Changes in microbial community composition, activity, and greenhouse gas production upon inundation of drained iron-rich peat soils

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A R T I C L E   I N F O

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A B S T R A C T

Globally, large-scale land drainage has severely deteriorated the functioning and services of peatlands, making restoration plans of the utmost importance. Rewetting is essential for the restoration of drained peatlands, but the level of success including greenhouse gas (GHG) mitigation largely depends on the soil microbiome in interactions under the prevailing biogeochemical conditions. Here, we investigated the effects of inundation of drained iron (Fe) -rich peat topsoils on nutrient release, surface water quality, GHG production and consumption, and on the composition and activity of the microbial community. The effect of the addition of different potential electron acceptors on methane (CH\textsubscript{4}) production and consumption were studied in incubation experiments. In response to inundation, porewater concentrations of Fe, total inorganic carbon, ammonium, and phosphorus increased. CH\textsubscript{4} emissions increased in the control (i.e. without any additions) and Fe(III) oxide amended incubations upon inundation. This could be explained by the increase in the relative abundance of methanogens even though Fe(III) was previously hypothesized to lower methanogenic activity. In contrast, nitrite, nitrate, and sulfate-rich incubations inhibited methanogenesis. The prolonged exposure to nitrogen oxides stimulated denitrification with nitrous oxide (N\textsubscript{2}O) as the main gaseous product, together with an increase in the relative abundance of denitrifying microbiota. Our results demonstrate that insights into the changes in microbial communities in relation to soil geochemistry explain differences in responses observed in different peat soils observed upon inundation. The increase in emissions of the potent GHGs CH\textsubscript{4} and N\textsubscript{2}O from Fe-rich peat topsoils are a major adverse effect in the early stage of inundation.

1. Introduction

Widespread drainage is compromising the capacity of the world’s peatlands to serve as sinks for nutrients and carbon (C) (Lamers et al., 2015). During the first half of the 20th century, large areas (worldwide approximately 15%) of peatlands have been lost due to drainage, thereby enhancing the aerobic decomposition of organic matter (OM) (Joosten, 2010; Joosten and Clarke, 2002). As a result, drained peatlands have become net C sources, with an annual global emission of approximately 2 Gt carbon dioxide (CO\textsubscript{2}). This is around 5% of all anthropogenic greenhouse gas (GHG) emissions originating from only 0.3% of the global land surface (Joosten et al., 2016). Simultaneously,
this drainage has also caused severe land subsidence (2–150 mm yr\(^{-1}\), depending on the region) (Leifeld et al., 2011; Syvitski et al., 2009), which may lead to high costs for infrastructure and, together with the anticipated sea-level rise, poses a severe inundation risk to often highly populated riverine areas and coastal deltas.

To counteract subsidence and restore ecosystem services, large areas of drained peatlands are being rewetted to restore the hydrology to historic water levels (Geurts et al., 2019). Although the positive effect of rewetting on carbon sequestration is undisputed, there are also several studies suggesting that there may be a considerable amount of CH\(_4\) released after rewetting (Franz et al., 2016; Günther et al., 2019; Hahn et al., 2015; Harpenslager et al., 2015; Hemes et al., 2018; Knox et al., 2015; Vanselow-Algan et al., 2015; Wilson et al., 2009). In addition, nitrous oxide (N\(_2\)O) emission may increase due to denitrification of accumulated nitrogen oxides (Beare et al., 2009; Harrison-Kirk et al., 2013; Liu et al., 2018; Snider et al., 2015). Since the global warming potential of CH\(_4\) is about 34 and N\(_2\)O is 298 times higher than that of CO\(_2\) over 100 years (Myhre et al., 2013), rewetting could have an (initial) negative effect on the GHG emissions. The evolution of GHG emission patterns following rewetting can vary substantially (from years to decades) depending on previous land use, restoration methods applied, and local climate conditions (Baird et al., 2013; Beetz et al., 2013; Juottonen et al., 2012; Mohamed Abdalla et al., 2016; Samaritani et al., 2011; Strack and Zuback, 2013; Wen et al., 2018).

The shift in the GHG emissions is triggered by the large changes in the composition and activity of the microbial community upon rewetting. The increase in water table lowers the oxygen (O\(_2\)) concentrations in the formerly drained soil, thereby stimulating anaerobic microbial processes. OM will be decomposed in an anaerobic syntrophic food chain ultimately providing hydrogen, CO\(_2\), and acetate to methanogenic archaea (Kotsyurbenko et al., 1995; Schink, 1997). The produced CH\(_4\) will diffuse upwards in the soil column. A fraction of that CH\(_4\) can then be oxidized by anaerobic methanotrophs to CO\(_2\) under anoxic conditions using several alternative electron acceptors including nitrite (NO\(_2\)), nitrate (NO\(_3\)), sulfate (SO\(_4^{2-}\)), or iron (Fe) (III) oxides (Bhattacharjee et al., 2016; Boetius et al., 2000; Cai et al., 2018; Chang et al., 2012; Crowe et al., 2011; Egger et al., 2015; Ettwig et al., 2016, 2010; Haroon et al., 2013; Milucka et al., 2012; Raghoebarsing et al., 2006; Saunois et al., 2016; Scheller et al., 2016; Torres et al., 2014; Vaksmaa et al., 2017, 2016). CH\(_4\) ultimately reaches oxic zones in the soil where it can be converted to CO\(_2\) by aerobic methane oxidizing bacteria (MOB) (Semrau et al., 2010). Depending on the prevailing conditions, residual CH\(_4\) can also escape biological oxidation and will be emitted to the atmosphere (Dean et al., 2018).

In order to establish optimal management strategies for peat restoration, the microbial processes governing the increase of CH\(_4\) emissions upon rewetting need to be better understood. The microbial community composition and interactions controlling the fluxes of GHGs will be directly influenced by factors such as water table levels, presence of oxidizing agents (e.g. Fe(III) oxides and SO\(_4^{2-}\)) that have accumulated during years of oxic conditions in the soil vegetation productivity, soil temperature, and presence of labile carbon (Dean et al., 2018). The aim of our research was to study the effect of ecosystem relevant electron acceptors (NO\(_2\), NO\(_3\), SO\(_4^{2-}\), Fe(III) oxides) on the microbial community composition and fluxes of CH\(_4\) and N\(_2\)O in anaerobic incubations of inundated soils. Previous field studies showed that inundation of Fe-rich peat soils still may result in high CH\(_4\) emissions, despite the predicted inhibition of methanogenesis at the presence of Fe(III) oxides (Bodegom et al., 2019).
Table 1

| Soil  | Location   | Latitude | Longitude | OM  | Total Fe | Total P | Bulk density | Texture  |
|-------|------------|----------|-----------|-----|----------|---------|--------------|----------|
| IRS1  | Abcoude    | 52.266   | 4.954     | 27  | 435      | 61      | 0.52         | clayey peat |
| IRS2  | Oude water | 52.002   | 4.847     | 40  | 641      | 95      | 0.40         | peat      |
| IRS3  | Wilnis     | 52.166   | 4.900     | 49  | 1691     | 119     | 0.37         | peat      |
| IPS   | Loosdrecht | 52.192   | 5.135     | 6.4 | 51       | 26      | 1.20         | sandy peat |

Soil cores were collected in March 2016 from four Dutch agricultural fields (three Fe-rich soils named IRS1-3 with increasing number indicating increasing total Fe content and one Fe poor soil named IPS) that are now in use as grasslands (with cattle) situated on top of drained peatlands (so-called peat meadows; Fig. 1, Table 1). They were used as agricultural lands for decades to centuries. None of the fields were currently unknown. We collected topsoils from four Dutch agricultural locations and properties of the four sampled soils. Total iron (Fe) and phosphorus (P) are in mmol kg dry weight⁻¹, the bulk density is in kg dry weight L⁻¹ soil, and OM (organic matter content) is given in %.

