Nature development in degraded landscapes: How pioneer bioturbators and water level control soil subsidence, nutrient chemistry and greenhouse gas emission

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ABSTRACT

The restoration of degraded ecosystems and landscapes is challenging, because returning to the original state is often socio-economically unfeasible. A novel approach is to construct new ecosystems to improve the functioning of degraded landscapes. However, the development of novel ecosystems is largely driven by the pre-construction hydrogeophysical and ecological conditions of the soil. In Lake Markermeer, a deteriorating freshwater lake in the Netherlands, a large archipelago is currently being constructed to boost the ecological functioning of the lake. Hence, islands – with wetlands and with more elevated and dryer areas – have been created to sustain biodiversity and key biogeochemical functions such as nutrient cycling. The islands are constructed from lake-bottom sediments. To study how two potentially important drivers, water level and bioturbation, affect soil characteristics in a novel wetland ecosystem, we experimentally tested the effects of water level (-30, -10 and 5 cm), and bioturbation by earthworms (*Lumbricus rubellus*) and *Tubifex* spp. in a microcosm experiment. We demonstrate that a high water level prevents soil subsidence, soil crack formation and carbon dioxide (CO_2) emissions, and affects nitrogen cycling. In dryer soils, the presence of earthworms strongly increases CO_2 emissions next to reducing soil crack formation, while *Tubifex* spp. in wetter soils hardly affect soil characteristics. Our findings highlight the important roles of both water level and bioturbation for the functioning of novel soils, which likely affects vegetation development in novel ecosystems. This knowledge can be used to aid the construction and nature development of novel wetlands.

1. Introduction

Anthropogenic disturbance is causing massive losses to ecosystems; including the degradation of iconic ecosystems such as wetlands, tropical rainforests, savannahs, peatlands and coral reefs (Halpern et al., 2007; Leifeld et al., 2019; da Cruz et al., 2020). The losses are particularly large in wetlands, as 50–87 % of their global extent is currently degraded or has been lost since 1700 AD (Mitsch and Gosselink, 2007; Davidson, 2014). These losses not only result in the extinction of species and typical biodiversity, but also in the loss of vital ecosystem services (Stirling et al., 2020). For example, natural wetlands store a great amount of soil carbon, provide food, fibre and clean

drinking water, mitigate flood and drought, prevent downstream pollution, cool their surroundings via evapotranspiration and provide protection against flooding and or storms (Maltby and Acreman, 2011; Mitsch et al., 2015).

Governments and non-governmental stakeholders have invested hundreds of millions of dollars in protecting threatened wetlands, yet they remain under continuous threat (Díaz et al., 2020). Conservation practitioners and policy makers are searching for new practises to halt the decline in wetland habitat. Recently, attention has been directed towards habitat restoration as a tool for conservation (Suding et al., 2015). However, the restoration of degraded wetlands is often challenging and expensive, because many stressors (e.g. overgrazing, eutrophication, unfavourable environmental conditions for establishment) frustrate restoration (Zedler, 2000; Temmink et al., 2020). Furthermore it is often challenging or impossible to define the historical reference point for restoration (Kentula, 2000) and the role of history in restoration ecology is currently changing (Higgs et al., 2014). A novel approach is the creation of entirely new ecosystems to enhance the functioning of severely altered and degraded landscapes (Hobbs et al., 2009), because many ecosystem services and functions operate at large scale, comprising different ecosystems (van der Zee et al., 2012; van de Koppel et al., 2015).

The ecological development of these novel wetland ecosystems is, however, determined to a large extent by the initial soil conditions. Hence, it is important to understand the early formation and development of soil in newly created wetland areas. For instance, physical soil subsidence occurs through compaction and consolidation (Greensmith and Tucker, 1986). Compaction increases the density of the soil by reducing the volume of air in the voids. Consolidation is the compression of saturated soils and occurs through water expulsion from the soil voids (Chang et al., 2014). Subsidence can additionally occur via biological processes of which the decomposition of organic material and bioturbation are the most important (Krantzberg, 1985). Bioturbation is the reworking of soils by plants or animals and include burrowing, ingestion and defecation of sediment grains (Krantzberg, 1985; Meysman et al., 2006; Baranov et al., 2016a). It occurs in terrestrial and wet ecosystems, by a great variety of species ranging from small invertebrates to large mammals (Wilkinson et al., 2009). Interestingly, hydraulic engineers have recognised that bioturbation is a crucial component in models of sediment dynamics in coastal ecosystems (Paarlberg et al., 2005). In novel wetland ecosystems, the sediment may at first be deprived of soil communities, but these soils are eventually colonized by soil fauna. Bioturbation by soil fauna can have profound effects on soil properties, such as the aeration of soils and its effect on soil subsidence, nutrient cycling and greenhouse gas emissions (GHG) (Mermillod-Blondin and Rosenberg, 2006; Ernst et al., 2009; Creed et al., 2010; Baranov et al., 2016b). Wetland ecosystems in particular play a crucial role in nutrient cycling, notably the nitrogen cycle. For instance, water level and related oxygen availability in the soil drive processes such as denitrification and ammonification (Hefting et al., 2004; Tian and Lu, 2010). Moreover, wetlands play a vital role in global carbon dynamics (Bridgham et al., 2013). For example, wetlands store great amounts of carbon in the soil because of high water levels and low decomposition rates, but can simultaneously emit a lot of carbon as methane (Dalal and Allen, 2008). In novel wetland ecosystems, the interactive effects of water level and bioturbation with respect to nutrient cycling and GHG fluxes remain unclear.

