch., *Juncus* spp., *Plantago lanceolata* L., *Lotus pedunculatus* Cav., *Holcus lanatus* L. and the mosses *Brachythecium* spp. and *Rhytidiadelphus squarrosus* (Hedw.) Warnst.. The undrained and the long-term drained site are both located within the same ecohydrological system, and are historically fed by iron-rich groundwater that has percolated through the glauconiferous sandy aquifers of the formation of Diest (Augustijns 2019). The vegetation at both sites is mown every year (at the end of the growing season) by the site owner, after which hay and litter is removed.

At the time of peat monolith collection (mid-December 2016), groundwater levels were at peat surface level in the undrained site and approximately 20–30 cm below the surface in the drained site. At each site, 10 replicate peat monoliths were extracted from a central and homogeneous part of the site (in a 4 x 1 m grid with 1 m interspacing) by means of PVC tubes measuring 20 cm x 12.5 cm (depth x diameter). The tubes were sharpened on their downward-facing edge and were rotated while gently pressing down, which facilitated insertion into the peat soil. Once the top of the PVC tube was flush with the surface of the peat, the tube with peat monolith was carefully extracted from the fen. The bottom of the tube was immediately sealed after extraction to prevent water loss during transport, and aboveground vegetation (if any, composition as described above) was carefully cut to peat soil surface level and removed. We note that not much biomass had to be clipped since the monoliths were collected in winter, and the vegetation had already been mown (and litter removed) by the site owner a couple of months earlier.

Experimental design

The bare peat monoliths were placed in a basement in full darkness to prevent establishment or resprouting of vegetation. By doing so, we assured that potential differences in vegetation structure and composition, for example through radial oxygen loss,

chimney effects and photosynthesis, were not confounding factors in our experiment. The measured C fluxes thus represented peat soil respiration under standardised conditions, and were not a representation of net ecosystem exchange (NEE) under fully natural, vegetated conditions. Next, the monoliths were subjected to a permanent (re-)wetting or partial drying treatment in a balanced design (with random allocation of the individual monoliths), in which water levels were manually kept stable at 0 cm (= (re-)wetting treatment) or 15 cm (= partial drying treatment) below the peat surface. Water level manipulation was accomplished by placing each PVC tube (perforated at the bottom) in a separate larger tube that was prefilled with the required amount of stagnant artificial groundwater (as in Emsens *et al*. 2016), i.e. tap water that had been diluted with demineralised water to an electrical conductivity of 150 µS cm^{-1} and a pH of 7.0. To avoid confusion in terminology, we refer to the experimentally induced water level treatment as "Wet" and "Dry", and we refer to drainage history of the peat as "Drained" and "Undrained". This setup resulted in a total of 20 peat monoliths, with five replicate peat monoliths per treatment combination (5 replicate peat monoliths x 2 peat drainage histories x 2 water levels = 20 monoliths (Scheme in Figure A1 in the Appendix)). The experiment was run from mid-December 2016 until early May 2017 (total = 133 days). Ambient temperatures ranged between (min) 12.6 °C and (max) 19.8 °C (mean ± SD = 17.0 ± 1.4 °C). At the end of the experiment, peat samples were collected from each monolith at the depths of 0–5 cm and 10–15 cm using a soil corer. Sampling equipment was thoroughly rinsed between samples. Samples were stored at -18 °C until further use for physicochemical and molecular analyses.

Characterisation of peat properties

We used the acid-detergent fiber (ADF) method to analyse all peat samples (= two depths per monolith) for ADF-cellulose and ADF-lignin fractions (Rowland & Roberts 1994). The method was initially developed to determine litter and forage quality (Van Soest 1963), which we extended for use in peatlands as peat soils are primarily composed of plant organic matter. From previous research we know that the cumulative fractions of cellulose and lignin (% ADF-cellulose + % ADF-lignin) in fen peat correlate strongly with total soil carbon (% C) contents as determined by standard C analysis through combustion on a CN-analyser (Pearson's r = 0.88, P < 0.001, based on analysis of 117 peat samples collected at 3 depths in 39 different fen sites (unpublished data from Emsens *et al*. (2020), presented in Figure A2). In short, the first step of the ADF method was to add cetyltrimethyl-ammonium-bromide (CTAB) to 0.5–1 g of air-dried (45 °C) peat material. The mixture was then boiled to, amongst others, hydrolyse and dissolve hemicellulose, proteins and possibly also a fraction of the easily dissolvable minerals contained within the peat matrix. This step left a fibrous residue of (alpha-) cellulose, lignin-like matter and insoluble minerals. Samples were then rinsed, dried at 105 °C, and weighed. Next, 72 % sulfuric acid (H_2SO_4) was added to dissolve ADF-cellulose, after which samples were again rinsed, dried, and weighed. Mass loss, corrected for initial mass of the sample, was used to calculate ADF-cellulose fractions. Finally, ADF-lignin was removed from the samples by ashing the remaining material at 550 °C for 2 h, after which only the insoluble mineral fraction remained. Again, mass loss was used to calculate ADF-lignin fractions. All fractions (g g^{-1}) were converted to percentages of dry soil.

Carbon flux measurements

CO_2 and CH_4 fluxes from the peat monoliths were measured every two weeks using an Ultraportable Greenhouse Gas Analyzer (UGGA, Los Gatos Research, San Jose, California). As the monoliths had no vegetation and were kept in the dark, carbon fluxes related to plant physiology were excluded. The first measuring session was initiated at t = 49 days in the experiment, after leaving time for peat monolith stabilisation, and the last measuring session was conducted at t = 133 days (thus spanning a total of 84 monitoring days). To perform the measurement, a cylindrical air-tight chamber (with a diameter of 12.5 cm and a total volume of 1.8 L, with a fan for internal air-cycling) was connected to the UGGA and placed securely over a monolith. The concentration of C (separated into CH_4 and CO_2) in the chambers was then logged every second for the duration of two minutes. This process was replicated three consecutive times for each monolith during each biweekly session, with clearing of the chamber between each (sub-) replicate measuring series. Post-hoc, we selected time frames for which the change in C concentration (= slope) was clearly linear and constant, and we only included time-series for which the R^2 of the linear relationship exceeded 0.75. This approach was to ensure that potential bursts of non-linear C release (e.g. due to ebullition, often triggered by monolith disturbance) were not incorporated, and it also implies that individual (sub-) replicate measurement series were sometimes omitted due to not meeting this standard. Overall, most steady fluxes were measured in all cores of drained origin and in the cores of undrained origin under low water levels