2. Materials and methods

2.1. Site description and sampling

Soil cores were collected in March 2016 from four Dutch agricultural fields (three Fe-rich soils named IRS1-3 with increasing number indicating increasing total Fe content and one Fe poor soil named IPS) that are now in use as grasslands (with cattle) situated on top of drained peatlands (so-called peat meadows; Fig. 1, Table 1). They were used as agricultural lands for decades to centuries. None of the fields were located in a nature reserve. Multiple sub-samples from the topsoil layer (0–10 cm below the root zone) were taken at each location with a gouge auger and placed into a glass cylinder (diameter of 14.5 cm). After sample collection, all soils were inundated separately with 1 L of artificial surface water (1.5 M CaCl₂, 1 M MgCl₂-6H₂O, and 2 M NaHCO₃), creating a water layer of approximately 10 cm above the soil surface. The inundated soils were placed in a dark climate room with controlled conditions at 15 °C (B-WARE, Nijmegen, Netherlands) to study the mobilization of nutrients over a period of 18 months. Porewater samples (30–40 mL) were taken anoxically using rhizon samplers (Eijkelkamp Agrisearch Equipment) after a 1-day (taken as time point 0), 2 weeks, 6 weeks, 12 weeks and 69 weeks of inundation. Samples were analyzed by inductively coupled plasma-optical emission spectrometry (ICP-OES) for Al, Ca, Fe, Mg, Mn, P, and S (iCap 6300, Thermo Scientific, Waltham MA, USA) with a sea spray nebulizer combined with a cyclone chamber. The plasma had a power of 1150 W and was generated by argon gas, with an auxiliary gas flow of 0.5 L min⁻¹ and a nebulizer flow of 0.6 L min⁻¹. The porewater samples were also analyzed by continuous flow analysis (CFA) for NO₃, NH₄, PO₄, Na, K, and Cl (Bran + Luebbe Auto Analyzer, SX Flow, Norderstedt, Germany; Seal Analytical Auto-Analyzer 3, Seal Analytical, Southampton, UK). Gravimetric water content and bulk density of the soil were determined by drying a fixed volume for 48 h at 70 °C. OM content was estimated by loss on ignition for 4 h at 550 °C. To determine total concentrations of Al, Ca, Mg, Fe, P, S, and N, a homogenized portion of 200 mg dry soil was digested in 5 mL HNO₃ (65%) and 2 mL H₂O₂ (30%) using an Ethos 1 Advanced microdigestion system (Milestone microwave type ml 1200 mega). Digestates were diluted and stored at 4 °C until elemental analysis by ICP-OES (IRIS Intrepid II XDL, Thermo Scientific, Waltham MA, USA). The pH was measured using an Ag/AgCl2 electrode connected to a radiometer (Copenhagen, type TIM840). Alkalinity of surface waters was determined by titration with 0.01 mol L⁻¹ HCl until pH 4.2 and concentrations dissolved inorganic carbon (CO₂ and HCO₃⁻) in surface and pore water were determined using an infrared gas analyzer (ABB Advance Optima IRGA). Electric conductivity (EC) was measured using an EC-probe (HACH) connected to an HQD-meter.

2.2. Batch incubation experiments

The batch incubation experiments were started in November 2017. For each of the four locations, the water layer on top of the soil was removed and the soil was sieved through a metal sieve (0.42 mm pore size) to remove the remnants of the plant material. Soil slurries were prepared by mixing the soil in a 1:4 weight/volume ratio with anoxic freshwater mineral medium (DSMZ 503) composed of KH₂PO₄ (0.20 g L⁻¹), NH₄Cl (0.25 g L⁻¹), NaCl (1.00 g L⁻¹), MgCl₂-6H₂O (0.40 g L⁻¹), KCl (0.50 g L⁻¹) and CaCl₂-2H₂O (0.15 g L⁻¹) in grade 3 demineralized water; 1 mL L⁻¹ trace element solution SL-10 (DSMZ), with CeCl₃-7H₂O 24 mg L⁻¹, Na₂SeO₃-5H₂O 30 mg L⁻¹ and Na₂WO₄-2H₂O 40 mg L⁻¹; and 1 mL L⁻¹ of vitamin solution (DSMZ 141). The pH of the slurries was measured (Hanna Instruments, Betuwehaven, the Netherlands) and was between 6.3 and 6.6. Homogenized soil slurry samples of 2.5 mL were taken from all four soils (T0), centrifuged for 10 min at 10,000 × g (Eppendorf, Nijmegen, The Netherlands) and pellets were stored at −18 °C for subsequent DNA extraction. Activity assays were performed in duplicate in 120 mL glass serum bottles with 53 mL of soil slurry and an end volume of 60 mL after the addition of substrates and/or grade 3 demineralized water. The incubation bottles were sealed with airtight right butyl rubber stoppers and secured with open-top aluminium crimp caps.

2.2.1. Aerobic methanotrophic incubations

For the aerobic methanotrophic incubations 20% CH₄ v/v (final concentration) was added to theoxic headspace filled with air. When the CH₄ concentration was lower than 4 μmol CH₄ mL⁻¹ slurry or O₂ was depleted, the headspace of the bottles was three times flushed with 0.2 μM filter-sterilized air before 20% CH₄ v/v, or 21% O₂ v/v was added again, respectively. The serum bottles were incubated by continuous shaking at 100 rpm in the dark for 120 days (platform shaker innova2300, New Brunswick Scientific Europe, The Netherlands). Homogenized soil slurry samples of 2.5 mL were taken aseptically from all incubations after 120 days (T1), centrifuged for 10 min at 10,000 × g (Eppendorf, Nijmegen, The Netherlands) and pellets were stored at −18 °C for subsequent DNA extraction.

2.2.2. Anaerobic methanotrophic incubations

Anoxic conditions were created in the incubation bottles by three cycles of 15 min vacuuming and 3 min gassing with Argon (Ar). A final overpressure of 0.5 bar was applied in all bottles. Subsequently, 5% CO₂ v/v was added to the headspace. All treatments were performed in duplicates. We used SO₄²⁻ to mimic the actual or geological marine or estuarine settings, N oxides as a result of intense N fertilization, and Fe (III) oxides which naturally accumulate in highly degraded peat soils with Fe-rich topsoil. Following treatments with respective starting concentrations were prepared for each soil: SO₄²⁻ (2 mM), NO₃⁻ (0.25 mM), NO₂⁻ (2 mM), ferricydrate (Fe(OH)₈, 10 mM) and goethite (FeOOH, 10 mM). Each treatment was prepared with and without added ¹³C-CH₄ (20% v/v final concentration). Control batch incubations of soil slurry were prepared with and without the addition of ¹³C-CH₄ (20% v/v).
final concentration (Supplemental Table S1).