Our study area – Lake Markermeer in the Netherlands – was historically a marine ecosystem ("de Zuiderzee", Fig. S1). Due to embankment, together with its adjacent neighbour Lake IJsselmeer, is now one of the largest European inland freshwater lakes. To improve water transparency by reducing wind-driven resuspension, and to increase wetland biodiversity, an artificial archipelago (700 ha land) is being created, named Marker Wadden. The islands are being constructed from Markermeer lake bottom sediments (Saaltink et al., 2016). The soil of these newly created islands typically subsides through both physical and biological processes, which will have strong effects on key environmental factors such as water level, nutrient availability, and soil crack formation, which in turn affect the composition of plant communities, greenhouse gas fluxes and the habitat for fauna.

In this study, we determine the effects of water level and bioturbation by early soil colonizers on soil processes, notably subsidence, crack formation, nutrient availably and greenhouse gas emissions. We studied these variables, as they interact through for instance oxygenation of the soil (water level, crack formation, burrowing activity), and resulting microbial-dependent mobilisation, fixation and cycling of nutrients, as well as decomposition of organic material. These processes ultimately control the biogeochemical functioning, including greenhouse gas emissions, of newly constructed wetlands. We focus on burrowing invertebrates with contrasting habitat preferences (wet and dry), as these are pioneer soil-fauna that are important biotic drivers for wetland soil formation (Krantzberg, 1985; Judd and Mason, 1995). The novel archipelago Marker Wadden will consist of wetlands and dryer areas by design and shaped by soil subsidence, which results in habitats preferred by different soil fauna species. We chose the earthworm (*Lumbricus rubellus*) and *Tubifex* spp. as model species, as representative bioturbators of respectively dryer and wetter habitats, and we expect that they can colonize the new soil relatively fast. *Tubifex* spp. are already present in high densities in lake Marker sediment (Saaltink et al., 2019), while earthworms are known to colonize new areas via passive dispersal as cocoons in mud that is attached to birds (Eijsackers, 2010, 2011). The earthworm creates large horizontal and vertical hollows, moves more sediment and occurs in relatively dry sediment (Lee, 1985), compared to *Tubifex* spp., which make shallow hollows and occur in wet environments (Plum and Filser, 2005, Nogaro et al., 2006, Eijsackers, 2010, Tian and Lu, 2010).

Using new sediment from the recently constructed novel ecosystem Marker Wadden, we performed a series of experiments to (1) assess the role of water level on aforementioned soil processes; (2) elucidate and compare the role of two common pioneer bioturbators within similar environmental context; (3) assess the effect of water level and the role of bioturbators in their preferred environment on soil processes. By conducting three complementary experiments, we gain insight in the effect of water level, early bioturbators and the effect of each species on soil processes, which provide new information on the processes in newly constructed wetlands. We hypothesize that (1) higher water levels prevent soil subsidence, soil crack formation and CO_2 emissions but increase CH_4 emissions. Water fills up the pore space and prevents consolidation and compaction of the soil and this prevents oxygen entering the soil decreasing oxidation of organic material thereby leading to lower CO_2 emissions. However, water-level-induced soil anoxia stimulates methanogenesis, which results in higher CH_4 emissions to the atmosphere (2) Bioturbation increases overall soil subsidence, crack formation and CO_2 emissions but lowers CH_4 emissions. By moving and aggregating the soil, and creating burrows, bioturbators aerate the sediment and thereby stimulate oxidation of organic material resulting in increased CO_2 emissions and decreased CH_4 production and emissions (3) Earthworms have a larger impact on the soil processes than *Tubifex* spp., because they move more sediment and create deeper and larger burrows than *Tubifex* spp.

2. Materials and methods

2.1. Novel ecosystem

The Marker Wadden are artificial islands located in Lake Markermeer, the Netherlands (coordinates 52°35′30.2″N 5°22′43.6″E). The archipelago was constructed between 2016–2020 to create new wetlands and to boost the natural values of the lake. The islands were constructed by building ring dikes of sand (ranging from 1.5 to 4 km in length, width of 15 m), in which layers of Holocene marine clay were pumped from the bottom of the lake at a depth of -20 m. The lake bottom

itself is located at -3.6 m.
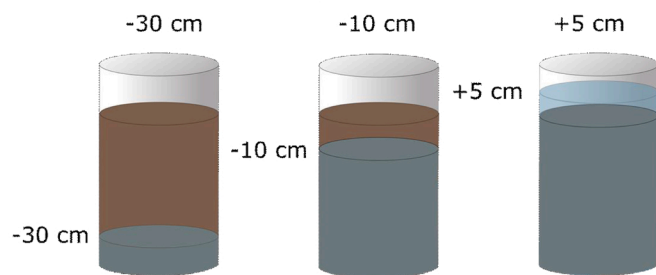
2.2. Experimental design

To answer the three research questions regarding the effects of water level, bioturbation, and species-specific effects on soil processes, we performed three complementary laboratory experiments (Fig. 1).

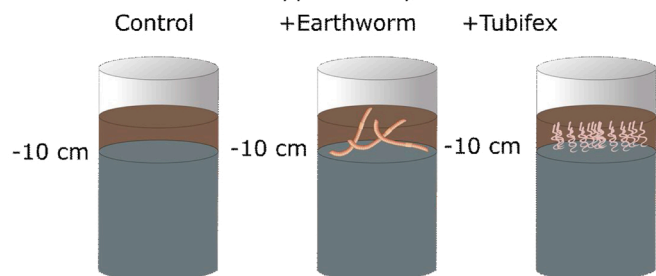#### 2.2.1. Experiment 1: Effect of water level on soil processes

To investigate the effect of water level on soil processes, we performed a greenhouse microcosm experiment in which we manipulated the water level in 12 columns filled with sediment (Fig. 1). Late November 2017, sediment was collected from a basin of the Marker Wadden that was recently filled with Holocene lake Markermeer sediment from pits up to 20 m depth in the summer of 2017 (52°35′1.52″N, 5°21′54.22″E) and transported to the greenhouse facilities of the