(5 % of sub-replicate series omitted), while the cores of undrained origin under high water levels had least stable fluxes (35 % of sub-replicate series omitted). After subsetting, the average fit of the linear relationship was very good for both CH_4 ($R^2 = 0.98 \pm 0.04$ (mean ± SD)) and CO_2 ($R^2 = 0.97 \pm 0.05$ (mean ± SD)). Carbon fluxes were estimated from the change in concentration in the chamber using the following formula:

$$Flux = \frac{(\frac{dc}{dt})*p*V}{R*T*A} \quad [1]$$

In which dc/dt is the concentration change of CH_4 or CO_2 over time (ppm s^{-1}), V is the volume of the chamber (m^3), A is the peat monolith surface (m^2), p is the partial air pressure (Pa, here assumed to be 1), R is the gas constant ($8.205*10^{-5}$ m^3 Pa K^{-1} mol^{-1}), and T is the temperature in the chamber (K). Units were converted and reported in mmol m^{-2} d^{-1}. The three (sub-)replicate measurements per soil monolith per session were averaged into one "true" replicate for further statistical analysis.

DNA extraction and qPCR

Peat subsamples (from the samples taken at the end of the experiment) were thoroughly homogenised through crushing with a chisel. DNA was extracted from a 0.25 g subsample of peat using the PowerSoil DNA Isolation Kit (MO BIO, Carlsbad, California), following the manufacturer's protocol, and quantified using the HS dsDNA Assay Kit and Qubit fluorometer (Invitrogen). All DNA isolations were additionally cleaned using a one-step PCR inhibitor removal kit (Zymo research, Irvine, CA, USA) and re-quantified using Qubit. We selected the mcrAf-mcrAr primer pair targeting the methyl coenzyme-M reductase (mcrA) gene indicative for methanogens (Luton *et al*. 2002). Total prokaryote abundance was additionally estimated by targeting the 16S rRNA gene using primers 515F–806R (Caporaso *et al*. 2011). qPCR was performed in duplicate reactions using KAPA SYBR fast (Roche, Basel, Switzerland) on a Bio-Rad CFX96 Real-Time Thermal Cycler (Bio=Rad, Hercules, CA, USA) with the following cycling conditions: 95 °C for 180 sec followed by 40 cycles at 95 ° C for 3 sec, 57 °C for 20 sec and 72 °C for 12 sec. For standards, diluted cleaned-up PCR products were used and accuracy was determined to be high based on a linear model of log-transformed concentrations on Cq's ($R^2 > 0.99$). Melting curve analysis was performed to confirm specific amplification. Gene copy numbers were estimated through Qubit measures of standards assuming a length in bp's of 292 for 16S and 478 for mcrA. mcrA and 16S copy numbers were expressed per gram of air-dried (45 °C) soil by correcting for sample moisture contents.

Data analysis

Data were analysed in R 3.5.3 (R Core Team 2020) and significance was accepted at $P < 0.05$.

Per replicate peat monolith we averaged the data on ADF and mcrA abundance from the two depths (0–5 cm and 10–15 cm) to have one mean value per monolith, to which C fluxes could then be related.

First, we then ran three two-way ANOVA tests to examine the interactive effect of site drainage history (factor "Undrained" or "Drained") and water level treatment (factor "Wet" or "Dry") on (i) peat ADF-cellulose content, (ii) peat ADF-lignin content and (iii) methanogen (mcrA gene) abundance. If interactions were non-significant, we reran the models including only main effects. We also tested whether peat ADF-cellulose and ADF-lignin contents were correlated using a Pearson's correlation test.

Next, we ran two separate linear mixed-effect models to assess the effects of drainage history (factor "Drained" or "Undrained"), time since start of the experiment (days), and water level treatment (factor "Wet" or "Dry") on the fluxes of respectively CH_4 and CO_2, in which peat monolith ID was incorporated as a random factor.

We estimated the total cumulative amount of CH_4 and CO_2 released from each peat monolith during the 84-day monitoring period using auc-curves (interpolation type = spline) using the MESS package (Ekstrøm 2020). Next, we used multiple linear regression to investigate how much of the variation in the total release of CH_4 and CO_2 could be explained by peat properties (i.e. ADF-cellulose and ADF-lignin contents) and by water level treatment. Here, we only included independent variables that significantly contributed to the model, with backward stepwise selection of parameters.

Finally, simple linear regression was used to test whether absolute methanogen abundance (mcrA gene copy numbers, measured at the end of the experiment) and relative methanogen abundance (mcrA:16S rRNA fraction) related positively to the cumulative amount of CH_4 that was released throughout the experiment. To conform to model assumptions of normality and homoscedasticity of residuals, we log10-transformed the CH_4 fluxes, the cumulative CH_4 emissions, and absolute methanogen abundances prior to statistical analysis. However, to allow for a meaningful interpretation we report the actual (non-transformed) mean CH_4 values of the different experimental groups in the results section.

RESULTS

Peat properties

The drainage history of the monoliths (i.e. from undrained or long-term drained origin) was reflected in the properties of the peat: the monoliths of drained origin were characterised by significantly lower contents of ADF-cellulose ($F_{1,17}$ = 55.01; P < 0.001) and ADF-lignin ($F_{1,17}$ = 40.94; P < 0.001) (Figure 1). Water level treatment (wet or dry, i.e. experimental water levels at 0 cm and 15 cm below the peat surface) had led to minor differences in peat characteristics at the end of the experiment, evidenced by slightly lower mean ADF-cellulose and ADF-lignin contents in the dry monoliths. However, these differences were not significant (P > 0.05 for all variables, Figure 1). There was a strong positive correlation between ADF-lignin and ADF-cellulose fractions of the peat soil (Pearson's r = 0.83, df = 18, P < 0.001).

