

Isoetid mediated radial oxygen loss prevents iron reduction and the related mobilisation of ammonium and methane: an experimental approach.

Jelmer van Doorn (✉ j.vandoorn@b-ware.eu)

B-WARE Research Centre <https://orcid.org/0000-0002-4966-1593>

Esther Lucassen

B-WARE Research Centre

Sarah Faye Harpenslager

B-WARE Research Centre

Lennart Santbergen

B-WARE Research Centre

Jan Roelofs

B-WARE Research Centre

Fons Smolders

Radboud Universiteit Nijmegen: Radboud Universiteit

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Abstract

Isoetids are slow growing ecosystem-engineers that maintain an oligotrophic environment through high radial oxygen losses (ROL) from their roots, which could potentially reduce methane (CH₄) emission by promoting CH₄ oxidation. Isoetid vegetations have declined in many European countries as an effect of eutrophication and inorganic carbon enrichment. To assess the effect of sudden disappearance of isoetids on biogeochemical carbon and nutrient cycling in organically rich sediments, we set up a greenhouse experiment. Intact vegetated sediment patches with *Lobelia dortmanna* and *Littorella uniflora* were collected from ten boreal Norwegian softwater lakes. For half of the patches, isoetids were manually removed. We determined the effect of removal on sediment redox potential, nitrogen (N), phosphorus (P), inorganic carbon availability and CH₄ emission. In addition, germination success of *L. dortmanna* seeds was tested on the sediments where isoetids were removed. The absence of ROL after isoetid removal led to a drop in sediment redox potential, resulting in anaerobic breakdown of organic matter using alternative electron acceptors, especially iron oxides. Consequently, increased pore water concentrations of ammonium (NH₄⁺), P and CH₄ were observed in sediments where isoetids had been removed compared to the isoetid vegetated sediments. Moreover, over 60 times higher emission of CH₄ was observed from the sediments where isoetids had been removed. On the highly reduced sediments, the germination of *L. dortmanna* was hampered. When isoetids are unable to recolonize after disturbance events, it is expected that the absence of ROL eventually results in a longterm increase in lake productivity and CH₄ emission.

1. Introduction

Isoetid plant species, such as *Littorella uniflora* and *Lobelia dortmanna*, dominate softwater lakes in boreal and temperate regions and at higher elevation in the subtropics. During the past century, isoetid vegetations have declined considerably in many European countries as an effect of eutrophication and inorganic carbon enrichment (Roelofs, 1983; Arts, 2002; Lucassen et al., 2016; Klimaszuk et al., 2020). Higher nutrient and inorganic carbon availability increases competition by favouring faster growing rooted macrophytes (Grahn, 1977; Roelofs, 1983; Lucassen et al., 2009) and alters the morphology of isoetids. It leads to an increased shoot and root weight and a decreased shoot to root ratio increasing the risk of plants becoming uprooted (Spierenburg et al., 2013; Lucassen et al., 2016). Mass uprooting events have already been observed in softwater lakes, for example in the Netherlands and Norway due to storm events (Spierenburg et al., 2013) and bottom freezing (Fig. 1), respectively. Since isoetids are considered ecosystem engineers, their sudden disappearance may strongly affect sediment processes of these soft water lakes.

The isoetid environment primarily consists of softwater lakes situated on poorly buffered siliceous bedrock or on non-calcareous sandy soils (Garcia et al., 1994; Murphy, 2002). These lakes are typically fed by rainwater and shallow groundwater resulting in nutrient-poor, weakly buffered conditions with very low concentrations of dissolved inorganic carbon (Smolders et al., 2002). The growth form of isoetids is

typical for species from unproductive environments (Hutchinson, 1975; Chapin, 1980; Vitousek, 1982) and they are characterized by a (very) slow growth rate (Roelofs et al., 1984; Hartog and Velde, 1988; Nielsen and Sand-Jensen, 1997). In the carbon limited surface waters occupied by isoetid species, carbon dioxide (CO_2) levels in the sediments are usually up to a 100-fold higher than in the water layer (Smolders et al., 2002). In contrast to most other aquatic macrophytes, isoetid species have developed the ability to take up CO_2 from the sediment for photosynthetic use (Boston et al., 1987; Nielsen et al., 1991; Pedersen and Sand-Jensen, 1992; Madsen et al., 2002). Specific adaptations of the plants that enable CO_2 uptake from the sediments include a thick leaf cuticle, often without stomata, a well-developed root system (high root to shoot ratio) and the presence of a continuous system of gas-filled interconnected lacunae that run throughout the entire plant. These adaptations enable the effective diffusion of CO_2 from the sediment into the plants where the loss of CO_2 to the water layer is prevented by the leaf cuticle (Sand-Jensen et al., 1982; Smolders et al., 2002). These morphological adaptations that allow isoetids to take up CO_2 from the sediment, also cause a high radial oxygen loss (ROL) from the roots. Isoetids plants are therefore badly adapted to growth on (highly) reductive sediments as the strong oxygen sink formed by these sediments would lead to a rapid extraction of oxygen from the permeable isoetid roots, thereby endangering the oxygen supply to the apical parts of the roots. ROL stimulates aerobic mineralization, thereby not only providing additional CO_2 for plant growth, but also reducing nitrogen (N) availability, by promoting the oxidation of ammonium (NH_4^+) and stimulating coupled nitrification-denitrification (Risgaard-Petersen and Jensen, 1997; Smolders et al., 2002). In addition, ROL lowers the phosphorus (P) availability by oxidizing ferrous iron and preventing the reduction of ferric iron(hydr)oxides, which efficiently bind P (Tessenow and Baynes, 1975; Christensen, 1997; Smolders et al., 2002). Therefore, isoetids help maintain the oligotrophic status of their habitat and can be considered true 'ecosystem-engineers' (Smolders et al., 2002).