Radboud University. After a four-week acclimation period in the dark at 5 °C, the sediment was carefully homogenized. In this process, during which no living soil fauna was observed by visually inspecting the soil, we removed dead wood and shells. Subsequently, the sediments were placed in 12 transparent columns (15 cm diameter, 50 cm height, hereafter are called microcosms). Each microcosm first received a five cm layer of gravel layer that acted as a water reservoir, which then was covered with cloth. Subsequently, a 35 cm layer of sediment was added. The 12 microcosms were then randomly allocated to receive one of the following hydrological treatments ($n = 4$): 5 cm, where the sediment was permanently inundated by a 5 cm layer of rainwater (which is continuously collected from the roof of the greenhouse); -10 cm, where the water level was 10 cm below the surface to mimic shallow drawdown; -30 cm, where the water level was 30 cm below the surface to mimic deep drawdown. A thin monitoring well of HDPE with a filter gauze perforated PVC-tube (5 cm diameter, 50 cm in height) was placed
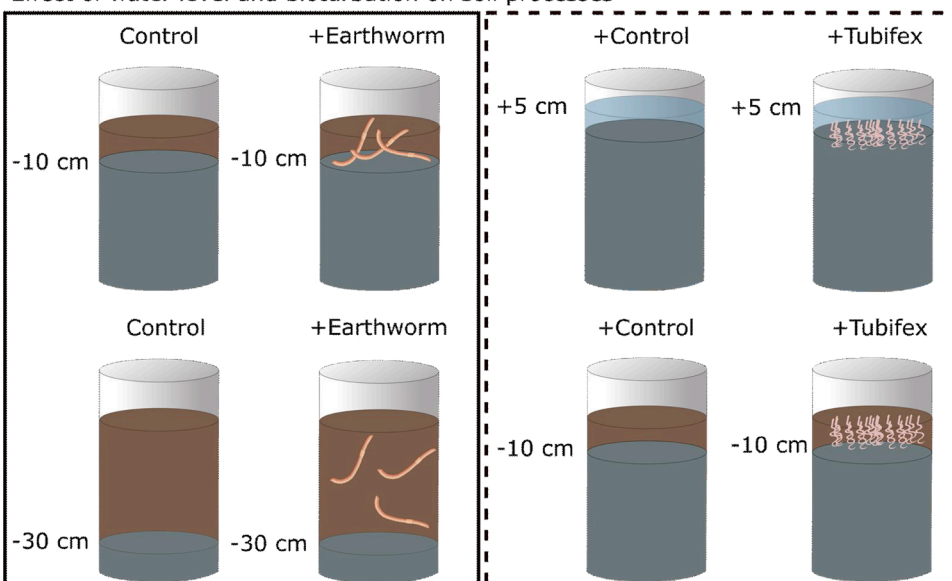

Fig. 1. Experimental design. Schematic of the experimental treatments for the three main research questions explored in this study. The solid (earthworm) and dotted (Tubifex) line indicate the two experiments for each bioturbating species. Note the different water levels in the two treatments, which correspond to the preferred water level of the studied species, as earthworms prefer dryer soils than Tubifex spp. Each set-up had 4 replicates. Symbols of the bioturbators courtesy of the Integration and Application Network, IAN Image Library (ian.umces.edu/imagelibrary/).

in the center of each microcosm, reaching the bottom. The well was used to measure and control the experimental water levels. The sides of the microcosms were kept in the dark to mimic natural conditions and were irrigated twice per week with 150 mL rainwater to compensate evaporation and maintain the target water level. The experimental treatment lasted for 10 weeks.

2.2.2. Experiment 2: Effect of two soil fauna species on soil processes

To examine the importance of contrasting soil fauna on soil processes, a set of eight microcosms was added to the set-up as described above (Fig. 1). The earthworm *Lumbricus rubellus* and *Tubifex* spp. were then added separately to four of these microcosms, respectively. As a control, the four -10 cm water level microcosms from experiment 1 were used and these microcosms had no soil fauna. Water level in all microcosms was maintained at -10 cm; this water level was chosen as both species can inhabit these conditions.

For the earthworm treatment, we added 11 earthworms (total ca. 4 g; 226 g/m^2; Regenwuermerkaufen.ch, Switzerland, Bohlen et al., 2004; Zorn et al., 2005). For the *Tubifex* treatment, we added ca. 200 individuals (ca. 1 g; 57 g/m^2; ca 12,000 individuals m^2; de Maanvis, Nijmegen, the Netherlands, Hurley et al., 2017; Saaltink et al., 2019). To mimic natural conditions for *Tubifex*, we used a flow system to ensure a constant and soft water flow for all treatments with a water level of 5 cm. To provide a source of food for the soil fauna, we added 10 g of dried cattail (*Typha latifolia*) to the sediment surface, including all microcosms of experiment 1 to ensure comparability. Data on survival collected at the end of the experiment indicates that most earthworms survived the experimental treatment (9 ± 0.5 individuals, this did not differ between the -10 and -30 cm water level treatment, $p = 0.4$). It proved impossible to assess *Tubifex* spp. survival due to its small size and clay soil, yet visual inspection indicated they thrived.

2.2.3. Experiment 3: Effect of bioturbation and water level on soil processes

To assess the combined effect of bioturbators and hydrology under conditions that represent the preferred environmental conditions of the bioturbators, we performed a third experiment (Fig. 1). Because earthworms prefer dryer soils than *Tubifex* spp., we treated them with different water level treatments: earthworms, -10 cm; earthworms, -30; Tubifex 5 cm; Tubifex, -10 cm ($n = 4$). As controls (i.e., no soil fauna) with the corresponding water level, the treatments from experiment 1 were used as a reference. The bioturbators were added in the same densities as used in experiment 2.