Carbon emissions

There was no effect of time on the CO_2 efflux ($F_{1,115}$ = 0.49; P = 0.566), so time was further excluded from the mixed-effect model as an explanatory factor, nor was there an interaction effect between peat drainage history and water levels ($F_{1,16}$ = 0.67; P = 0.424). CO_2 emissions were primarily determined by peat drainage history ($F_{1,17}$ = 35.21; P < 0.001): emissions were on average 307.1 mmol m^{-2} d^{-1} higher (= 141 %) from the originally undrained peat than from the originally drained peat (Figure2c,d). Water level had an additional effect on the CO_2 efflux ($F_{1,17}$ = 6.31; P = 0.022): emissions were on average 130.6 mmol m^{-2} d^{-1} higher from the monoliths subjected to low water levels than from monoliths with high water levels, corresponding to 42 % higher emissions under low water levels. Overall, the lowest CO_2 emissions were measured from the originally drained peat under high water levels (mean (± SE) = 176.5 (± 19.04) mmol m^{-2} d^{-1}, corresponding to 7.77 g (± 0.84) m^{-2} d^{-1}), while the highest CO_2 emissions were measured from the originally undrained peat under low water levels (mean (± SE) = 607.4 (± 30.8) mmol m^{-2} d^{-1}, corresponding to 26.73 (± 1.36) g m^{-2} d^{-1}).

CH_4 emissions increased significantly over time ($F_{1,115}$ = 12.36; P < 0.001), particularly in the originally drained peat under high water levels (Fig 2a). Here, CH_4 emissions seemed to stabilise after approximately 120 days. There was no interaction between peat drainage history and water levels on CH_4 emissions ($F_{1,16}$ = 1.39; P = 0.256). CH_4 emissions were primarily determined by water level treatment ($F_{1,17}$ = 59.17; P < 0.001): emissions were on average 69.2 mmol m^{-2} d^{-1} higher (= 430 %) from the monoliths subjected to high water levels than from monoliths with low water levels (Figure 2a,b). Peat drainage history had an additional effect on the CH_4 efflux ($F_{1,17}$ = 30.96; P < 0.001): emissions were on average 47.9 mmol m^{-2} d^{-1} higher from the originally undrained peat than from the originally

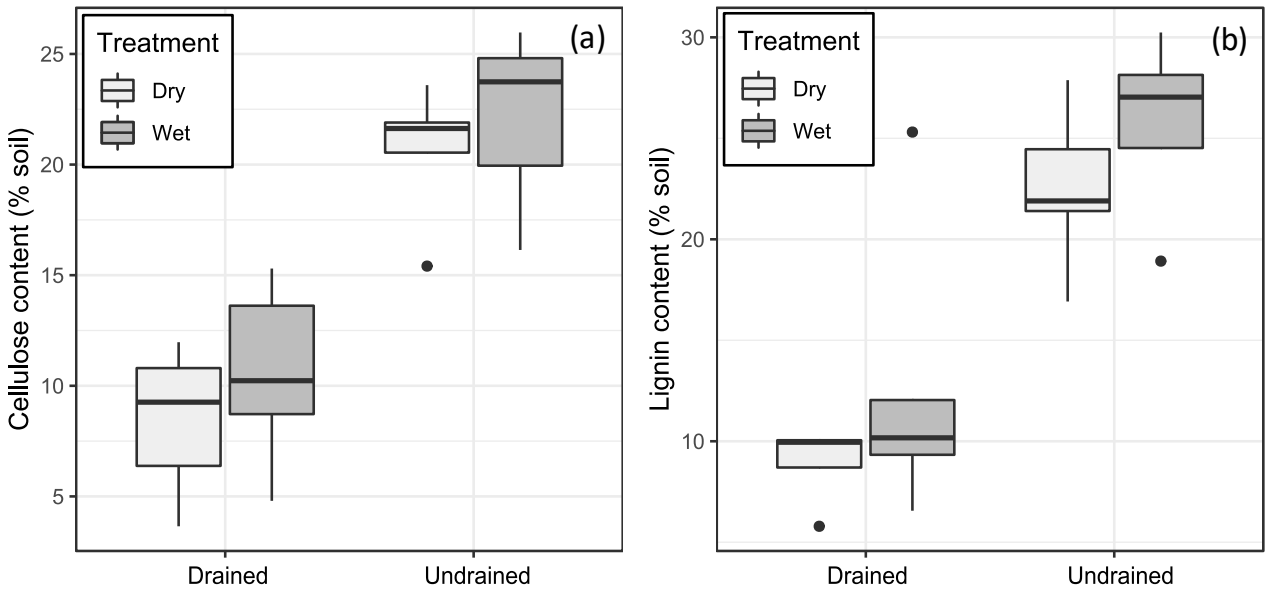

Figure 1. Properties of 20 peat monoliths collected in two fen sites with different drainage histories (historically "drained" or "undrained"), after being experimentally exposed to two water level treatments ("wet" or "dry", i.e. water levels at 0 cm and 15 cm below the peat surface). Peat properties were characterised by (a) cellulose content and (b) lignin content.

drained peat Figure 2a,b), corresponding to approximately 180 % higher methane emissions. As a result, CH_4 emissions varied orders of magnitude between the treatment extremes. Overall, the highest CH_4 emissions were measured from the originally undrained peat under high water levels (mean (± SE) = 123.6 (± 21.3) m^{-2} d^{-1}, corresponding to 1.98 g (± 0.34) m^{-2} d^{-1}), while the lowest emissions were measured from the originally drained peat under low water levels (mean (± SE) = 1.47 (± 0.26) mmol m^{-2} d^{-1}, corresponding to 0.024 (± 0.004) g m^{-2} d^{-1}).