Ferrihydrite was synthesized by dissolving 35 g of ferric citrate in 300 mL distilled water (80 °C) under vigorous stirring (adapted from Leibl et al., 1999). After cooling to room temperature, the pH was adjusted to 8.0 by the dropwise addition of 10 M sodium hydroxide solution and stirring was continued for 30 min. The resulting ferrihydrite particle suspension was centrifuged at 10,000×g at 4 °C for 60 min, the supernatant was discarded and the pellet was resuspended in distilled water. After five cycles of centrifugation, removing the supernatant and resuspension in distilled water, the pellet was resuspended in a small volume of distilled water. Ferrihydrite concentration was determined by measuring the dry weight of an aliquot. Goethite was prepared from an alkaline Fe(III) system according to the protocol by Böhm (1925) as described in Schwertmann and Cornell (1991) with a 72 h incubation at 70 °C.

The serum bottles were incubated horizontally by continuous shaking at 100 rpm in the dark for 240 days (platform shaker innova2300, New Brunswick Scientific Europe, The Netherlands). Liquid samples (0.4 mL) were taken during the experiment from the NO$_2$, NO$_3$, and SO$_4^2-$ treatments with a syringe. The samples were centrifuged for 10 min at 10,000×g (Eppendorf, Nijmegen, The Netherlands) and the supernatant was collected and stored at −18 °C until further processing. All electron acceptors, except Fe(III) oxides, were regularly re-added when necessary from anoxic stock solutions. As one bottle with slurry from IRS2 amended with SO$_4^2-$ and CH$_4$ broke during the experiment, we have a single incubation for this amendment. Homogenized soil slurry samples of 2.5 mL were taken aseptically from all incubations after 120 days (T1) and 240 days (T2), centrifuged at room temperature for 10 min at 10,000×g (Eppendorf, Nijmegen, The Netherlands) and pellets were stored at −18 °C for subsequent DNA extraction. The pH of all incubations was measured after 120 and 240 days (Supplemental Table S2).

2.2.3. Denitrification potential

To study the denitrification potential of soil slurries, we added around 2 mM $^{15}$N-NaNO$_2$ to NO$_2$ amended incubations at a point when non-labeled NO$_2$ was depleted (<4.3 μM, using Merckosquant test strips, Merck, Germany). NO$_2$ which is the first intermediate of NO$_3$ reduction under anoxic conditions can be converted to N$_2$O, N$_2$ or ammonium (NH$_4^+$) as an end product. The $^{15}$N label was followed over time by gas measurements and by colorimetric assay for NO$_2$ and expressed as rates of N-atom conversion from NO$_2$. The use of $^{15}$N-NaNO$_2$ in the incubations enabled the distinction between $^{14}$N/1-$^{15}$N-N$_2$O and $^{15}$N/15-$^{15}$N-N$_2$O produced from the $^{15}$N-NaNO$_2$, as well as the distinction between 14/15-$^{15}$N-N$_2$O, 15/18-$^{15}$N-N$_2$O, and 15/15-$^{15}$N-N$_2$O. $^{15}$N-NaNO$_2$ was amended to all bottles except for IRS3 and IPS where one bottle amended with CH$_4$ and NO$_2$ was not depleted yet in NO$_2$. From IRS1, one of the bottles amended with only NO$_2$ was excluded from analysis, as it was amended with 1 mM $^{15}$N-NaNO$_2$.

2.3. Analytical methods

2.3.1. Gas measurements

Gas samples (50 μL) were taken with a glass syringe (Hamilton, Reno NE, USA). CH$_4$ was quantified by triplicate injections to an HP 5890 gas chromatograph (Hewlett Packard, Palo Alto CA, USA) equipped with a Porapak Q 100/120 mesh column (Sigma Aldrich, Saint Louis MI, USA) and a flame ionization detector (FID). The quantification of CO$_2$, O$_2$, and gaseous N compounds was performed by duplicate injections to a gas chromatograph (6890 series; Agilent, United States) using a Porapak Q column at 90 °C with helium as the carrier gas (flow rate, 24 mL min$^{-1}$) that was coupled to a mass spectrometer (Agilent 5975C inert MSD; Agilent, United States). Quantification was performed based on calibration curves which were calculated from different volume injections of a standard gas (Linde Gas Benelux BV, The Netherlands). Gas concentration in liquids was calculated using the Ostwald coefficient (Wilhelm et al., 1977). For better comparison of activities and concentrations between the different soils, activities and concentrations are expressed per mL of soil slurry.

2.3.2. NO$_2$ and NO$_3$ concentration measurements

During the course of incubation experiments, concentrations of NO$_2$ and NO$_3$ were first estimated using Merckosquant Nitrate test strips (0–113 mg L$^{-1}$, Merck, Germany). Aliquots (0.4 mL) were taken and stored at −18 °C until analysis. Concentrations of both NO$_2$ and NO$_3$ were measured in triplicate using the Griess assay (Griess-Romijn van Eck, 1996) on the microplate spectrophotometer (Spectramax 190, Molecular Devices, The Netherlands). For the determination of NO$_3$ concentration an additional step was performed: vanadium(III) was added to reduce NO$_3$ to NO$_2$, and the concentration of NO$_2$ was subtracted from the total NO$_3$ measured (Miranda et al., 2001).

2.3.3. SO$_4^{2-}$ determination

Aliquots of liquid samples (0.4 mL) were diluted 20 to 50 times with 0.1% nitric acid to a final volume of 4 mL. The SO$_4^{2-}$ concentration was determined by measuring the total S concentration in the sample by ICP-OES (iCap 6300, Thermo Scientific, Bremen, Germany) with a sea spray nebulizer combined with a cyclone chamber. The plasma had a power of 1150 W and was generated by argon gas, with an auxiliary gas flow of 0.5 L min$^{-1}$ and a nebulizer flow of 0.6 L min$^{-1}$.

2.4. Molecular analysis

2.4.1. DNA extraction

DNA was extracted with the PowerSoil DNA Isolation Kit (MO BIO, Qiagen, Venlo, the Netherlands) following the manufacturer’s instructions with the modification of adding a 3 min bead beating step (Mixer Mill MM 400, Retsch GmbH, Germany) at 30 bps instead of PowerBead Tube vortexing step. Quantity and quality of the DNA were measured by the NanoDrop 1000 (Invitrogen, Thermo Fisher, Carlsbad CA, USA) and the Qubit spectrophotometer using the dsDNA HS Assay Kit (Invitrogen, Thermo Fisher, Carlsbad CA, USA) according to the manufacturer’s instructions. Extracted DNA from duplicate incubations was pooled in equimolar concentration. DNA was stored at −18 °C until further processing.