Freshwater lakes are a major source of greenhouse gas (GHG) emissions via microbially mediated anaerobic decomposition of organic matter (Bastviken et al., 2011). Isoetids are potential mitigators of GHG emissions by promoting methane (CH_4) oxidation and carbon sequestration in sandy sediments (Ribaud et al., 2011; 2017). A low CH_4 emission as a result of soil oxygenation was also observed in wetlands with the cushion plants *Astelia pumila* and *Donatia fascicularis*, which have similar extensively developed root systems (Fritz et al., 2011). Hence, isoetids not only maintain the oligotrophic status of their habitat, they can also regulate carbon sequestration and release in freshwater lakes. Especially, northern freshwater lakes and ponds ($> 50^\circ\text{N}$) are a considerable source of atmospheric CH_4 because of their large areal extent (Wik et al., 2016). Therefore, elucidating the role of isoetid species as potential mitigator of CH_4 emissions is highly desirable.

Although isoetids are most common on mineral sediments they can also grow on more organic soils (Woodhead, 1951). In softwater Norwegian lakes, for instance, isoetids grow on sediments with organic matter contents up to 20–40% (Roelofs et al., 1994; Lucassen et al., 2016). These soils, however, consist of relatively inert organic matter with a very low availability of nutrients and are a weak sink for oxygen. On

these organic sediments isoetids have the same growth form as on mineral sediments with small sprouts and a relatively extensive root system.

To assess the role of isoetids in the biogeochemical carbon and nutrient cycling in organically rich sediments, we set up a greenhouse experiment. Intact vegetated sediment patches with *L. dortmanna* and *L. uniflora* were collected from ten boreal Norwegian softwater lakes. After removal of isoetid vegetation from half of the patches, we determined the effect of this removal on sediment N, P and inorganic carbon availability and CH₄ emission. Moreover, to determine whether softwater lakes that have lost their isoetid vegetation could theoretically recover, we tested germination success of *Lobelia dortmanna* seeds on the bare sediments where isoetids were manually removed. We hypothesized that isoetid removal promotes anaerobic breakdown of organic matter due to the absence of ROL by isoetid roots, resulting in increased (inorganic) carbon (internal alkalization) and nutrient concentrations. Moreover, we hypothesized that the absence of ROL after isoetid removal creates favorable conditions for methanogenesis while reducing the CH₄ oxidation potential, thereby increasing production and emission of CH₄ from these organically rich Norwegian lake sediments.

2. Methods

Isoetid removal experiment

In May 2010, intact vegetated sediment patches with isoetids (*L. dortmanna* and *L. uniflora*) were collected in ten lakes, situated along Nodlandsveien, Heggdalsveien and Vind-Birkedalsveien in Egersund (SW Norway). In these 10 lakes, the patches were collected in duplo (n = 20) with a spade and cut to fit into dark plastic containers (35x25x8 cm). Additional samples were collected in the 10 lakes to determine the sediment composition. The containers were transported to the greenhouse facilities of Radboud University Nijmegen (the Netherlands). In half of the containers all plants were carefully removed (bare sediments) while the isoetid vegetation in the other half of the containers remained intact (vegetated sediments). In all containers, two rhizon soil pore water samplers (length 10 cm; Eijkelkamp Agrisearch, Giesbeek, The Netherlands) were installed vertically in the sediment. The containers were placed into larger transparent plastic basins (40x30x30 cm) and inundated by adding artificial rainwater. Sediment pore water was collected by connecting the rhizons to vacuumed syringes, and redox potential was measured in the sediment top layer (see Chemical analyses).

Germination experiment

At the end of December 2010, a plastic open ring (r = 4 cm; h = 4 cm) was partly pushed into the top layer of the sediment of the containers in which the vegetation had been removed (n = 10), after which 40 *L. dortmanna* seeds were added to each ring. Germination of the seeds was monitored weekly until mid February 2011. After that, the redox potential was measured in the sediment top layer.

Chemical analyses

Sediment composition was determined from the samples that had been collected separately at each of the 10 lakes. Organic matter content was determined by loss on ignition, by combusting a known amount of dried (homogenized) sediment for 4 hours at 550°C. Sediment elemental composition was determined by digestion of 200 mg of homogenized, dried sediment with 4 mL of nitric acid (65%) and 1 mL of hydrogen peroxide (30%) using a microwave (Milestone microwave type mls 1200 mega), after which samples were analysed by Inductively Coupled Plasma (ICP) spectroscopy (IRIS-OES model Intrepid II XDL; Thermo Fisher Scientific, Waltham, MA, U.S.A). Plant-available (Olsen) P was determined by soil extraction after shaking 3 g of dried sediment with 60 mL of sodium carbonate (0.5 mol/l) for 30 minutes. Extracts were analysed for total P content using ICP.

Total Oxygen Demand (TOD) of the sediment was determined by filling 250 mL pre-weighed bottles with 5 grams of fresh sediment and 20 mL of demineralized water ($n = 3$). The water layer was aerated overnight after which the bottles were closed and placed on a shaker at 100 rpm. The dissolved oxygen concentration in the water layer was determined before closing the bottles and subsequently three times (using the three replicates) within 30 hours. The water layer was removed and the bottles with sediment were dried in the oven to determine the sediment dry weight. The Biological Oxygen Demand (BOD) was determined using the same procedure but without overnight aeration of the water layer. The Chemical Oxygen Demand (COD) was calculated by subtracting BOD from TOD.