To investigate the drivers of C emission in more detail, we constructed multiple linear regression models including the estimated 84-day cumulative emission of CO_2 and CH_4 from the monoliths as dependent variables and water level treatment ("dry" or "wet") and edaphic properties (peat soil ADF-cellulose and ADF-lignin fractions) as explanatory factors. The combination of water level treatment and peat ADF-cellulose content explained 52 % of the variation in cumulative CO_2 emission ($F_{2,17}$ = 9.093, R^2 = 0.52, P = 0.002, Figure 3a). Higher cumulative CO_2 emissions were primarily related to higher soil ADF-cellulose contents (standardised β = 0.65, P = 0.001), followed by lowered water levels (standardised β = -0.40, P = 0.03). Peat soil ADF-

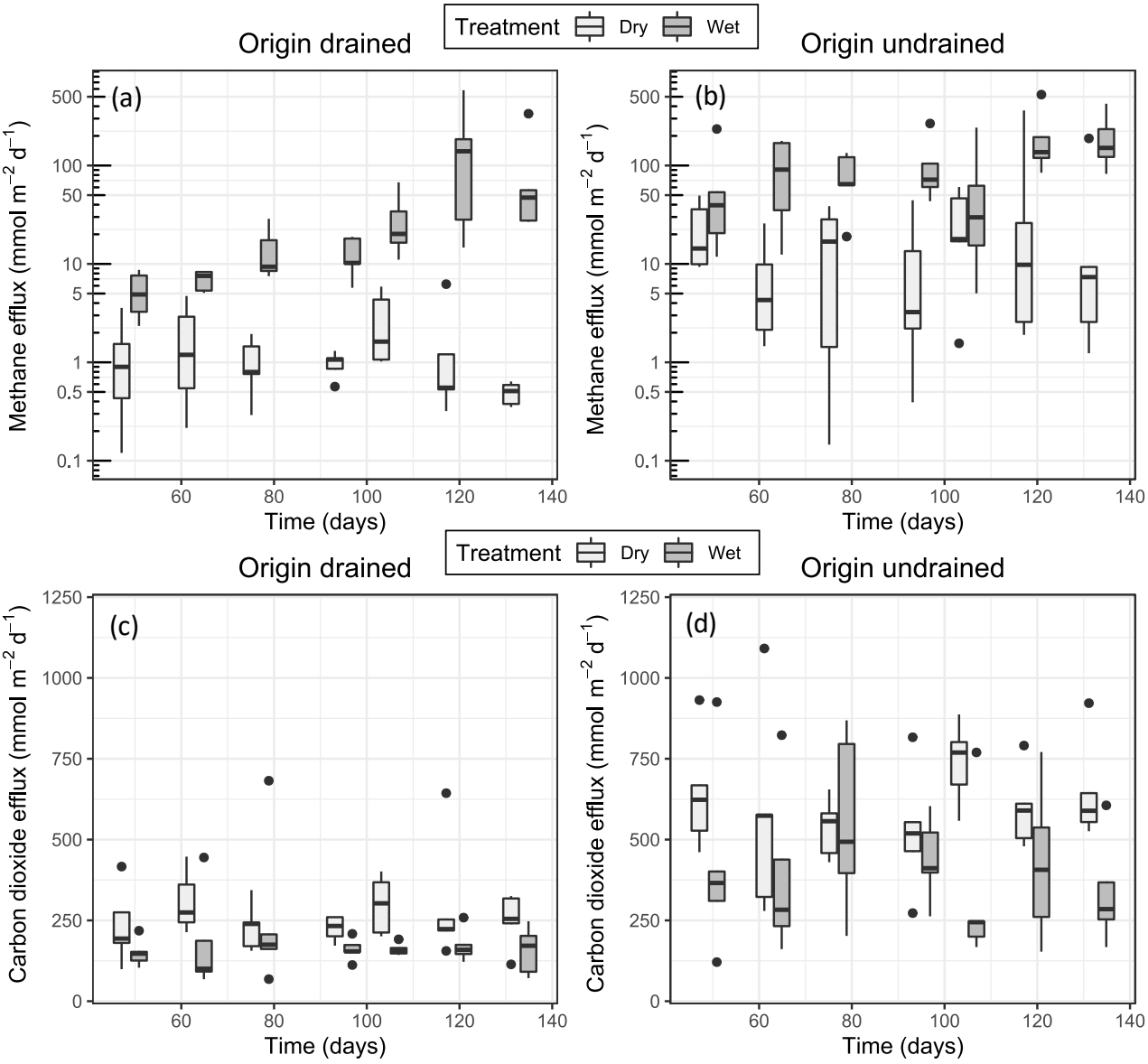

Figure 2. Emission rates of (a,b) CH_4 [log10 scale] and (c,d) CO_2 from unvegetated peat monoliths collected in two fen sites with different drainage histories (historically "drained" or "undrained") over time and in response to two experimentally induced water levels ("wet" or "dry", i.e. water levels at 0 cm and 15 cm below the peat surface).

lignin content had no significant additional explanatory power and was omitted from the model.

A similar but stronger pattern was found for the log10-transformed cumulative emission of CH_4: water level treatment and peat soil ADF-cellulose content together explained 78 % of the cumulative CH_4 emission ($F_{2,17}$ = 29.63, R^2 = 0.78, P < 0.001, Figure 3b). Again in line with previous results on CH_4 emissions, higher cumulative CH_4 emissions were primarily related to higher water levels (standardised β = 0.68, P < 0.001), and secondarily by increasing ADF-cellulose contents (standardised β = 0.48, P < 0.001). Again, there was no significant additional explanatory effect of ADF-lignin content on C emissions.

Methanogen abundance

Absolute and relative methanogen abundances, as respectively estimated by mcrA gene copy numbers and mcrA:16S rRNA copy number fractions, were highest in the wet peat monoliths of undrained origin and lowest in the dry peat monoliths of drained origin (Figure 4a,b). Methanogen abundances were thus controlled by the water level treatment (Absolute abundance: $F_{1,17}$ = 13.86, P = 0.002; Relative abundance: $F_{1,17}$ = 23.53, P < 0.001) and by peat drainage history (Absolute abundance: $F_{1,17}$ = 13.02, P = 0.002; Relative abundance: $F_{1,17}$ = 17.31, P < 0.001). Furthermore, there was a positive relationship between the estimated cumulative CH_4 emissions from the peat monoliths and the absolute and relative abundances of methanogens (Absolute abundance: R^2 = 0.64, $F_{1,18}$ = 32.55, P < 0.001, Figure 4c; Relative abundance: R^2 = 0.60, $F_{1,18}$ = 27.69, P < 0.001, Figure 4d).