2.4.2. Phylogenetic analysis

The community composition and diversity of Bacteria and Archaea were assessed by the 16S rRNA gene amplicon sequencing. Sequencing was performed on an Illumina MiSeq Next Generation Sequencer by Macrogen, Korea. Primers used for amplification were Bac341F (5′- CCTACGGGNGGCWGGCAG-3′) (Herlemann et al., 2011) and Bac806R (5′- GGAATACHVGGGTWTCTAAAT-3′) (Caporaso et al., 2012) for Bacteria and Arch349F (5′-GYGCASCAGCGGCATCA-3′) and Arch806R (5′-GGACTACVSGGGTATCTAAT-3′) (Takai and Horikoshi, 2000) for Archaea. A two-step PCR was performed with 12.5 ng of genomic DNA (2.5 μL), 5 μL of forward and reverse primer (1 μM), and 12.5 μL of 2x KAPA HiFi Hotstart ready mix using the following thermal program: 3 min at 95 °C, 30 s at 95 °C, 30 s at 55 °C, and 30 s at 72 °C (these last three steps were repeated for 25 cycles), finished by 5 min at 72 °C. PCR products were purified using AMPure XP beads. The purified PCR products (5 μL) were used for the second PCR (same PCR protocol with only 8 cycles) in combination with sample-specific barcoded primers (Nextera XT index kit, Illumina). The produced PCR library was cleaned by AMPure XP beads and quantified by using the Bioanalyzer DNA 1000 chip. Subsequently the libraries were sequenced on an Illumina MiSeq with 2 × 300bp chemistry. The number of reads generated is stated in Supplemental Tables S3 and S4.

The first steps of data analysis were conducted in CLC Genomics Workbench v. 11 (QIAGEN Aarhus A/S, Denmark) by removing the primers by trimming the first 20 nucleotides of all sequences. Further processing was performed with the DADA2 Pipeline (1.8) (Callahan et al., 2016) and the DADA2 Pipeline (1.8) (Callahan et al., 2016).
et al., 2016) following the standard operating procedure in Rstudio (RStudio Team, 2015). Reads were quality filtered (default settings) and trimmed to 280 base pairs for the forward read and to 220 base pairs for the reverse reads. Subsequently, the error rates in the sequences were learned and the DADA2 algorithm was applied. Reads were merged, chimeras were removed, and taxonomy was assigned. Taxonomic classification was performed by using the SILVA v132 16S rRNA gene non-redundant database (SSURef_NR99_132_SILVA) as a reference. Reads identified as ‘Bacteria’ and ‘Archaea’ were selected for the bacterial and archaeal sequence datasets, respectively, and ‘Mitochondria’ were removed from the bacterial sequence datasets (‘Chloroplasts’ were not detected). The number of sequences after each step of processing can be found in Supplemental Tables S3 and S4. Archaeal reads were rarefied on 720 reads (discarding sequences from IRS1 sample with NO\(_2\) and CH\(_4\) from the time point of 240 days with 420 sequences) and bacterial reads on 2560 reads (Supplemental Fig. S1). Data was visualized using R packages phyloseq (McMurdie and Holmes, 2013), ggplot2 (Wickham, 2016) and vegan (Oksanen et al., 2018). All sequencing data was submitted to the GenBank databases under the BioProject with accession number PRJNA562618.

3. Results

We studied the effect of inundating in four peat topsoils (Fig. 1) with respect to porewater quality, microbial activity and community composition upon different substrate amendments, and potential GHG fluxes. The soils varied in OM content, texture, and total Fe concentration (Table 1).

3.1. Changes in nutrient release in relation to porewater biogeochemistry upon inundation

The four topsoils were artificially inundated for 18 months, which led to profound changes in the porewater chemistry. These changes were related to the soil type and affected the initial availability of the substrates present (Supplemental Tables S3 and S4). Experimental inundation resulted in a rapid decrease of NO\(_3^-\) concentration within two weeks in all soils. Concentrations of porewater Fe, total inorganic carbon (TIC), NH\(_4^+\), and P increased in all incubations in response to water saturation. The magnitude of this effect was the strongest in the Fe-rich soils IRS1, IRS2, and IRS3 (Fig. 2). The NH\(_4^+\) concentration increased up to approximately 1 mmol L\(^{-1}\) in all three Fe-rich soils while it remained an order of magnitude lower (maximum 0.1 mmol L\(^{-1}\)) in the IPS (Fig. 2). Total dissolved P reached the highest concentration in IRS3, which had the highest total Fe concentration, followed by the IRS2 (Fig. 2).

3.2. Methanogenic activity

The effect of electron acceptor availability (NO\(_3^-\), NO\(_2^-\), SO\(_4^{2-}\), Fe(III) mineral forms ferrihydrite and goethite) on the CH\(_4\) dynamics in the different soils was investigated in batch incubations (Fig. 3, Table 2). In the presence of both Fe(III) oxide forms the net methanogenic activity was neither significantly suppressed nor enhanced in any of the soil
slurry incubations in comparison to control incubations. The presence of NO$_3^-$, NO$_2^-$, and SO$_4^{2-}$ inhibited the methanogenic activity. NO$_3^-$ and NO$_2^-$ were provided regularly during the experiment to avoid exhaustion due to the high denitrification rates in the soils (Supplemental Figure S2, S3, and S4). However, time periods with lower concentrations or depletion of these substrates, especially during the first 100 days of the experiment for NO$_2^-$, still occurred, resulting in temporal CH$_4$ production (Fig. 3).

3.3. Methanotrophic activity

The CH$_4$ produced by the methanogens can be oxidized by both aerobic and anaerobic methanotrophs when suitable electron acceptors are sufficiently present. The potential for AOM was assessed by the conversion of $^{13}$C-CH$_4$ into $^{13}$C-CO$_2$ (Supplemental Fig. S5). The production of $^{13}$C-CO$_2$ was only detected in the incubations amended with Fe(III) oxides and controls without added electron acceptors. The $^{13}$C-CO$_2$ production followed the same trend as the CH$_4$ production, and the presence of NO$_2^-$, NO$_3^-$, and SO$_4^{2-}$ inhibited both $^{13}$C-CO$_2$ production and methanogenesis. The concentration of $^{13}$C-CO$_2$ in the headspace did not exceed 0.08 μmol per mL slurry and formed up less than 0.9% of the total cumulative $^{13}$C-CH$_4$ amended into the bottles. Aerobic methanotrophy became active in all soils after a lag phase of around 30 days from the time the slurries were exposed to O$_2$. Their activity was similar for all soils oxidizing ~196 μmol CH$_4$ mL$^{-1}$ slurry in 120 days (Table 3).