During the isoetid removal experiment, the redox potential was measured monthly at 5 cm depth in the sediment with a multimeter (p901, Consort, Belgium), a platinum electrode and an Ag/AgCl reference electrode (Metrohm, Switzerland). The measured electrical potentials were converted to redox potentials relative to the standard hydrogen potential (E_h) by adding the reference (210 mV) and correcting for temperature and pH of the pore water. The collected pore water was determined for pH using a titration workstation (TitraLab 840, Radiometer analytical SAS, Villeurbanne, France) with a double Ag/AgCl pH electrode (Orion 9156BNWP, Thermo Scientific, USA). The concentration of total inorganic carbon (TIC) was measured on an infrared carbon analyzer (Advance Optima, ABB, Cary, NC, U.S.A.). Based on the pH and TIC concentrations, the CO_2 and bicarbonate (HCO_3^-) concentrations were calculated according to Stumm and Morgan (1996). The total elemental composition (P, Ca, Na, K, S, Al, Fe, Mg, Mn, and Zn) of the porewater samples from the bare and vegetated sediments were determined in acidified samples (65% HNO_3) using ICP. The NH_4^+ , NO_3^- and PO_4^{3-} content was analyzed by an Auto Analyser system (model III, Bran and Luebbe, Nordstedt, Germany) using a salicylate method for NH_4^+ , a hydrazine sulphate method for NO_3^- and an NH_4^+ molybdate method for PO_4^{3-} . Chloride was analysed by flame photometry.

CH_4 concentrations in the pore water were measured in glass bottles in which a 90% volume headspace was created using oxygen-free dinitrogen gas. CH_4 emission was quantified at the end of the isoetid removal experiment using upside-down funnels ($r = 5$ cm; $h = 4$ cm) closed on top with a rubber stop. Gas samples were collected through the rubber stop by using a syringe (1 ml) and a needle. Four gas samples were collected over the course of two days. Linear regression analysis was used to determine emission

rates per area. CH₄ gas samples were analysed with ethane as an internal standard on a Pye Unicam gas chromatograph Porapak Q (80/100 mesh) column (Water Chromatography, Etten-Leur, the Netherlands).

Statistical analyses

The difference in pore water chemistry, COD concentration and CH₄ emission between vegetated and bare sediments was tested using linear mixed effect models with lake as random effect. For this, only pore water data for the last six months were used to prevent artefacts caused by sediment disturbance. Before all statistical analyses were performed, data was evaluated for normality (QQ-plots) and homoscedasticity (Levene's test). All data were analysed using Microsoft Excel 2016 and R software version 3.4.3 (R Core Team, 2017). For R, the following packages were used: "gvlma" (Pena and Slate, 2006); "ggplot2" (Wickham, 2016); "agricolae" (De Mendiburu, 2019); "lawstat" (Gastwirth et al., 2017); "PMCMRplus" (Pohlert, 2018) and "nlme" (Pinheiro et al., 2021). Means with standard deviation are given throughout the text.

3. Results

Sediment chemistry

Sediment composition from all 10 lakes is presented in Table 1. The sediments were rich in organic matter (OM), which ranged from 10 to 66% (mean: $50 \pm 20\%$). In addition, high concentrations of tot-Al (817 ± 303 mmol/kg DW) and tot-Fe (653 ± 544 mmol/kg DW) were measured in the sediment. Plant available P (Olsen-P) was low and ranged from 16 to 92 $\mu\text{mol/L}$ FW, with a mean of 45 ± 23 $\mu\text{mol/L}$ FW. The mean tot-P concentration, on the other hand, was very high (31 ± 12 mmol/kg DW) due to the presence of apatite ($\text{Ca}(\text{PO}_4)_x$) in these sediments (Baar et al., 2011). There was a positive linear correlation between tot-Fe and tot-P concentrations ($F_{(1,8)} = 8.1$, $p = 0.02$, $R^2 = 0.5$) and between tot-Ca and tot-P concentrations ($F_{(1,8)} = 8.2$, $p = 0.02$, $R^2 = 0.5$) in the sediment, whereas a weak correlation was found between OM content and tot-P concentration ($F_{(1,8)} = 5.1$, $p = 0.05$, $R^2 = 0.4$).

Table 1
Composition of sediments (dry weight) collected from the ten lakes (tot = total; Olsen-P based on fresh weight) before isoetid removal.

parameter	lake										
	1	2	3	4	5	6	7	8	9	10	mean ± SD
moist (%)	66	87	86	100	100	100	97	100	89	89	91 ± 10
organic matter (%)	10	57	66	63	53	59	65	62	18	47	50 ± 20
density (mg/dw/L)	144	63	90	41	26	23	26	30	120	118	68 ± 46
tot-Al (mmol/kg)	685	801	453	395	986	850	1315	743	696	1267	817 ± 303
tot-Fe (mmol/kg)	308	472	65	335	1999	601	785	1014	339	611	653 ± 544
tot-P (mmol/kg)	11	25	17	37	46	36	49	38	21	31	31 ± 12
Olsen-P (μmol/L FW)	92	50	70	16	30	28	31	27	43	60	45 ± 23
tot-Ca (mmol/kg)	14	47	30	105	49	62	86	47	15	28	48 ± 29
tot-K (mmol/kg)	4.2	11	6.0	9.0	7.5	17	10	13	4.1	8.5	9.1 ± 4
tot-S (mmol/kg)	26	87	91	112	113	121	142	134	80	107	101 ± 33
tot-Si (mmol/kg)	29	13	8.0	8.0	12	12	17	8.0	56	36	20 ± 16
tot-Mg (mmol/kg)	64	31	38	60	44	45	41	40	26	42	43 ± 12
tot-Mn (mmol/kg)	1.1	1.9	0.3	3.7	28	1.3	1.6	0.7	9.6	1.0	4.9 ± 9
tot-Zn (mmol/kg)	0.7	1.1	0.6	1.9	1.5	0.9	1.4	1.1	0.1	0.2	1.0 ± 1