DISCUSSION

By measuring biweekly gas effluxes of carbon dioxide (CO_2) and methane (CH_4) from unvegetated fen peat monoliths, we found that carbon emissions were strongly determined by current water levels and by quantities of easily degradable organic matter in the peat soil, which related to the drainage history of the site (i.e. historically undrained or long-term drained with degradation legacy). Moreover, we observed a strong response of methanogens to the experimental treatments, and found that methanogen abundance was a good predictor of cumulative CH_4 emissions.

Controls on carbon dioxide emission

The lowest CO_2 emissions were measured in the originally drained peat under high water levels (mean (± SE) = 176.5 (± 19.04) mmol m^{-2} d^{-1}, corresponding to 7.77 g (± 0.84) m^{-2} d^{-1}) and the highest CO_2 emissions came from the originally undrained peat under low water levels (mean (± SE) = 607.4 (± 30.8) mmol m^{-2} d^{-1}, corresponding to 26.73 (± 1.36) g m^{-2}

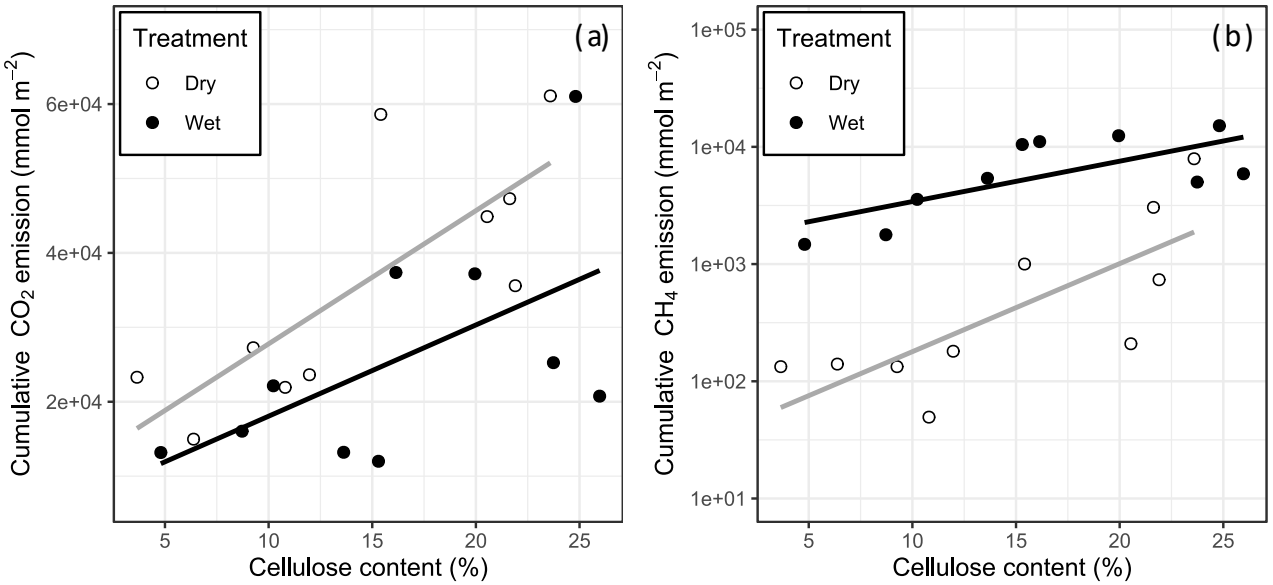

Figure 3. The cumulative emission of (a) CO_2 and (b) CH_4 [log10 scale] from peat monoliths versus peat ADF-cellulose contents under two experimentally induced water levels ("wet" or "dry", i.e. water levels at 0 cm and 15 cm below the peat surface). Cumulative CO_2 and CH_4 emissions were estimated over a 84-day period by constructing AUC curves using biweekly flux data.

d^{-1}, here 95 % of the total weight-based mean C efflux). These emission estimates were in the higher range of what is generally reported in literature (although similar ranges have been reported, e.g. by Lohila *et al*. (2003) or Chimner & Cooper (2003)). Most likely our high numbers were caused by (i) the inevitable disturbance of the peat matrix upon peat monolith extraction and defoliation, which may have triggered increased root decay and decomposition rates, (ii) the continuously moderate temperatures throughout the experiment (12.6–19.8 °C), and (iii) emissions reflecting soil respiration rather than net ecosystem exchange (due to the lack of light and vegetation). The lack of vegetation in our study is important to keep in mind: living plants may stimulate or inhibit carbon losses through processes such as photosynthesis and C assimilation, root exudation, gas transport and radial oxygen loss (Dunn *et al*. 2016, Rupp *et al*. 2019).

Nonetheless, our standardised experimental approach allowed us to disentangle the relative contribution of our two main factors of interest,