2.3. Measurements

For all experiments, we determined soil subsidence, soil crack formation, bulk density, soil organic matter, porewater nutrient concentrations and greenhouse gas fluxes during the last week of the experiment. To measure soil subsidence, we measured soil height on fixed locations at the start (34.8 ± 0.2 cm; average ± SE) and end of the experimental period. Next, we determined the total surface area of cracks at the side of each microcosm. To do so, we folded transparent plastic sheets around the transparent microcosm and traced visible cracks manually with markers. We then digitalized the plastic sheets and loaded them into ImageJ. To measure the crack surface, we first set a scale of a known length, and then set the color scale to black and white. Finally, we measured the crack surface area.

To measure bulk density and organic matter, we sampled the soil at 0–5, 5–10 cm depth and at the bottom (bottom 5 cm), using a cut 60 mL syringe. Soil samples were stored at −20 °C until further analysis. Bulk density was determined by weighing the soil and calculating the weight/volume based on a dry soil. This was determined by weighing samples before and after drying at 70°C for 72 h. Organic matter was measured by determining the loss on ignition at 500 °C. Data of the three depths were pooled, as we were interested in the effect of treatments on the microcosm-level.

Porewater samples were collected anaerobically using 5-cm rhizons samplers (Eijkelkamp, Giesbeek, the Netherlands) connected to vacuum syringes at 0–5, 5−10 and 10−15 cm depth. Next, all samples were divided and i) stored at 4 °C in vials (10 mL) containing 0.1 ml of 65 % nitric acid (HNO$_3$) (ISO 17294-2,2016) or ii) frozen and stored at −20 °C (10 mL) until further analysis (Vroom et al., 2020). Using samples stored at −20 °C, Concentrations of nitrate (NO$_3^-$) and ammonium (NH$_4^+$) were determined by colorimetric methods (Auto Analyser III, Bran and Luebbe GmbH, Norderstedt, Germany) and chloride (Cl$^-$) using flame photometry (FLM3Flame Photometer, Radiometer, Copenhagen, Denmark). In the acidified subsamples, sulphur (S) was measured using inductively coupled plasma optical emission spectrometry (ICP-OES) (Thermo Fischer Scientific, Bremen, Germany). Data of the three depths were pooled, as we were interested in the effect of treatments on the microcosm-level.

To determine greenhouse gas fluxes from the soil, we measured carbon dioxide (CO$_2$), nitrous oxide (N$_2$O) and methane (CH$_4$) diffusion using a closed chamber that was sealed gas-tight to a microcosm and connected to a NIRS-CRD greenhouse gas analyser in a closed loop on 30-1-2018, 1-3-2018, and 8-3-2018 (Fig. S2, Picarro G2508 Greenhouse Gas Analyzer; Picarro Inc., Santa Clara, CA, USA). Measurements were conducted until we observed a 3-min linear change in CO$_2$ and CH$_4$ gas concentration. The change in gas concentration in the chamber over time was used to calculate the diffusive flux of CO$_2$, and CH$_4$ as in Oliveira Junior et al. (2019). For N$_2$O we did not observe an increase in concentration (R^2 < 0.9), and we therefore considered the flux to be 0 mg m^{-2} d^{-1}. As a proxy for potential ebullition rates, we once manually shook each microcosm until the CH$_4$ concentration stabilized in the headspace (maximum 60 s) at the end of the experiment. We then measured the released CH$_4$. Released CH$_4$ after shaking was very low with a maximum of 0.6 mg m^{-2} and a median of 0.0009 mg m^{-2}.

2.4. Statistical analyses

A Principal Component Analyses (PCA) was performed to examine clustering of the data by treatments, using the following variables: subsidence, crack surface, bulk density, soil organic matter, porewater NH$_4^+$ and NO$_3^-$ concentrations, CO$_2$, CH$_4$ and N$_2$O fluxes. As N$_2$O fluxes were 0 in all treatments, we did not further analyze these data.

To assess the effect of water level on soil processes (experiment 1), we analyzed the effect of water level on all variables using Analysis of Variance, followed by a Tukey post-hoc test. Residuals were tested for normality and homogeneity of variance. As a result, crack surface and CH$_4$ flux data were analyzed non-parametrically using a Kruskal-Wallis test followed by Dunn tests with Benjamini-Hochberg corrections of the significance level for multiple comparisons, as assumptions for normality could not be met.

To assess the effect of bioturbation on soil processes (experiment 2), we analyzed the effect of bioturbation on all aforementioned variables, using Analysis of Variance, followed by a Tukey post-hoc test. Residuals were tested for normality and homogeneity of variance. Porewater NO$_3^-$ concentrations and CH$_4$ flux data were log transformed to meet the normality assumption. Soil subsidence and crack surface data were analyzed non-parametrically using a Kruskal-Wallis test followed by Dunn tests with Benjamini-Hochberg corrections of the significance level for multiple comparisons, as assumptions for normality could not be met.

To assess the effect of bioturbation and water level – earthworms prefer dryer soils than *Tubifex* spp., hence the different water level treatments; -30 cm for the earthworm and 5 cm for the *Tubifex* spp. – on soil processes (experiment 3), we analyzed the effect of species (bioturbator – control) and water level (higher – lower) on all aforementioned variables, using a two-way ANOVA including the interaction. For fitting the models, we started by testing the complete model with all treatments and interactions and stepwise reduced the model by excluding nonsignificant interactions. Data of each species were

analyzed separately. Residuals were tested for normality and homogeneity of variance. For earthworms, porewater NO_3^- concentrations were log-transformed and crack surface square root-transformed to meet the normality assumption. For *Tubifex* spp., data on porewater NO_3^- and CO_2 fluxes were square root and crack surface and CH_4 fluxes log-transformed. Lastly, to test the differences between earthworm survival in the -10 and -30 treatment, we performed a Student's T-test. Data were analysed and visualized with R version 3.6.0 (R Core Team, 2020) using packages "car" (Fox et al., 2012), "emmeans" (Lenth et al., 2018), and "ggplot2" (Wickham, 2011). All data depicted are means ± SEM.