Pore water chemistry

Sediments vegetated by isoetids had a higher redox potential (92 to 242 mV) than sediments from which isoetids had been removed (134 to -79 mV; $t_{(18)} = 6.8$, $p < 0.001$; Table 2). Bare sediments had higher porewater concentrations of NH_4^+ ($t_{(18)} = 3.4$, $p = 0.008$), Fe^{2+} ($t_{(18)} = 4.2$, $p = 0.002$), HCO_3^- ($t_{(18)} = 3.6$, $p = 0.006$), TIC ($t_{(18)} = 3.3$, $p = 0.01$), pH ($t_{(18)} = 7.0$, $p < 0.001$) and COD ($t_{(18)} = 3.4$, $p = 0.009$) than vegetated sediments (Table 2) whereas porewater SO_4^{2-} concentrations were lower in the bare sediments ($t_{(18)} = -3.6$, $p = 0.006$). Pore water pH was negatively correlated with redox potential in both bare ($F_{(1,8)} = 40$, $p < 0.001$, $R^2 = 0.8$) and vegetated sediments ($F_{(1,8)} = 16.9$, $p = 0.003$, $R^2 = 0.7$; Fig. 2). Similar negative correlations with redox potential were observed for HCO_3^- ($F_{(1,8)} = 27.4$, $p < 0.001$, $R^2 = 0.8$; $F_{(1,8)} = 12.6$, $p = 0.008$, $R^2 = 0.6$) and Fe^{2+} ($F_{(1,8)} = 32.7$, $p < 0.001$, $R^2 = 0.8$; $F_{(1,8)} = 8.2$, $p = 0.02$, $R^2 = 0.5$) concentrations in the pore water of bare and vegetated sediments, respectively (Fig. 2). The pore water concentrations of CO_2 ($F_{(1,8)} = 5.3$, $p = 0.04$, $R^2 = 0.4$), TIC ($F_{(1,8)} = 17.2$, $p = 0.003$, $R^2 = 0.7$), NH_4^+ ($F_{(1,8)} = 14.5$, $p = 0.005$, $R^2 = 0.6$) and COD ($F_{(1,8)} = 20$, $p = 0.004$, $R^2 = 0.7$) were only correlated with redox potential in the bare sediments (Fig. 2). The pore water concentration of P tended to increase with isoetid removal, whereas NO_3^- concentrations tended to decrease with isoetid removal (Fig. 2).

Table 2
Pore water (pw) chemistry (mean $\pm$ SD) based on monthly values of the ten lakes 3–9 months after isoetid removal.

parameter	treatment		
	<i>p</i>	with isoetids	isoetids removed
pw-pH ($\mu\text{mol/l}$)	< 0.001	5.5 $\pm$ 0.3	6.3 $\pm$ 0.4
pw-redox (mV)	< 0.001	158 $\pm$ 46	6.2 $\pm$ 63
pw-CO ₂ ($\mu\text{mol/l}$)	0.01	321 $\pm$ 129	564 $\pm$ 326
pw-HCO ₃ ($\mu\text{mol/l}$)	0.006	53 $\pm$ 41	484 $\pm$ 399
pw-TIC ($\mu\text{mol/l}$)	0.01	324 $\pm$ 124	913 $\pm$ 641
pw-CH ₄ ($\mu\text{mol/l}$)	< 0.001	6.2 $\pm$ 9.8	318 $\pm$ 183
pw-NO ₃ ($\mu\text{mol/l}$)	0.07	7.6 $\pm$ 9.8	1.3 $\pm$ 0.6
pw-NH ₄ ($\mu\text{mol/l}$)	0.008	6.5 $\pm$ 7.7	110 $\pm$ 99
pw-Ca ($\mu\text{mol/l}$)	0.22	63 $\pm$ 25	54 $\pm$ 22
pw-Fe ($\mu\text{mol/l}$)	0.002	25 $\pm$ 33	433 $\pm$ 325
pw-Mg ($\mu\text{mol/l}$)	0.81	73 $\pm$ 8.8	75 $\pm$ 23
pw-Mn ($\mu\text{mol/l}$)	0.15	1.7 $\pm$ 2.1	58 $\pm$ 10
pw-P ($\mu\text{mol/l}$)	0.09	0.4 $\pm$ 0.1	1.0 $\pm$ 1.0
pw-S ($\mu\text{mol/l}$)	0.006	32 $\pm$ 16	11 $\pm$ 10
CH ₄ emission (mg/day/m ²)	0.04	0.2 $\pm$ 0.4	13 $\pm$ 17
COD (mg/cm ³ /h)	0.009	3544 $\pm$ 4208	9862 $\pm$ 4911

In addition, pore water HCO₃⁻ and NH₄⁺ concentrations were positively correlated with Fe²⁺ concentration in bare ($F_{(1,8)} = 27.4$, $p < 0.001$, $R^2 = 0.8$; $F_{(1,8)} = 44.7$, $p < 0.001$, $R^2 = 0.9$) and vegetated sediments ($F_{(1,8)} = 58.7$, $p < 0.001$, $R^2 = 0.9$; $F_{(1,8)} = 25.7$, $p < 0.001$, $R^2 = 0.8$), respectively (Fig. 3). A similar pattern between pore water Fe²⁺ concentrations and TIC ($F_{(1,8)} = 22.4$, $p = 0.001$, $R^2 = 0.7$) was observed, but only for the bare sediments (Fig. 2). In the bare sediments, pore water Fe²⁺ concentrations tended to increase with total iron contents of the sediment (Fig. 3).