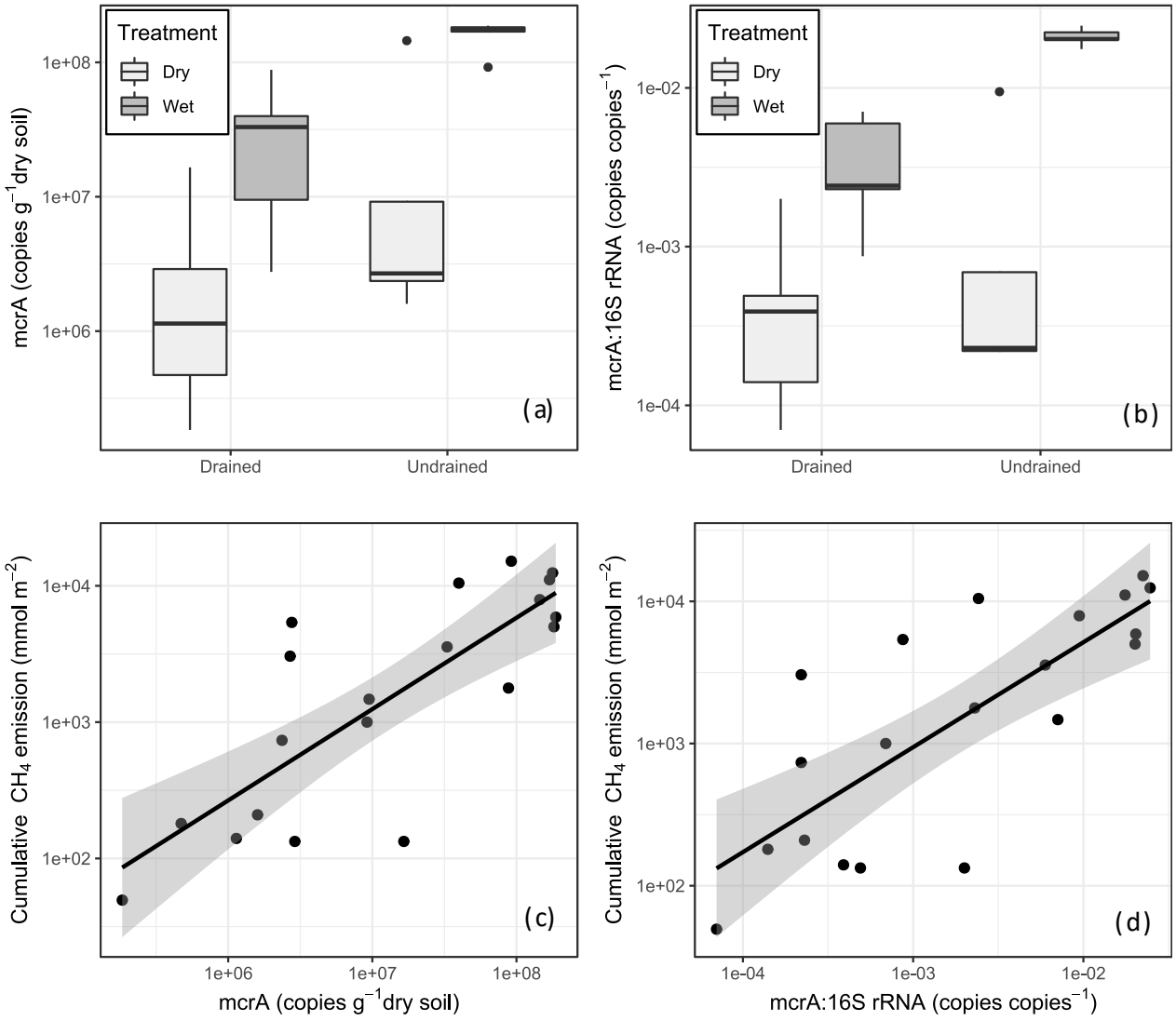

Figure 4. Absolute and relative methanogen abundances in unvegetated peat monoliths in relation to experimental treatments and cumulative CH_4 emissions. Panel (a): absolute methanogen (mcrA) abundance in relation to peat drainage history and water level treatment; panel (b): relative methanogen abundance (mcrA:16S rRNA) in relation to peat drainage history and water level treatment; panel (c): absolute methanogen (mcrA) abundance as a predictor of cumulative CH_4 emissions; panel (d): relative methanogen (mcrA:16S rRNA) abundance as a predictor of cumulative CH_4 emissions. All panels are on a log10-scale, panels c and d show regressions line with 95 % CI's. Methanogen abundances were calculated as the mean values of two depths per monolith. Cumulative CH_4 emissions were estimated over a 84-day period by constructing AUC curves using biweekly flux data.

current water levels and peat properties. Overall, it has been well established that current water level is a strong determinant of CO_2 emissions from organic soils, with a generally steep increase in emissions following water level drawdown and concomitant aerobic peat oxidation (Moore & Knowles 1989, Silvola *et al*. 1996, Chimner & Cooper 2003). In our experiment, even a minor (15 cm) decrease in water levels indeed resulted in, on average, 42 % higher CO_2 emissions. However, it was surprising that current water level was not the primary determinant of CO_2 emissions. CO_2 effluxes were more strongly related to peat drainage history and corresponding peat properties, with 141 % higher fluxes from the peat monoliths of undrained origin. These monoliths were characterised by higher contents of cellulose- and lignin-like structures as compared to the monoliths of drained origin. Here, we cannot fully rule out that these structural differences in lignin and cellulose contents (and thus of C fluxes) that we relate to the actual peat matrix, may in fact also be – at least partially– due to potential differences in fresh root biomass allocation between the two sites. Since it is impossible to separate living roots from the surrounding fen peat matrix (which is also largely composed of (dead) roots), we were unable to test for potential site differences in (fresh) root biomass. However, since drainage often leads to (i) an increase rather than a decrease in fresh root biomass and (ii) deeper rooting (Laiho & Finér 1996, Murphy *et al*. 2009), it is unlikely that the drained site would have had significantly lower quantities of fresh root biomass than the undrained site.

Regardless of the exact origin of the organic matter, multiple linear regression models indicated that the cumulative CO_2 efflux related best to the ADF-cellulose fraction of the peat matrix, which varied between 1.4 % and 29.3 % in our monoliths across drainage histories. Cellulose, a polysaccharide, is the most abundant carbohydrate in plants and is therefore also an important constituent of peat. It decomposes more easily than lignin, and both cellulose and its breakdown products are important sources of energy and carbon for a diverse consortium of peatland microbes (Schmidt *et al*. 2015). Although it seems evident that higher contents of easily-degradable carbon in the soil correlate with higher heterotrophic soil respiration, as it is the main C substrate, ample studies report unclear, weak or even no relationships between quantity and quality of soil organic matter and CO_2 emissions from organic soils (Eickenscheidt *et al*. 2015, Tiemeyer *et al*. 2016, Bader *et al*. 2018). At the same time, other studies have reported results that are in line with our findings. For example, Lohila *et al*. (2003) found that peaty soils had 2–3x higher respiration rates than soils with higher mineral contents, which they mainly attributed to the differences in soil C content. Also, Leifeld *et al*. (2012) showed that soil respiration rates of a disturbed temperate peatland were strongly governed by the peat's quality, as defined by a high availability of polysaccharides. From this, they inferred that respiration rates would be highest at the onset of peat drainage (due to a high initial peat quality), but would decrease through time due to the concomitant decomposition-driven decrease in peat quality. We concur with the latter hypothesis, and we postulate that this process is highly relevant to our long-term drained study site with its degradation legacy, where centuries of drainage and concomitant loss of organic matter have led to much lower quantities and quality of remnant soil carbon. Taken together, current water levels and peat ADF-cellulose content explained approximately 52 % of the variation in cumulative CO_2 emissions from our peat monoliths.