3. Results

3.1. Effect of water level on soil processes

Water level had a major influence on overall soil processes and structure, while biotic treatments seemed less important, as illustrated by a PCA (Fig. 2). High water level, caused permanent inundation, preventing soil subsidence and reducing the area of cracked surface and CO_2 emissions, while it increased porewater NH_4^+ and NO_3^- concentrations. Specifically, soil subsidence was lower at a water level of 5 cm than of -30 cm ($F_{2,9} = 4.92$, $p = 0.036$, Fig. 3a). The area of cracked surface was 43 ± 5 cm^2 at a water level of -30 cm, while no cracks were observed at a water level of 5 cm ($\chi^2 = 10.2$, df = 2, $p = 0.006$, Fig. 3b). There was no effect of water level on bulk density (1.34 ± 0.02 g cm^{-3}; average of all treatments, $F_{2,9} = 1.1$, $p = 0.37$) and soil organic matter (9 ± 0.14 %; average of all treatments, $F_{2,9} = 1.67$, $p = 0.24$). Nitrate concentrations were highest at -10 cm and undetectable at -30 and 5 cm, while porewater NH_4^+ concentrations were highest at a water level of 5 cm (280 ± 20 µmol L^{-1}, $F_{2,9} = 22.05$, $p = 0.0003$) and lowest at -30 cm (30 ± 15 µmol L^{-1}, $F_{2,9} = 46.07$, $p < 0.0001$, Fig. 3c-d). A water level of 5 cm decreased CO_2 emissions, which were 12 times lower compared to emissions at a water level of -30 cm (3700 ± 430 vs 300 ± 330 mg m^{-2} d^{-1}, $F_{2,9} = 31.05$, $p < 0.0001$, Fig. 4a). In general, CH_4 (6.9 ± 6 mg m^{-2} d^{-1}, Fig. 4b) and N_2O (0 ± 0 mg m^{-2} d^{-1}) fluxes were very low or undetectable ($\chi^2 = 9.07$, df = 2, $p = 0.011$ for CH_4). In addition, a medium water level of -10 cm typically resulted in intermediate effects on all soil processes.
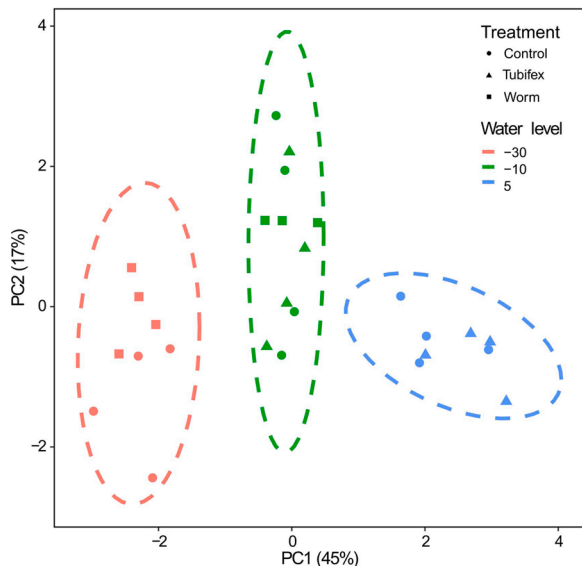
Fig. 2. Principal Component Analyses. Principal Component Analyses with data of subsidence, crack surface, bulk density, organic matter, porewater NH_4^+ and NO_3^- concentrations, and fluxes of CO_2, CH_4 and N_2O. The treatments water level (-30, -10 and 5 cm) and bioturbation (control, *Tubifex* spp. and earthworms) are used to visualize groups.

### 3.2. Effect of two soil bioturbator species on soil processes

There was no treatment effect on soil subsidence ($\chi^2 = 1.12$, df = 2, $p = 0.55$) and soil organic matter ($p = 0.55$, Fig. 3a). However, bioturbating earthworms reduced the area of cracked surface compared to *Tubifex* and control treatments at a water level of -10 cm in this experiment (Fig. 3). Specifically, when earthworms were present, no cracked surface was observed (0 ± 0 cm^2), while it was 8 ± 4 and 9.7 ± 1 cm^2 for *Tubifex* and controls, respectively ($\chi^2 = 7.69$, df = 2, $p = 0.02$, Fig. 3b). Bulk density was lower in the *Tubifex* (1.16 ± 0.06 g cm^{-3}) than in the control treatment (1.35 ± 0.02 g cm^{-3}, $F_{9,2} = 4.7$, $p = 0.04$). NH_4^+ and NO3- were not affected by the treatments at -10 cm ($F_{9,2} = 1.76$, $p = 0.24$ and $F_{9,2} = 6.75$, $p = 0.36$ for NH_4^+ and NO3-, respectively, Fig. 3c-d). Earthworms increased CO_2 emissions ($F_{9,2} = 6.75$, $p = 0.02$, Fig. 4a), while there were no detectable N_2O emissions. Specifically, in the control and *Tubifex* treatments, CO_2 emissions were lowest with 2800 ± 90 and 2700 ± 250 mg m^{-2} d^{-1}, respectively, while the emissions are ~1.5 times higher in the earthworm treatment with 4000 ± 370 mg m^{-2} d^{-1}. Although there was a treatment effect on CH_4 emissions ($F_{9,2} = 4.79$, $p = 0.038$), a post-hoc test showed no significant differences (Fig. 4b).