CH₄ production and emission

CH₄ concentrations in the pore water were higher in the bare sediments ($318 \pm 183 \mu\text{mol/L}$) compared to the vegetated sediments ($6.2 \pm 9.8 \mu\text{mol/L}$; $t_{(18)} = 5.4$; $p < 0.001$; Table 2). Pore water CH₄ concentrations were negatively correlated with the redox potential of the pore water in both bare ($F_{(1,8)} = 16.3$, $p = 0.004$, $R^2 = 0.7$) and vegetated sediments ($F_{(1,8)} = 9$, $p = 0.02$, $R^2 = 0.5$; Fig. 4). A negative correlation was found between porewater CH₄ and TIC concentrations in bare sediments ($F_{(1,8)} = 14.1$, $p = 0.006$, $R^2 = 0.6$; Fig. 4). The CH₄ emission from bare sediments was more than 60 times higher ($13.1 \pm 17 \text{ mg CH}_4/\text{m}^2/\text{day}$) than from vegetated sediments, where almost no emission was measured ($0.2 \pm 0.4 \text{ mg}/\text{m}^2/\text{day}$; $t_{(18)} = 2.5$; $p = 0.04$). The CH₄ emission tended to increase with CH₄ concentration in the sediment pore water (Fig. 4).

Germination success L. dortmanna

Germination of *L. dortmanna* varied between 0 and 70 % of the added seeds and only occurred on sediments with a redox potential above -4 mV (Fig. 5).

4. Discussion

In our experiment, we revealed that the manual removal of isoetids led to a drop in redox potential and to higher inorganic carbon, N and CH₄ concentrations in the sediment pore water (Fig. 6). CH₄ emission also strongly increased with the removal of isoetids. In strongly reduced sediments, the germination of isoetids was hampered.

The effects of isoetid removal on inorganic carbon and nutrient availability

Due to the high ROL along their entire root system, isoetids have a strong influence on nutrient and carbon cycling in softwater lakes (Wium-Andersen and Andersen, 1973; Sand-Jensen et al., 1982; Smolders and Roelofs, 1996; Pedersen et al., 2011). Oxygenating the sediment elevates the redox potential, even on more organic soil types, thereby stimulating N losses and the P binding capacity of the sediment (Smolders et al., 2002; Lucassen et al., 2009).

Our results show that the absence of ROL following manual removal of isoetids leads to anaerobic sediment conditions and a drop in redox potential. Without ROL, labile organic matter is oxidized through alternative terminal electron acceptors, i.e. nitrate, manganese oxides, iron oxides or sulphate (Knorr and Blodau, 2009). During this anaerobic degradation of labile organic matter, protons are consumed resulting in a raised pH and internal alkalinisation through the conversion of CO₂ to HCO₃⁻ (Schindler, 1988). In reducing sediments of limed Norwegian softwater lakes, iron(III)(hydr)oxides seem to act as the dominant electron acceptor, resulting in iron mobilization (Roelofs et al., 1994; Lucassen et al., 2009; 2016). The reduction of iron(III)(hydr)oxides under anaerobic conditions was also evident in our experiment, with significantly increased ferrous iron (Fe²⁺) concentrations after isoetid removal. Furthermore, we found a strong negative correlation between Fe²⁺ concentrations and redox potential in the bare sediments. When iron is reduced, previously iron-bound P can be mobilised in the sediment

porewater (Tessenow and Baynes, 1975; Christensen, 1997; Smolders et al., 2006; Lucassen et al., 2009). We only found a small increase in P concentration after isoetid removal. This can be explained by the high total iron content of the sediments, which can still bind substantial amounts of P as suggested by the strong correlation between total iron and P contents of the sediments.

Besides P mobilisation, iron-mediated anaerobic degradation of organic matter also results in the conversion of organic N to inorganic N in the form of NH_4^+ (Jong et al., 2020). Due to the lack of oxygen after removal of isoetids, NH_4^+ accumulates in the pore water since it is not oxidized to nitrate. As a result, coupled nitrification–denitrification is limited in the absence of ROL (Risgaard-Petersen and Jensen, 1997; Smolders et al., 2002). In softwater lakes with relatively organically rich sediments, in-lake alkalinity generation may stimulate organic matter breakdown, contributing to even higher NH_4^+ concentrations in the sediment pore water (Lucassen et al., 2009). These processes explain the strong increase in NH_4^+ concentration in the sediments where isoetids had been removed.

The positive correlation between pore water NH_4^+ and Fe^{2+} concentrations in the bare sediments reveals that Fe^{2+} is the main electron acceptor under anaerobic conditions. These results are in line with other studies showing raised carbon and nutrient availability and increased Fe^{2+} concentrations after a drop in oxygen concentrations in the sediment, for example due to absence of ROL after massive uprooting of isoetids on organic soils (Spierenburg et al., 2013) or after rewetting of organically-rich (peat) soils (Jong et al., 2020).

The effects of isoetid removal on CH_4 production and emission

Manual removal of isoetids resulted in sharp increases in CH_4 concentrations in the pore water. In addition, CH_4 emission from the bare sediments was more than 60 times higher compared to the isoetid vegetated sediments (Fig. 6).