Table 2
Maximum methane (CH$_4$) production rates in nmol CH$_4$ mL$^{-1}$ slurry day$^{-1}$ in batch incubations amended with sulfate (SO$_4^{2-}$), nitrite (NO$_2^-$), nitrate (NO$_3^-$), ferrhydrite (Fe(OH)$_3$), goethite (FeOOH), and in the control without electron acceptor additions. Maximum CH$_4$ production rates were calculated after 100 days of incubation.

| Soil | SO$_4^{2-}$ | NO$_2^-$ | NO$_3^-$ | Fe(OH)$_3$ | FeOOH | Control |
|------|------------|----------|----------|------------|-------|---------|
| IRS1 | 1.3 (±0.2) | 8.3 (±0.3) | 2.9 (±0.6) | 59 (±11) | 65 (±8.2) | 59 (±6) |
| IRS2 | 0.5 (±0.0) | 0.5 (±0.7) | 0.6 (±1.9) | 37 (±6.1) | 30 (±4.6) | 32 (±0.6) |
| IRS3 | 0.03 (±0.004) | 2.8 (±0.1) | 0.009 (±0.1) | 20 (±6.1) | 16 (±4.6) | 15 (±0.6) |
| IPS  | 0.03 (±0.04) | 1.1 (±0.7) | 0.006 (±0.002) | 23 (±2.5) | 23 (±6.1) | 22 (±1.6) |

Table 3
Maximum methane (CH$_4$) oxidation rates and total CH$_4$ consumed in 120 days. Units in μmol CH$_4$ mL$^{-1}$ slurry.

| Soil | Max CH$_4$ oxidation | Total CH$_4$ oxidized |
|------|----------------------|-----------------------|
| IRS1 | 4.2 (±0.1)           | 192 (±1.8)            |
| IRS2 | 4.7 (±0.1)           | 189 (±6.8)            |
| IRS3 | 3.6 (±0.01)          | 208 (±1.1)            |
| IPS  | 3.5 (±0.01)          | 194 (±0.6)            |

Fig. 3. Methane (CH$_4$) production rates in batch incubations for the four investigated soils amended with nitrite (NO$_2^-$), nitrate (NO$_3^-$), sulfate (SO$_4^{2-}$), ferrhydrite (Fe(OH)$_3$), goethite (FeOOH), and the control without added electron acceptors. The graphs show the average of duplicate batch incubation.

Fig. 4. Rates of $^{15}$N-N$_2$ and $^{15}$N-N$_2$O production in the batch incubations amended with $^{15}$N-NO$_2$ in the four investigated soils.
3.4. Denitrification potential

The reduction of NO₂ and NO₃ occurred in all slurries and the rates differed per soil. Highest rates were detected in the IRS1 and IRS2 (172 ± 60 and 191 ± 17 nmol NO₂ mL⁻¹ slurry day⁻¹, and 136 ± 11 and 49 ± 7 nmol NO₃ mL⁻¹ slurry day⁻¹ respectively), whereas the rates in IRS3 and IPS were nearly 3 times lower (53 ± 4 and 52 ± 4 nmol NO₂ mL⁻¹ slurry day⁻¹, and 35 ± 2 and 25 ± 5 nmol NO₃ mL⁻¹ slurry day⁻¹ respectively). To quantify the denitrification products, the incubations that previously received NO₂ were amended with ¹⁵N-NO₂ (~2 mM) after the unlabeled pool of NO₂ was exhausted. N₂O was detected in all soils (Fig. 4). In IRS1 and IRS2, only 20% and 10% respectively of the ¹⁵N label from ¹⁵N-NO₂ could be recovered in N₂O (¹⁴/¹⁵N₂O and ¹⁵/¹⁵N₂O respectively) in a period between 30 h and 126 h after the

Fig. 5. Taxonomic distribution of archaeal 16S rRNA gene amplicon sequences on family level for the four investigated soils a) IRS1, b) IRS2, c) IRS3, and d) IPS at three time points of the batch incubation experiment (days 0, 120, 240) with and without additions of substrates.
addition of $^{15}$N-NO$_2$. For IRS3 and IPS, both exhibited lower NO$_2^-$ reduction rates, the recovery of the $^{15}$N label in N$_2$O was 40% and 20% of the consumed $^{15}$N-NO$_2$ respectively. The evolution of N$_2$ ($^{14}/^{15}$N$_2$ and $^{15}/^{15}$N$_2$) was only observed in IRS1 (between 4.4 and 19 nmol N$_2$ mL$^{-1}$ slurry day$^{-1}$).

Fig. 6. Taxonomic distribution of bacterial 16S rRNA gene amplicon sequences on order level for the four investigated soils a) IRS1, b) IRS2, c) IRS3, and d) IPS at three time points of the batch incubation experiment (days 0, 120, 240) with and without additions of substrates.
3.5. Changes in the archaeal community composition

Biomarkers of putative methanogenic archaea showed the highest relative abundance in the incubations amended with Fe(III) oxides and controls without additions (Fig. 5), coinciding with the observed CH₄ production rates (Fig. 3). The methanogenic taxa consisted of Methanoregulaceae and Methanosaetaceae (recently reclassified as Methanotrichaceae (Paruls et al., 2017)). Methanoregulaceae dominated in the IRS2 at both Fe(III) oxide amended and control incubations (relative archaeal abundance to 9% and 26%), while Methanosaetaceae were more abundant in the soil prior to batch incubations (relative abundance of 18%) and decreased in relative abundance during the incubations (to 7%–18%). In the IRS1, IRS3, and IPS, Methanosaetaceae were the most abundant methanogens (relative archaeal abundance of 17%–46%), which was in line with their presence in the soils of IRS1 and IRS3 prior to the batch incubations (15% and 10% relative abundance respectively). This family was not detected in the IPS prior to the batch incubations. Here, Methanocellaceae was the most abundant methanogenic family (relative abundance of 5%). Methanocellaceae increased in relative abundance in incubations with goethite and the controls (after 120 days relative abundance of 11%–15%, after 240 days 15%–21%). IPS had the most diverse methanogenic community at the end of the incubations. In all incubations, Methanosarcinaceae methanogens became outcompeted despite the initial presence in all soils (relative abundance of 2%–10%).

The known anaerobic CH₄ oxidizers classified as ‘Candidatus Methanoperedens nitroreducens’ were only detected in the NO₃ and ¹³C-CH₄ amended incubations after 240 days in IRS2, reaching a relative abundance of 9% of total archaeal reads (Fig. 5). Despite of their enrichment in the IRS2, no AOM activity could be detected in these incubations. Neither NO₃ nor SO₄²⁻ dependent AOM microorganisms known, could be detected in the 16S rRNA gene amplicon data.

3.6. Changes in the bacterial community composition

The bacterial community structure changed over the course of incubation with the different substrates (Fig. 6). In incubations amended with NO₂ and NO₃, Betaproteobacteria became the most dominant class of Bacteria after both 120 and 240 days of incubation (Fig. 6). The detected betaproteobacteria were dominated by the families Burkholderiaceae and Hydrogenophilaceae at all sampling points (Supplemental Fig. S6). Rhodanobacteriaceae family dominated the bacterial community in NO₂ incubations of IRS2 after 240 days (relative abundance of 35% and 47% without and with CH₄ addition, respectively, Supplemental Fig. S6), while it was under the detection limit at the other time points. An enrichment of Gallionellaceae was detected in IRS1, IRS3, and IPS in incubations amended with both NO₂ and NO₃ and in IRS2 only in NO₃ amended incubations after both 120 and 240 days of incubation (relative abundance between 2% and 27%) (Supplemental Table S7). Also, the presence of ferrihydrite triggered changes in bacterial community composition in comparison to goethite amended and control incubations, which remained more similar to one another (Supplemental Fig. S6). The amendment of ferrihydrite triggered the enrichment of Geobacteraceae (relative bacterial abundance between 3% and 18%) in all soil slurries (Supplemental Table S7). Geobacteraceae sequences were below the detection limit prior to incubation.