### 3.3. Effect of bioturbation and water level on soil processes

In general, *Tubifex* spp. were most active in the top 2.5 cm and created burrows up to 5 cm, while earthworms where mainly active in the 15 cm top layer and created burrows up to 30 cm deep. As *Tubifex* spp. and earthworms naturally occur in different environments – *Tubifex* spp. occur in wet conditions, while worms typically occur in dryer conditions –, we analysed the effect for each species in their environment separately (-30 and -10 for worms and -10 and 5 for *Tubifex* spp.). In their preferred environment at a water level of -30 cm, earthworms reduced crack surface ($F_{1,12} = 72.4$, $p < 0.0001$) and increased CO_2 emissions ($F_{1,13} = 12.1$, $p = 0.0054$), but did not affect subsidence ($F_{1,12} = 1.87$, $p = 0.2$), NH_4^+ ($F_{1,13} = 0.7$, $p = 0.42$), NO_3^- ($F_{1,13} = 0.035$, $p = 0.86$) and CH_4 fluxes ($F_{1,13} = 3.67$, $p = 0.08$, Figs. 3,4). Specifically, the area of cracked surface in controls (43 ± 5 cm^2) at a water depth of -30 cm was on average 14 times higher than when earthworms were present (3 ± 3 cm^2, Fig. 3b). *Tubifex* had hardly any effect on their environment with respect to the measured parameters ($p > 0.05$, see Table S1 for the exact statistical output). *Tubifex* affected bulk density ($F_{1,12} = 14.8$, $p = 0.0024$) and was lower (1.16 ± 0.05 g cm^{-3}) at -10 cm compared to its respective control (1.35 ± 0.02 g cm^{-3}). Nitrogen cycling, reflected by porewater NH_4^+ and NO_3^-, showed to be strongly affected by water level under earthworm ($F_{1,13} = 95.1$, $p < 0.0001$ and $F_{1,13} = 31.03$, $p < 0.0001$ for NH_4^+ and NO3-, respectively) and Tubifex treatments ($F_{1,13} = 6.4$, $p = 0.025$ and $F_{1,13} = 32.5$, $p < 0.0001$ for NH_4^+ and NO3-, respectively) while soil fauna presence had only minor effects ($p > 0.05$, see Table S1 for the exact statistical output Fig. 3c-d). At the same time, CO_2 emissions were on average 1.7 times higher when earthworms were present, with values of 6400 ± 940 and 3700 ± 430 mg m^{-2} d^{-1} for the earthworm and control treatment, respectively ($F_{1,13} = 3.67$, $p = 0.0054$, Fig. 4a).

4. Discussion

In this paper, we tested the effects of water level and presence of two types of bioturbating macrofauna on physical and biogeochemical functioning of a single soil type used to create a novel wetland. In agreement with our hypotheses, our experiments demonstrate that a high water level and presence of bioturbating earthworms reduced soil subsidence and crack formation. Earthworms strongly increased CO_2 emissions, without affecting emissions of CH_4 and N_2O, while *Tubifex* spp. presence hardly affected the measured soil parameters. CH_4 emissions were low irrespective of water level and bioturbation, opposite to our expectations. We argue that the presence and composition of early soil colonizers plays an important role in both subsidence and CO_2