With the removal of isoetids and the absence of ROL, favourable conditions arise for methanogenesis (Bodegom and Stams, 1999; Lai, 2009). In wetlands, CH_4 is mainly produced by methanogenic archaea following microbially mediated anaerobic decomposition of organic matter via the conversion of acetate (acetoclastic methanogenesis) or CO_2 and hydrogen (hydrogenotrophic methanogenesis) to CH_4 (Schink, 1997; Bastviken et al., 2011). The produced CH_4 can be emitted to the atmosphere via plant-mediated transport, ebullition or diffusion, or it can be oxidized by methanotrophs in aerobic niches of the soil or in the water layer (Semrau et al., 2010; Torres et al., 2014; Dean et al., 2018). In theory, part of the produced CH_4 in the sediment may already be oxidized before being emitted to the atmosphere, which may play a role in the fact that only a weak correlation between pore water CH_4 production and CH_4 emission was observed in the bare sediments.

Due to continuous and complete soil oxidation by isoetids, anaerobic respiration and related CH_4 production is remarkably reduced (Pinardi et al., 2009; Ribaudou et al., 2011; Ribaudou et al., 2017).

Oxygenation of the sediment through ROL stimulates methanotrophic activity, leading to oxidation of CH₄ produced in anaerobic soil niches (Popp et al., 2000) and to reoxidation of reduced alternative electron acceptors, thereby suppressing CH₄ production (Laanbroek, 2010). A similar result was observed in pristine bogs in Patagonia with a high density of cushion plants, which thoroughly oxidized the soil (Fritz et al., 2011). We measured higher CH₄ concentrations after isoetid removal compared to the study by Ribauda *et al.* (2017) from bare sandy soils. This can be attributed to the higher organic matter content of the sediments in our experiment. CH₄ production and emission are stimulated by higher organic carbon availability, resulting in higher methanogenic activity (Ribauda *et al.*, 2011; Grassat *et al.*, 2018; Beaulieu et al., 2019).

Methanogenesis generally initiates after depletion of other alternative electron acceptors when the production of CH₄ becomes an energetically favourable respiration pathway (Peters and Conrad, 1996; Teh et al., 2007; Minderlein and Blodau, 2010; Yamada et al., 2014). Nevertheless, substantial CH₄ production and emission were measured from the iron-rich sediments used in our experiment, which implies that methanogenesis was not inhibited by the presence of iron oxides. Similar results were found by Jong et al. (2020) for rewetted peat soils. This can be attributed to the fact that methanogenesis can still take place in micro-environments of the upper sediment where substantial amounts of CH₄ can build up before alternative electron acceptors are entirely consumed (Knorr et al., 2008; Knorr and Blodau, 2009) or that iron reducing bacteria and methanogenic archaea do not entirely compete for the same organic substrates.

Conclusions

Summed up, our experiment shows that isoetids are effective in maintaining oligotrophic conditions and in mitigating CH₄ emission, at least in softwater lake sediments with a more organic character. When isoetids disappear, through disturbances following eutrophication or inorganic carbon enrichment, sediments become reductive and anoxic. This hampers recolonization of isoetids due to low germination success (Arts and Heijden, 1990; Bellemakers et al., 1996). Here, we also found that *L. dortmanna* failed to germinate on sediments with a redox potential < -4 mV. When, a new vegetation of faster-growing species establishes permanently, these systems can switch from oligotrophic softwater lakes to more mesotrophic lakes (Lucassen et al., 2016). Hence, longterm absence of isoetids can promote nutrient mobilization, increase lake productivity, and cause these systems to transform into net CH₄ sources.

Declarations

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Conflict of interest

The authors E.C.H.E.T. Lucassen, J.G.M. Roelofs and A.J.P. Smolders have a personal relationship with Kaj Sand-Jensen (Professor Freshwater Biology, University of Copenhagen) that does not allow K. Sand-Jensen to evaluate the manuscript objectively (for example as potential reviewer). The authors have no relevant financial interests to disclose.

Data availability

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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Figures



Figure 1

Massive uprooting of the isoetids *Lobelia dortmanna* and *Littorella uniflora* in a pristine shallow Norwegian softwater lake during winter 2009-2010 (photo: Esther Lucassen)