The most abundant aerobic methanotrophs detected in all oxic incubations belonged to the Methyllococcales with Methylophilaceae as the dominant family (Fig. 6, Supplemental Table S7). It reached the highest relative abundance in IPS (47% of the bacterial reads) and lowest in soil IRS2 (16%), showing that the abundance in the soils did not correlate with the detectable activity (Table 3). In addition to Methylphilaceae, the IRS3 incubations also showed a relative enrichment in Methylcoccales after 120 days (2% of total bacterial amplicon sequence variants (ASVs), Supplemental Table S7). This group of methanotrophs was below the detection limit in the soils prior to batch incubations. Sequences belonging to Methylophilaceae family were detected in all the batch incubations with relative abundance above 2% (Supplemental Table S7).

4. Discussion

This study investigated the effects of the addition of different electron acceptors mimicking diverse conditions, on the activity and composition of the microbial community of Fe-rich drained peatlands subject to inundation as land restoration management strategy. Topsoil samples were collected from four different locations in the Netherlands, with a gradient in total Fe concentrations (low-rich-very rich).

4.1. Effect of inundation and Fe concentrations on carbon and nutrient biogeochemistry

Inundation was simulated for 1.5 years in the laboratory by the use of artificial freshwater resulting in increased concentrations of Fe, NH₄ and P in the porewater. Similar results were reported by other studies (Enzens et al., 2016; Harpenslager et al., 2015; Zak et al., 2018). The observed accumulation of NH₃ as well as total dissolved Fe and TIC in all Fe-rich soils can be explained by higher rates of Fe-mediated anaerobic degradation of OM, which triggers the conversion of organic-N to inorganic-N and releases NH₄. The high concentrations of TIC in the Fe-rich soils were mainly related to anaerobic reduction processes coupled to OM decomposition, in combination with lower CO₂ diffusion rates as a result of flooding. The high concentration of dissolved P in IRS2 could be related to higher S loads at this site, since SO₄²⁻, and particularly sulfide produced by microbial reduction, can enhance internal eutrophication (Lamers et al., 2002; Smolders et al., 2006). This change in porewater quality will affect ecosystem functioning and could very well lead to eutrophication.

4.2. Ferric iron does not suppress methanogenesis in inundated peat soils

To investigate how the microbial community in inundated soils would respond to the exposure of ecosystem-relevant oxidants such as NO₂, NO₃, SO₄²⁻ and Fe(III) oxides, activity measurements and the succession of microbial communities were investigated. Interestingly, neither forms of Fe(III) oxide (ferrihydrite and goethite) applied suppressed methanogenesis in inundated peat soils. These Fe(III) oxides are abundant in soils, but differ greatly in their crystallinity and bioavailability. In the presence of heterotrophic Fe(III) oxide reduction (Bodegom et al., 2004; Chidthaisong, 2000; Jackel and Schnell, 2000; Liu et al., 2011; Qu et al., 2004; Teh et al., 2007; Yamada et al., 2014). However, recent studies have reported stimulation of CH₄ production by (semi)conductive Fe(III) oxides (Cruz Viggiti et al., 2014; Kato et al., 2012; Kato and Igarashi, 2019; Li et al., 2015; Tang et al., 2016; Zhou et al., 2014; Zhuang et al., 2015). Here, we did not observe a detectable effect on methanogenic activity.

4.3. Higher relative abundance of methanogens and Fe(III)-reducing bacteria induced by ferrihydrite

The ferrihydrite addition led to the higher relative abundances of methanogens and Fe(III) reducing Bacteria. The methanogenic taxa consisted mainly of Methanoregulaceae (mostly hydrogenotrophic methanogens (Brauer et al., 2011)) and Methanosaetaceae (mostly acetoclastic methanogens (Jetten et al., 1992)). Abundant Methanosaetaceae
were also previously observed in rewetted fens that were characterized by high CH₄ emissions (Wen et al., 2018). A recent study has shown that acetoclastic methanogenesis can also reduce Fe(III) with acetate for respiratory growth (Prakash et al., 2019). This could be one of the possible reasons why acetoclastic Methanosetaeae increased in relative abundance in ferrihydrite amended incubations without higher CH₄ production rates compared to controls. Geobacteraceae biomarkers were only detected in the ferrihydrite amended batch incubations. Geobacteraceae bacteria are abundant in soils where they employ dissimilatory Fe(III) reduction with various organic substrates (Caccavo et al., 1994; Lovley et al., 2004, 1993). Our results show that the community composition of Fe(III) reducing bacteria depended on Fe(III) oxide mineralogy and availability. The enrichment of Geobacteraceae with additional ferrihydride could also lead to syntrophic associations with Methanosetaeae methanogens when acetate becomes scarce and magnetite accumulates upon reduction of ferrihydrite (Rotaru et al., 2014). Both organisms can cooperate via direct electron transfer where Methanosaeta scavenges the electrons from Geobacter and acts as an electron acceptor (Rotaru et al., 2014). During this syntrophic metabolism, Methanosaetaeae methanogens perform CO₂ reduction with electrons derived from Geobacter (Rotaru et al., 2014). Other factors such as temperature or pH can also influence the balance between methanogenesis and Fe(III) reduction in a system with Fe(III) oxide minerals was further shown to be dependent on temperature and pH (Aromokeye et al., 2018; Marquart et al., 2019).

4.4. Long-term exposure to nitrogen oxides suppresses methanogenesis but leads to the production of N₂O

Exposure of soil slurries to NO₂, NO₃, and SO₂⁻⁻ inhibited methanogenesis corroborating previous studies (Klüber and Conrad, 1998a, 1998b; Lovley et al., 1982; Roy and Conrad, 1999; Scholten and Stams, 1995). The long-term exposure to N oxides resulted in production of N₂O. The increase in N₂O production upon rewetting has been reported previously and is attributed to the increase in the denitrification activity when N oxides were present (Bear et al., 2009; Harrison-Kirk et al., 2013; Liu et al., 2018; Ruser et al., 2006; Snider et al., 2015). The nitrite reduction rates found (to 0.06–0.21 μmol N g⁻¹ dry weight h⁻¹) were in the range of the rates found in studies with comparable experimental set ups, ranging from 0.007 μmol N g⁻¹ dry weight h⁻¹ in clay loam soil to 0.6 μmol N g⁻¹ dry weight h⁻¹ in an arable field (Enwall et al., 2005; Holtan-Hartwig et al., 2000; Van Den Heuvel et al., 2011). Nonetheless, this resulted in lower N₂O:N₃ ratio compared to previous studies (Holtan-Hartwig et al., 2000; Rudaz et al., 1991; Van Den Heuvel et al., 2011). The biological denitrification depends on several factors including C content, pH, inorganic N content, copper availability, soil texture, and microbial community (Feldgate et al., 2012; Harrison-Kirk et al., 2013; Ruser et al., 2006; Van Den Heuvel et al., 2011, 2010), which could have contributed to the differences in N₂O production rates per soil. The evolution of N₂ (¹⁴/¹⁵N₂ and ¹⁵/¹⁷N₂) was only observed in IRS1, which points to differences in the composition and functionality of the microbial community in analyzed soils.