Controls on methane emission

Overall, the lowest CH_4 emissions were measured from the originally drained peat under low water levels (mean (± SE) = 1.47 (± 0.26) mmol m^{-2} d^{-1}, corresponding to 0.024 g (± 0.004) m^{-2} d^{-1}) and the highest fluxes were measured from the originally undrained peat under high water levels (mean (± SE) = 123.6 (± 21.3) mmol m^{-2} d^{-1}, corresponding to 1.98 g (± 0.34) m^{-2} d^{-1} , here accounting for approximately 23 % of the total mean C efflux). These values are much higher than what is generally reported from fens under field conditions, i.e. annual CH_4 emissions usually do not exceed maxima of approximately 100 g m^{-2} (Wilson *et al*. 2009, Günther *et al*. 2014), which approximately equates to less than 0.3 g m^{-2} d^{-1}. Although there are some scattered reports of disproportionately high CH_4 peak emissions from fen soils, e.g. 0.7 g m^{-2} h^{-1} (Hahn *et al*. 2015), > 1.5 g m^{-2} d^{-1} (Harpenslager *et al*. 2015), with extremes of up to 4.1 g m^{-2} d^{-1} (Turetsky *et al*. 2014), we suspect that the high CH_4 emissions in our experiment probably do not reflect emissions under field conditions and should thus be regarded as potential emissions; i.e. emissions from unvegetated (and initially disturbed) bare peat monoliths that are kept under stable water levels and relatively high temperatures.

It is well known that current water level is the primary determinant of CH_4 emissions (e.g. see Moore & Knowles 1989, Dise *et al*. 1993, Turetsky *et al*. 2014). In our experiment, we measured on average 430 % higher emissions from the peat monoliths that were subjected to the high water level treatment.

However, CH_4 emissions were not stable through time: despite a monolith stabilisation period of 49 days prior to taking the first measurement, emissions still increased significantly during the subsequent monitoring period and seemed to reach an equilibrium after approximately 120 days. This overall significant increase was primarily due to an increase in emissions from the monoliths under high water levels, as emissions from monoliths under low water levels appeared relatively stable (Figure 2a,b). As expected, the strongest relative increase (i.e. more than an order of magnitude) was observed in the monoliths of drained origin that were subjected to high water levels (= "rewetting" treatment). This result indicates that, after rewetting, methanogen communities require some time to reach their maximum potential. This is a generally well known mechanism, and it relates to the gradual depletion of electron acceptors that is needed to reach typical methanogenic conditions of low redox potential (Knorr & Blodau 2009).

More interesting however was that CH_4 production in the peat monoliths that were subjected to the lower water levels did not come to a standstill. Sustained CH_4 emissions in these monoliths may be due to the fact that a 15 cm drop in water levels only qualifies as minor water level drawdown. Here, it is likely that the high oxygen-depletion rates (intrinsic to organic soils) in combination with capillary water rise resulted in incomplete aeration and therefore sufficient anoxic micro-habitats in which methanogenic communities were able to persist. Methanogen communities are generally well adapted for survival in a dynamic redox environment (Brune *et al*. 2000), and they can withstand temporary drought (Knorr *et al*. 2009). This is supported by our results on methanogen abundance: although the 15 cm drop in water levels resulted in an order of magnitude decrease in mcrA copy numbers in the peat matrix, methanogen abundance was nowhere near zero, indicating that methanogens were able to persist upon minor water level drawdown. As expected, we also found that methanogen abundance was a good predictor of cumulative CH_4 emissions (see also Huth *et al*. (2020) for similar results).

In line with results on CO_2 emissions, CH_4 emissions were also higher in the peat monoliths of undrained origin: CH_4 emissions were on average 180 % higher from the peat monoliths of undrained origin than those of drained origin, regardless of current water levels. Again, multiple linear regression models indicated that the ADF-cellulose fraction of the peat soils related positively to cumulative CH_4 emissions, while peat ADF-lignin fractions had no significant additional explanatory power. Taken together, current water levels and peat ADF-cellulose content explained most (= 78 %) of the variation in cumulative CH_4 emissions from the peat monoliths. The pathways of CH_4 production are generally complex and interlinked with pathways of CO_2 production, and cannot be disentangled in our study: part of the CH_4 flux will stem from the reduction of CO_2, while part of the CO_2 flux will stem from the oxidation of CH_4. Regardless of the exact mechanisms, our results do show that higher contents of easily-degradable carbon in the peat and concomitant higher soil respiration rates – either directly or indirectly – also lead to higher CH_4 emissions, which supports conclusions from previous studies (Moore & Knowles 1989, Yavitt & Lang 1990, Reiche *et al*. 2010, Hahn-Schofl *et al*. 2011).

In wetlands, most CH_4 originates from acetate fermentation and CO_2 reduction (Avery *et al*. 2003, Christensen *et al*. 2003), for which polysaccharides such as hemicellulose and cellulose are important initial organic matter substrates (Corbett *et al*. 2015, Schmidt *et al*. 2015). These polysaccharides first undergo different steps of hydrolysis and transformation –mediated by a diverse microbiome– before the main products (acetate and CO_2) become available for methanogenesis. Hence, the observed positive correlation between soil ADF-cellulose contents and CO_2 and CH_4 emission rates suggests that a broad consortium of decomposers is active at the same time, readily using and transforming the high amounts of easily degradable polysaccharides. We therefore conclude that edaphic properties strongly affect the production potential of CH_4, with the lowest emissions from soils with low absolute quantities of easily degradable organic matter, i.e. as typically found in long-term drained and heavily degraded fens. Urbanova & Barta (2020) found similar results and showed that methanogenesis in degraded peat soils could be stimulated by manual addition of easily degradable organic substrates. Furthermore, our finding does not support the hypothesis that rewetting of drained fens leads to very high CH_4 emissions. CH_4 spikes in re-flooded peatlands often stem from a prompt dieback of the former vegetation that is not adapted to anaerobic conditions, resulting in a temporarily increased input of fresh and easily-decomposable organic matter (Hahn-Schofl *et al*. 2011, Hahn *et al*. 2015).