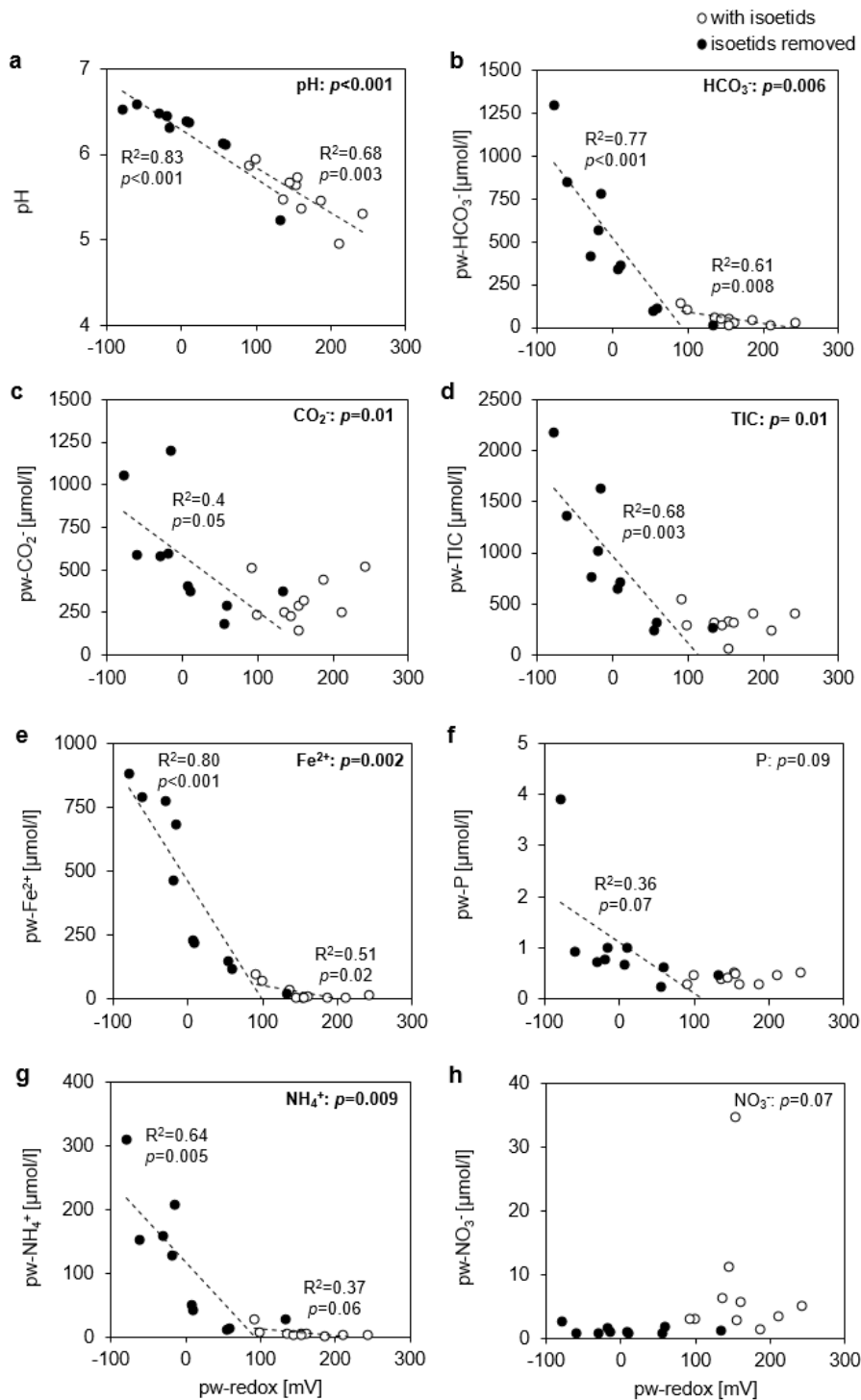


Figure 2

Correlations between the pore water (pw) redox potential and various chemical parameters in the pore water of the isoetid vegetated sediments (white dots) and sediments where isoetids were removed (black dots). Values represent mean monthly values of the ten lakes after 3-9 months. Note that COD was only measured once at the end of the experiment. Lines represent linear regressions. Bold p -values represent statistical differences of the parameter on the y-axis between vegetated and bare sediments (Table 2)