The incubations with added N oxides became dominated by bacterial groups putatively involved in autotrophic N oxide reduction with Fe(II) and S that were already present in the soils, and indicate a significant role of Fe(II) as electron donor compared to organic C. High relative abundance was detected for example for Burkholderiaeae, known to perform autotrophic NO₃ dependent Fe(II) oxidation (Carlson et al., 2013; Kiskira et al., 2017; Straub et al., 2004), and for Hydrogenophilaeeae which are able to oxidize ferrous sulphide (FeS) and various reduced inorganic S compounds in the presence of NO₃ (Ihdeirich et al., 2011; Straub et al., 1996). The studied IRS1-3 were rich in Fe(III) oxides that were already present in the soils, and indicate a significant role of Fe(III) as electron donor over organic C. Furthermore, the Rhodanobacteriaeae family which became highly abundant in IRS2 includes many members capable of complete denitrification (Kostka et al., 2012; Van Den Heuvel et al., 2010). However, no N₂ formation was detected in this soil, which could be the result of electron donor shortage due to exhaustion of labile organic C during incubation, low pH, or limitation of copper (Feldgate et al., 2012; Van Den Heuvel et al., 2011, 2010). Besides microbial denitrification, some abiotic chemodenitrification that involves NO₂ and Fe(II) could also be responsible for some of the N₂O formation at neutral pH (Grabb et al., 2017; Heil et al., 2014; Jones et al., 2015). The increase of N₂O production is an adverse effect of inundation and should be studied in more detail, taking also vegetation into account.

4.5. Potential for anaerobic methanotrophy

The electron acceptors NO₂, NO₃, and SO₂⁻ can all potentially be used by anaerobic CH₄ oxidizing Archaea and Bacteria (Boetius et al., 2000; Cai et al., 2018; Haroon et al., 2013; Raghoecharsing et al., 2006). The potential for AOM was assessed by the conversion of ¹³C-CH₄ to ¹³C-CO₂. The production of ¹³C-CO₂ was only detected in the incubations amended with Fe(III) oxides and control incubations without added electron acceptors. The ¹³C-CO₂ production followed the same trend as the CH₄ production, and the presence of NO₂, NO₃ and SO₂⁻ inhibited both ¹³C-CO₂ production and methanogenesis. The concentration of ¹³C-CO₂ in the headspace did not exceed 0.08 μmol per mL slurry and formed up to 0.9% of the total ¹³C-CH₄ amended to the bottles. With this small amount of ¹³C CO₂ we produced we cannot exclude the process of trace CH₄ oxidation (TMO) that has been demonstrated for pure cultures of active methanogens (Timmers et al., 2017). Known anaerobic CH₄ oxidizers classified as ‘Candidatus Methanoperedens nitroreducens’ were only detected in the NO₂ and ¹³C-CO₂ amended incubations after 240 days in IRS2. These organisms belong to the anaerobic methanotrophic archaea clade 2d (ANME-2d), which are known to perform NO₂ and Fe(III) dependent AOM in freshwater systems (Cai et al., 2018; Haroon et al., 2013). Similar enrichment timcxsdsxxxxxxxxxxxxxxx for NO₃ dependent AOM was reported by Vaksmaa et al. (2017) where significant growth was detected after 9 months of enrichment. Interestingly, despite the onset of ANME-2d enrichment, no AOM activity could be detected in these incubations that could be explained by a requirement of minimum population size for the onset of measurable activity. Neither NO₂ nor SO₂⁻ dependent known AOM microorganisms could be detected in the 16S RNA amplicon data. It is possible that AOM microorganisms need longer time frames for enrichment in inundated peat soils.

4.6. High potential for aerobic methanotrophy under oxic conditions

The potential for aerobic CH₄ oxidation is relevant for agricultural areas where rewetting occurs in short cycles or when the water table is below the soil surface level. The results showed that aerobic methanotrophs quickly become active and enriched in agricultural peat soils when O₂ and CH₄ are supplied. For rewetting practices, optimal conditions to establish a methanotrophic population would include a water table level just below the soil surface. Such conditions may minimize the diffusive fluxes of CH₄ to the atmosphere (Couwenberg, 2009).

5. Conclusions and outlook

Sustainable, climate-smart management strategies are needed to
restore drained peatlands that simultaneously would prevent the harmful GHG emissions from these soils. Large-scale rewetting is currently put in practice and planned for the near future to prevent further degradation of peat soils and subsidence, and to lower GHG (mainly CO₂) emissions. Although rewetting is essential for the restoration of drained peatlands, the reduction in GHG emissions after rewetting largely depends on the interactions between the geochemical conditions and the microbial community present in these soils. Permanent inundation of degraded fertilized peatlands with Fe-rich topsoils will lead to increases in methanogenic abundance and potentially activity, subsequently leading to higher CH₄ emissions when inhibitors such as N oxides and SO₄²⁻ become depleted. Unexpectedly, abundant Fe (III) oxide availability did not inhibit methanogenesis. None of the amended electron acceptors stimulated detectable AOM activity during the investigated period of 8 months. However, the onset of ANME-2d enrichment towards the end of the incubation period with added CH₄ and NO₃⁻ scenario shows that AOM communities may require even longer periods to establish and become significantly active. Moreover, the possible establishment of AOM populations will depend on the initial microbial community of a particular soil and sufficient supply of electron acceptors. Inundation with N-rich water will stimulate denitrification, which may lead to elevated short-term emissions of N₂O depending on prevailing soil geochemical conditions and microbial community. The potential processes that could play a major role upon inundation of Fe-rich peat topsoils are summarized in Fig. 7. Elevated emissions of CH₄ and increased production of N₂O are major adverse effects in the early stages of inundation. In the long run (years to decades), inundation is assumed to have a net GHG sequestration effect. The current strategies are idealistic in restoring exhausted peatlands and thereby combating CO₂ emissions, but may impose elevated emissions of even more potent greenhouse gases CH₄ and N₂O, at least on the short term. In essence, although we are convinced that rewetting is an essential step in order to prevent peatland ecosystems functioning and services, the management of these soils needs to apply additional and more site-specific strategies to minimize emissions of CH₄ and N₂O. Applicable inundation strategies of eutrophic Fe(III) oxide-rich peatlands could include the removal of the Fe-rich topsoil to prevent both eutrophication and increase in CH₄ emissions. Additionally, peat soils could be inundated with application of vegetation like Sphagnum or Typha latifolia species which decreases CH₄ emissions. Maintenance of an elevated water table just below the soil surface level to prevent anoxic conditions in the topsoil could also be used to combat the CH₄ emissions.

Declaration of competing interest

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

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Appendix A. Supplementary data

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References

Oksanen, A.J., Blanchet, F.G., Friendly, M., Kindt, R., Legendre, P., Mglínský, D., et al., 2018. Vegan: community ecology package. https://CRAN.R-Project.org/Ppackage--vegan.

Aromoekeye, D.A., Richter-Heitmann, T., Otis, O.E., Kulkarni, A., Yin, X., Kasten, S., et al., 2018. Temperature controls crystalline iron oxide utilization by microbial communities in methanic ferruginous marine sediment incubations. Frontiers in Microbiology 9, 2574. https://doi.org/10.3389/fmicb.2018.02574.