CONCLUSIONS

Our results indicate that degradation legacy and current water levels are strong predictors of carbon emission potential in fens. While present-day high

water levels correlate with relatively low and high emissions of CO_2 and CH_4 respectively, the highest potential C emissions (both as CO_2 and CH_4) are from historically undrained fens that contain large quantities of easily degradable organic matter. Hence, it appears that the present-day C-rich soils, including pristine fens, are also the largest potential present-day C leaks.

Long-term drained fens, on the other hand, have already lost much of the easily-decomposable peat fraction, and this legacy translates into significantly lower present-day baseline C emissions. Unfortunately, this has not come without a cost: in drained fens, most C has already been emitted into the atmosphere decades to centuries ago. This past loss of soil carbon is irreversible within a time span of centuries to even millennia, as fen peat accretion rates are very low even after rewetting. To mitigate climate change, we therefore conclude that all undrained fens must be preserved in order to prevent aeration and decomposition of the remnant and highly reactive peat matrix, while rewetting of currently drained fens has excellent prospects from a C balance point of view. Not only does the low quality and quantity of remnant soil carbon reduce the risk of high C emissions after rewetting, the re-establishment of peat-forming vegetation should also lead to a rapid shift to an ecosystem with renewed net C sequestration.

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AUTHOR CONTRIBUTIONS

WJE, EV, PS, MvR and RvD developed the methodology of the research; WJE, EV, PS, and YL collected the data, WJE and PS analysed the data, WJE wrote the first draft of the manuscript and all authors reviewed and gave critical feedback. All authors contributed to final editing of the manuscript and approved the final version.

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Appendix

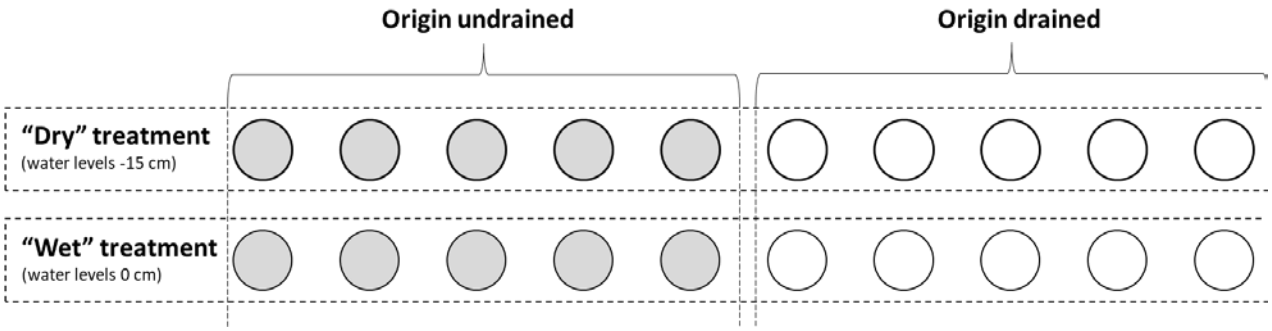

Figure A1. Experimental set-up. A total of 20 monoliths was collected in one originally undrained site (10 monoliths) and one long-term drained site (10 monoliths). The monoliths were then subjected to a permanent (re-)wetting "wet" or partial drying "Dry" treatment in a balanced design (random allocation of monoliths), with 5 replicate monoliths per treatment combination.

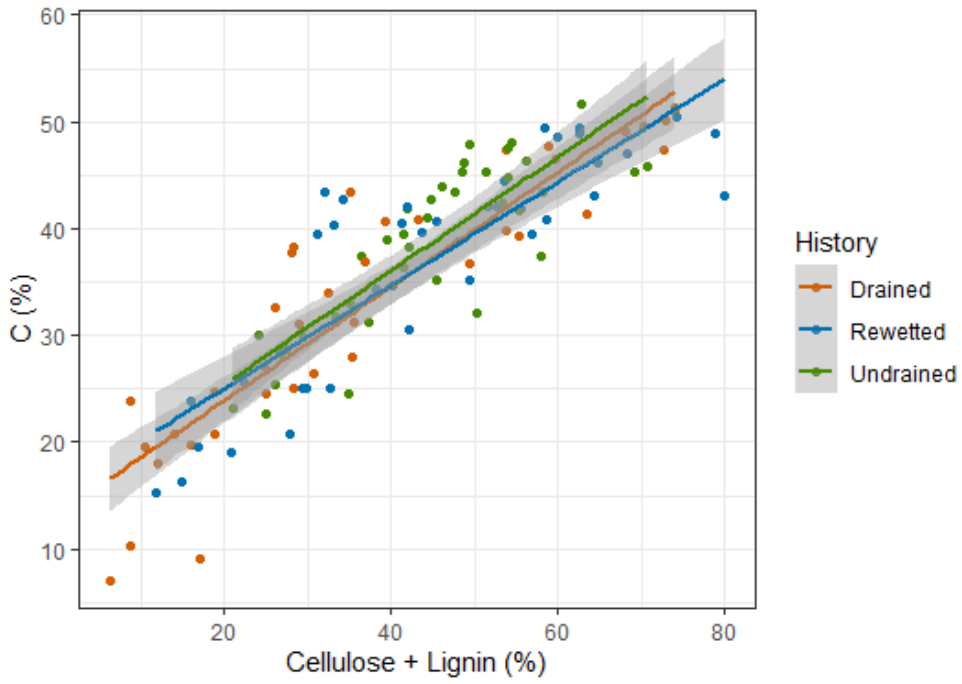

Figure A2. Positive relationship between the cumulative fractions of cellulose and lignin (% ADF-cellulose + % ADF-lignin) and total soil carbon (% C) contents as determined by standard C analysis through combustion on a CN-analyser (Pearson's r = 0.88, P < 0.001). Analyses were part of a different study (Emsens *et al*. 2020), in which 117 fen peat samples were collected at 3 depths (from 0 to 50 cm below the surface) in 39 different fen sites (including the study sites of the current study) that differed in drainage history (drained, rewetted or undrained).