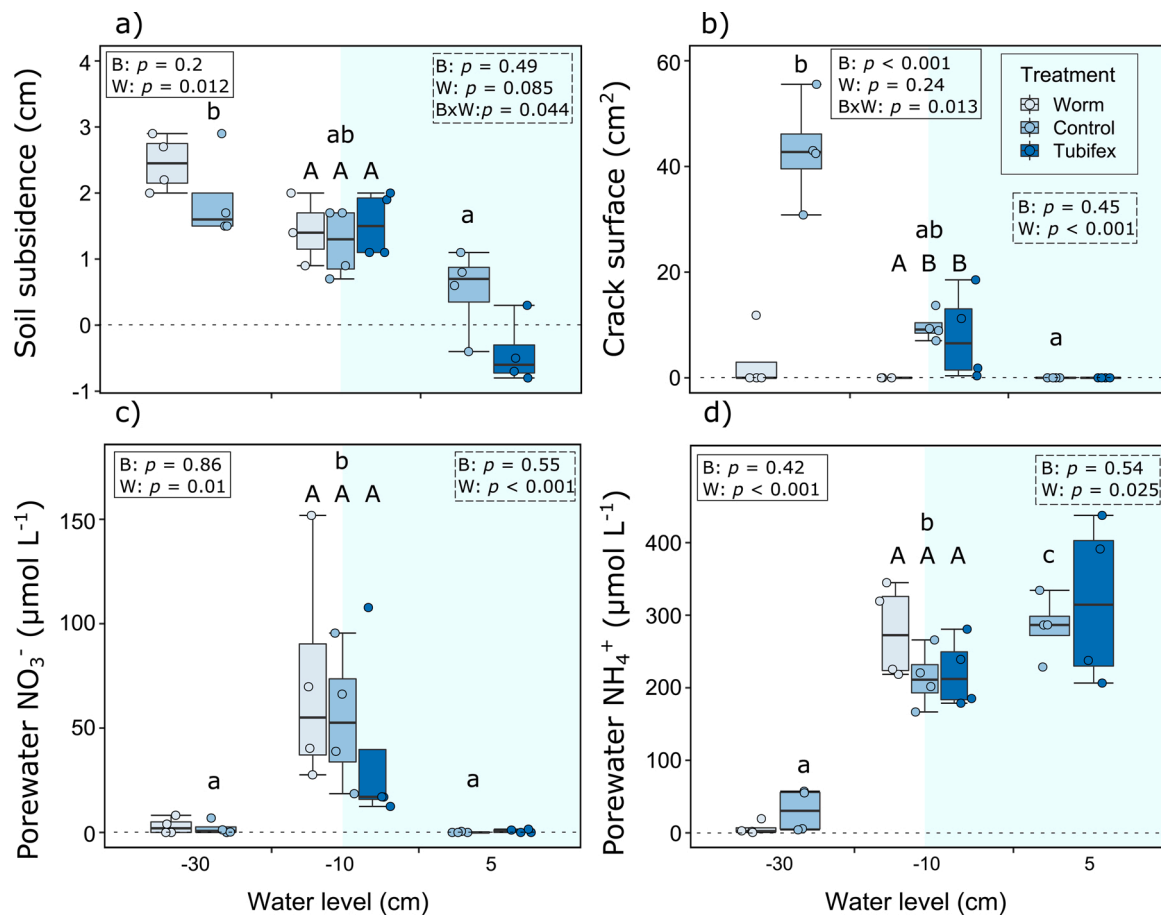
Fig. 3. **Effect of water level and bioturbation on physical and chemical soil conditions**. a) Soil subsidence (cm); negative numbers indicate an increase in soil level, b) Crack surface (cm^2), c) porewater NO_3^- and d) NH_4^+ concentrations ($\mu mol\ L^{-1}$), $n = 4$. The white area shows treatments with earthworms and light blue with Tubifex spp. Not all combinations were possible due to species-specific environmental requirements (e.g. earthworms do not survive under inundated conditions). Boxplots show the median (middle line), quartiles (boxes), 1.5 times the interquartile range (IQR) (whiskers), and the individual data values (dots). Dots outside the whiskers are extreme values. Different letters indicate differences (p ≤ 0.05) between treatments: experiment 1: small letters and experiment 2 capitalized letters. Main (B: bioturbation, W: water level) and interactive (BxW) effects are shown in boxes for each species for experiment 3: lined-box = earthworms, dotted-box = Tubifex spp. An overview of the statistical analyses and results are presented in appendix Table S1. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).
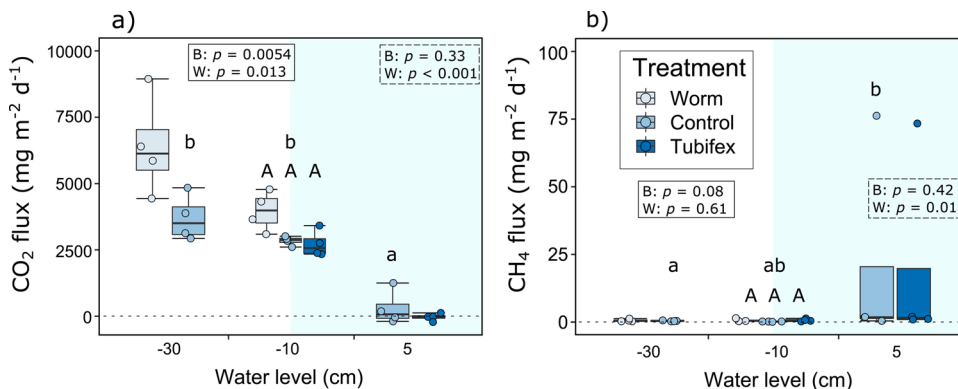
Fig. 4. **Effect of water level and bioturbation on greenhouse gas emissions**. Emissions of a) CO_2 ($m^2\ m^{-2}\ d^{-1}$) and b) CH_4 ($m^2\ m^{-2}\ d^{-1}$), $n = 4$ (for fluxes of earlier measurements, see Fig. S2). Positive values denote emissions, while negative values denote uptake. The white area shows treatments with earthworms and light blue with Tubifex spp. Not all combinations were possible due to species-specific environmental requirements (e.g., earthworms do not survive under inundated conditions). Boxplots show the median (middle line), quartiles (boxes), 1.5 times the interquartile range (IQR) (whiskers), and the individual data values (dots). Dots outside the whiskers are extreme values. Different letters depict statistical differences between treatments: experiment 1: small letters and experiment 2 capitalized letters. Main (B: bioturbation, W: water level) and interactive (BxW) effects are shown in boxes for each species for experiment 3: lined-box = earthworms, dotted-box = Tubifex spp. An overview of the statistical analyses and results are presented in appendix Table S1. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