Baird, A.J., Belyea, L.R., Morris, P.J., 2013. Upscaling of peatland-atmosphere fluxes of methane: small-scale heterogeneity in process rates and the pitfalls of ‘Bucket-and-Shlab’ models. In: Baird, Andrew J., Belyea, L.R., Conas, X., Reeve, A.S., Slater, L.B. (Eds.), Carbon Cycling in Northern Peatlands. American Geophysical Union, Washington, DC, pp. 37–53. https://doi.org/10.1029/2008GM000826.

Beare, M.H., Gregorich, E.G., Ste-Georges, P., 2009. Compaction effects on CO₂ and N₂O production during drying and rewetting of soil. Soil Biology and Biochemistry 41, 611–621. https://doi.org/10.1016/j.soilbio.2008.12.024.

Beets, S., Liebersbach, H., Glazel, S., Jurassinski, G., Buczko, U., Hoper, H., 2013. Effects of land use intensity on the full greenhouse gas balance in an Atlantic peat bog. Biogeosciences 10, 1067–1082. https://doi.org/10.5194/bg-10-1067-2013.

Bhattacherjee, A.S., Mottlach, A.M., Jetten, M.S.M., Geol, R., 2016. Methane dependent denitrification- from ecosystem to laboratory-scale enrichment for engineering applications. Water Research 99, 244–252. https://doi.org/10.1016/j.watres.2016.04.079.

Blothe, M., Roden, E.E., 2009. Composition and activity of an autotrophic Fe(II)-oxidizing, nitrate-reducing enrichment culture. Applied and Environmental Microbiology 75, 6937–6940. https://doi.org/10.1128/AEM.01742-09.

Bodegom, P.M., Scholten, J.C.M., Stams, A.J.M., 2004, Direct inhibition of methanogenesis by ferric iron. FEMS Microbiology Ecology 49, 261–268. https://doi.org/10.1016/j.femsec.2003.04.017.

Boettiger, A., Fierer, N., Loechte, K., 2000. Bacterial activity in sediments of the deep Arabian Sea in relation to vertical flux. Deep Sea Research Part II: Topical Studies in Oceanography 47, 2835–2875. https://doi.org/10.1016/S0967-0645(00)00051-5.

Böhm, J., 1925. Über Aluminium- und Eisenhydroxyde. I. Zeitschrift für Anorganische und Allgemeine Chemie 149, 203–216. https://doi.org/10.1002/zacz.19251490114.

Brauer, S.L., Cadillo-Quiroz, H., Ward, R.J., Yavitt, J.B., Zinder, S.H., 2011. Methanocorpusuloa bohaii gen. nov., sp. nov., an acidophilic methanogen isolated from an acidic peat bog. International Journal of Systematic and Evolutionary Microbiology 61, 45–52. https://doi.org/10.1099/ijs.0.021782-0.

Caccavo, F., Lonergan, D.J., Lovley, D.R., Davis, M., Stolz, J.F., McInerney, M.J., 1994. Archaelon couples anaerobic oxidation of methane to Fe(III) reduction. The ISME Journal 1, 2031–2042. https://doi.org/10.1023/A:1021652009742.

Cai, C., Leu, A.O., Xie, G.-J., Guo, J., Feng, Y., Zhao, J.-X., et al., 2018. A methanotrophic archaeon couples anaerobic oxidation of methane to Fe(II) reduction. The ISME Journal 12, 1929. https://doi.org/10.1038/s41396-018-0159-x.
fertilized Italian paddy soil. FEMS Microbiology Ecology 92, 1–11. https://doi.org/10.1093/femsec/fsw181.
Vaksmaa, A., Guerrero-Cruz, S., van Alen, T.A., Cremers, G., Ettwig, K.F., Lüke, C., et al., 2017. Enrichment of anaerobic nitrate-dependent methanotrophic ‘Candidatus Methanoperedens nitroreducens’ archaea from an Italian paddy field soil. Applied Microbiology and Biotechnology 101, 7075–7084. https://doi.org/10.1007/s00253-017-8416-0.
Van Den Heuvel, R.N., Van Der Biezen, E., Jetten, M.S.M., Hefting, M.M., Kartal, B., 2010. Denitrification at pH 4 by a soil-derived Rhodanobacter-dominated community. Environmental Microbiology 12, 3264–3271. https://doi.org/10.1111/j.1462-2920.2010.02301.x.
Van Den Heuvel, R.N., Bakker, S.E., Jetten, M.S.M., Hefting, M.M., 2011. Decreased N2O reduction by low soil pH causes high N2O emissions in a riparian ecosystem. Geobiology 9, 294–300. https://doi.org/10.1111/j.1472-4669.2011.00276.x.
Vanselow-Algan, M., Schmidt, S.R., Greven, M., Fiencke, C., Kutzbach, L., Pfeiffer, E.-M., 2015. High methane emissions dominated annual greenhouse gas balances 30 years after bog rewetting. Biogeosciences 12, 4361–4371. https://doi.org/10.5194/bg-12-4361-2015.
Wen, X., Unger, V., Jurasinski, G., Koebisch, F., Horn, F., Rehder, G., et al., 2018. Predominance of methanogens over methanotrophs contributes to high methane emissions in rewetted fens. Biogeosciences 15, 6519–6536. https://doi.org/10.5194/bg-2018-184.
Wickham, H., 2016. ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag, New York.
Wilhelm, E., Battino, R., Wilcock, R.J., 1977. Low-pressure solubility of gases in liquid water. Chemical Reviews 77, 219–262. https://doi.org/10.1021/cr60306a003.
Wilson, D., Alm, J., Laine, J., Byrne, K.A., Farrell, E.P., Tuittila, E.-S., 2009. Rewetting of cutaway peatlands: are we re-creating hot spots of methane emissions? Restoration Ecology 17, 796–806. https://doi.org/10.1111/j.1526-100X.2008.00416.x.
Yamada, C., Kato, S., Kimura, S., Ishii, M., Igarashi, Y., 2014. Reduction of Fe(III) oxides by phylogenetically and physiologically diverse thermophilic methanogens. FEMS Microbiology Ecology 89, 637–645. https://doi.org/10.1111/1574-6941.12365.
Zak, D., Goldhammer, T., Cabezas, A., Gelbrecht, J., Gurke, R., Wagner, C., et al., 2018. Top soil removal reduces water pollution from phosphorus and dissolved organic matter and lowers methane emissions from rewetted peatlands. Journal of Applied Ecology 55, 311–320. https://doi.org/10.1111/1365-2664.12901.
Zhou, S., Xu, J., Yang, G., Zhuang, L., 2014. Methanogenesis affected by the co-occurrence of iron(III) oxides and humic substances. FEMS Microbiology Ecology 88, 107–120. https://doi.org/10.1111/1574-6941.12274.
Zhuang, L., Xu, J., Tang, J., Zhou, S., 2015. Effect of ferrhydrite biomineralization on methanogenesis in an anaerobic incubation from paddy soil. Journal of Geophysical Research: Biogeosciences 120, 876–886. https://doi.org/10.1002/2014JG002893.