emissions of this particular soil and should be taken into account in the early planning phase and subsequent management of soils in novel wetland ecosystems.

4.1. Effect of water level on soil processes

In our experiment, high water level prevented soil subsidence, cracks

in the surface and CO_2 emissions, while increasing porewater NH_4^+ concentrations. While the water level affected crack formation, it did not affect bulk density, nor CH_4 emissions in our experiment, which is opposite to our hypothesis. Water level is known to be a driving factor for many soil processes (Tanner et al., 1999; Couwenberg et al., 2011; Evans et al., 2021), and consequently the establishment of new wetland vegetation. For instance, the plants *Phragmites australis* or *Typha* spp. require a specific water level for germination (Yu et al., 2012). Besides affecting germination, water level also affects nitrogen dynamics in wetlands. Our findings of higher porewater ammonium concentrations at a water level of -10 and 5 cm are in line with earlier observations that accumulation of nitrogen occurs at higher water levels because ammonification prevails over denitrification, resulting in ammonium accumulation in the porewater (Hefting et al., 2004). Furthermore, as hypothesized, water level greatly affected CO_2 emissions; an increasing water level resulted in lower CO_2 emissions, which is in agreement with other studies (Kosten et al., 2018; Almeida et al., 2019; Keller et al., 2020).

Contrasting to our expectation, we hardly observed diffusive nor potential ebullitive CH_4 emissions in our experiments, which typically are very high in freshwater wetlands (Aben et al., 2017; Rosentreter et al., 2021). Typically, methanogenesis primarily takes place under anoxic but fresh soil conditions with ample availability of labile organic material (Valentine et al., 1994; Segers, 1998; Wang et al., 2017a). As the soil consisted of just under 10 % organic material and was watered with rainwater, the conditions were expected to be suitable for methanogenesis and high CH_4 emissions in our experiment. However, the marine history of the sediment may have inhibited CH_4 production (Poffenbarger et al., 2011), which could have resulted in the observed near-absent CH_4 emissions. In sediments from marine origin, chloride and sulphate (SO_4^{2-}) are an important control for biogeochemical processes in wetlands (Wang et al., 2017b; van Dijk et al., 2019; Rosentreter et al., 2021). As SO_4^{2-} reduction is a thermodynamically more favourable process than methanogenesis, methanogens may be outcompeted by sulphate reducers that are favoured by more saline waters, resulting in low CH_4 emissions. Indeed, our data show porewater chloride concentrations of 7.5−20 mmol L^{-1} (average 15 mmol L^{-1}) suggesting brackish conditions, highlighting its former marine history. Furthermore, the high porewater SO_4^{2-} concentrations (range: 0.07–20.1 mmol L^{-1}, average: 7 mmol L^{-1}) indicate that the sulphuric nature of this soil may have supressed methanogenesis. In addition, under these conditions, sulphur-driven anaerobic oxidation of methane (AOM) may take place, further reducing emissions (Egger et al., 2017). Likewise, N_2O emissions were virtually absent in our experiment. Other studies demonstrated that high NO_3- concentrations may lead to elevated N_2O production, because these may hampering the reduction of N_2O during denitrification (Lind et al., 2013). However, NO_3- concentrations in the porewater of our microcosms were low.

4.2. Effect of two bioturbating soil organisms on soil processes

Results from the bioturbation experiments clearly demonstrate that earthworms increase CO_2 emissions, while they reduce cracks at the surface. Next to an increase in CO_2 emissions, the reduction of cracks can hamper the germination and establishment of seeds under dry conditions (Elberling, 2000; Burmeier et al., 2010; Song et al., 2012). Furthermore, earthworms can stimulate seedling establishment by reducing exposure to aboveground seed predators (Heithaus, 1981), and create nutrient rich patches favourable for seed germination (Edwards and Bohlen, 1996), while hamper germination by burying seeds at depths that prevent seedlings emerging from the soil (Regnier et al., 2008), or they may damage or digest seeds during gut passage (Eisenhauer et al., 2009). In addition to reducing the number of cracks, earthworms created burrows in the soil matrix, which is known to affect soil processes, such as nutrient cycling and resulting gas fluxes. The importance of these mechanism depends most likely on ecological

groups of earthworms (Asshoff et al., 2010). Similar to our results, Kladivko et al. (1986) demonstrated that worms reduced soil crusting/cracks by enhancing soil moisture in the topsoil (Ernst et al., 2009). In our experiments, CO_2 emissions were higher in treatments with earthworms, specifically at a water level of -30 cm. The burrows created by the earthworms can increase soil oxygenation, thereby stimulating aerobic oxidation – most favourable terminal electron acceptor – of organic material in a larger volume of the soil (Bundt et al., 2001; Stroud et al., 2016). These findings suggest that soil respiration due to a higher biological activity – including respiration by the worms – in the soil is causing the higher CO_2 emissions in the earthworm's treatment (Lubbers et al., 2013). This does not necessarily indicate that earthworms reduce soil C sequestration, because the worms may convert labile carbon into stable carbon (Zhang et al., 2013). However, enhanced decomposition of organic material by the aeration of the soil by earthworms apparently only plays a minor role in the subsidence of this clay soil, as the presence of earthworm did not lead to additional soil subsidence during a 10-week period. Furthermore, CH_4 emissions from the soil were low and we did not observe an effect of bioturbation on CH_4 fluxes, which might be explained by the low methanogenesis rates, observed across all bioturbation and water level treatments, due to the marine origin of the Holocene soils (Wang et al., 2017a; Rosentreter et al., 2021).

In accordance with our hypotheses, our results show that earthworms affected soil properties, while *Tubifex* spp. did not at the density we used and irrespectively of the water level. However, other studies demonstrate that they do affect soil properties because of their dense gallery network (Navel et al., 2012; Kang et al., 2017; Saaltink et al., 2019). In line with these findings, in our experiment we found lowest average NO_3^- concentrations in the presence of *Tubifex* at a water level of -10 (Fig. 3c), where N-cycling was most pronounced, likely due to good conditions for coupled nitrification-denitrification. In our experiment, we had a density of ~12.000 individuals m^{-2}, which is comparable to Kang et al. (2017) and Saaltink et al. (2019) and natural densities described by Mermillod-Blondin et al. (2013), and which can be found in lake Markermeer. However, these densities are lower than the densities of 20.000-70.000 individuals m^{-2} used by Pelegri and Blackburn (1995) and Lagauzère et al. (2009). Pelegri and Blackburn (1995) for instance, showed that high densities of *Tubifex* increased rates of denitrification, and lowered rates of nitrification. Therefore, there might be effects, such as stimulating nitrogen loss, of *Tubifex* spp. on their environment at higher densities.

5. Conclusions and implications

In a two-month experiment using a single soil type and two taxa of early soil colonizers, we demonstrate that a high water level reduces soil subsidence and CO_2 emissions, while bioturbating earthworms increase soil subsidence and reduce soil cracks in a soil that was used to construct an archipelago to harbour new wetland ecosystems. In Marker Wadden soils, we observed low CH_4 and N_2O emission, which implies that climate-smart water management is, in the early development stages, not complex, as there is no trade-off between CO_2 and CH_4. Field measurements to complement these experimental results should be a next step. The results from our microcosm study imply that it is possible to influence soil processes with the alteration of water management and introduction of soil fauna. In terrestrial ecosystems, large-scale field experiments showed that the application of soil biota promotes ecosystem restoration and that different soil inocula can steer plant community development towards different target communities (Wubs et al., 2016). This effect was particularly strong when the topsoil was removed (Wubs et al., 2016), which might be comparable to newly constructed ecosystems such as the Marker Wadden. To determine if the introduction of soil fauna can drive ecological development in newly constructed wetlands, comprehensive field studies under a wide range of realistic environmental conditions are now required. Management measures should be carefully chosen as not to interfere with restoration

goals and prevent negative side-effects. Ideally, these kinds of experiment should be done before construction starts to guide construction workers were to collect their sediment.

Data accessibility

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

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.pedobi.2021.150745.

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