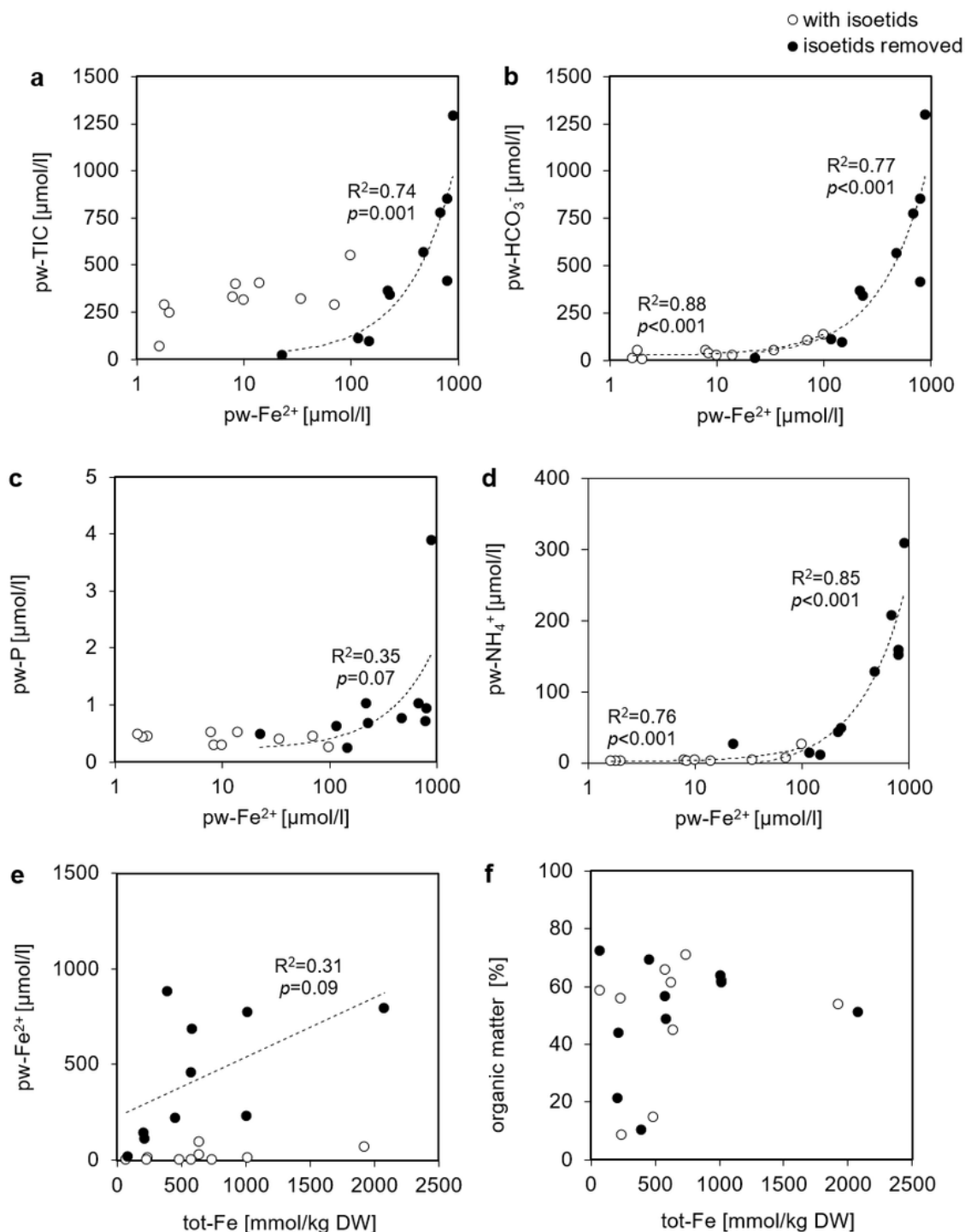


Figure 3

Correlations between pore water (pw) iron and inorganic carbon (a-b), nutrient (c-d) concentrations and between total (tot) iron content (sediment) and pore water iron concentration (e) and organic matter content (f) of the isoetids vegetated sediments (white dots) and sediments where isoetids were removed (black dots). Values represent mean monthly values of the ten lakes after 3-9 months. Note that organic

matter content and total iron content were measured only once at the start of the experiment. Lines represent linear regressions

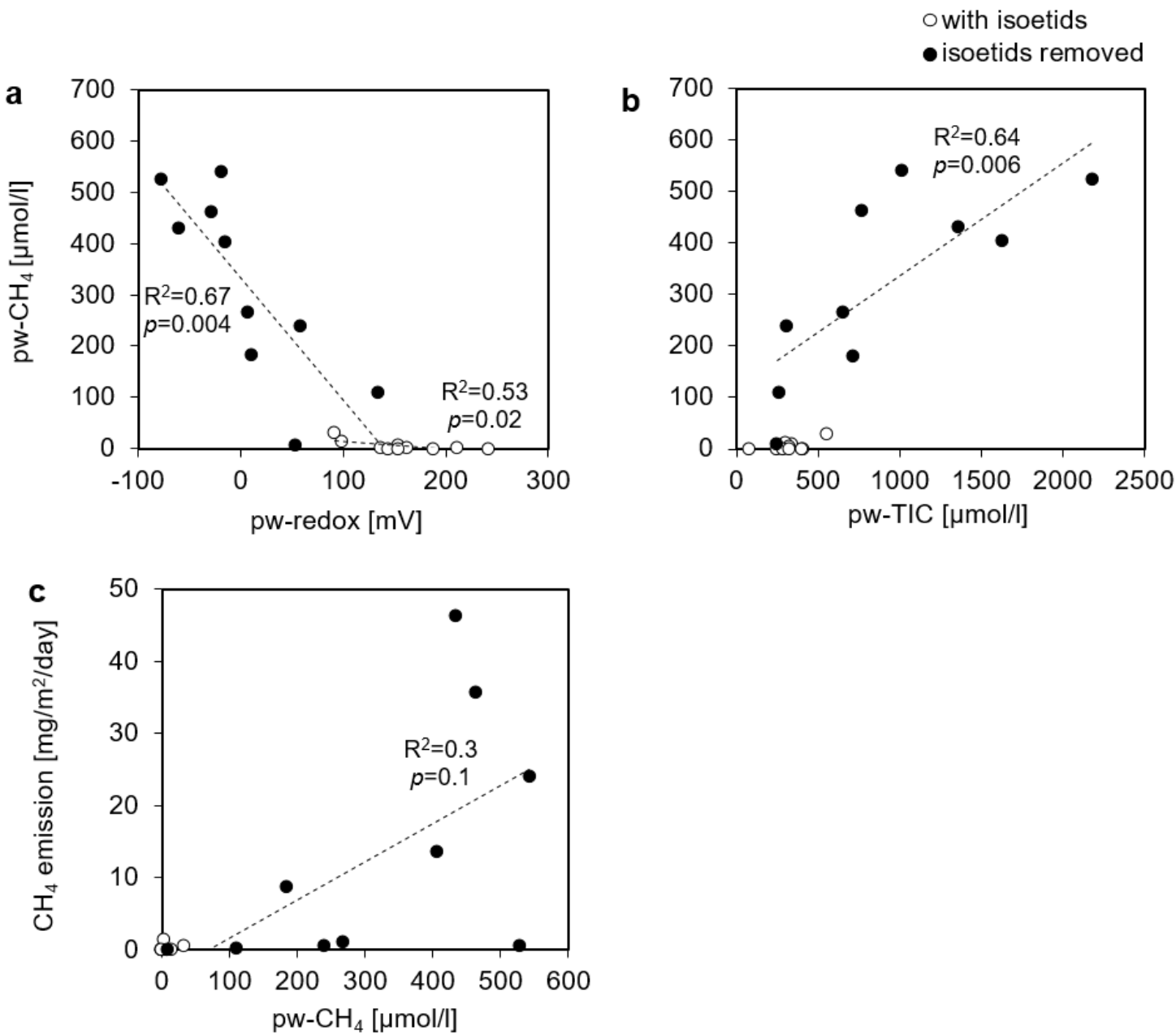


Figure 4

Correlations between pore water (pw) CH₄ concentration and redox potential (a), TIC (b) and between CH₄ emission and the concentration of pore water CH₄ (c) of the isoetids vegetated sediments (white dots) and sediments where isoetids were removed (black dots). Values represent mean monthly values of the ten lakes after 3-9 months (except for CH₄ emission rates). Lines represent linear regressions

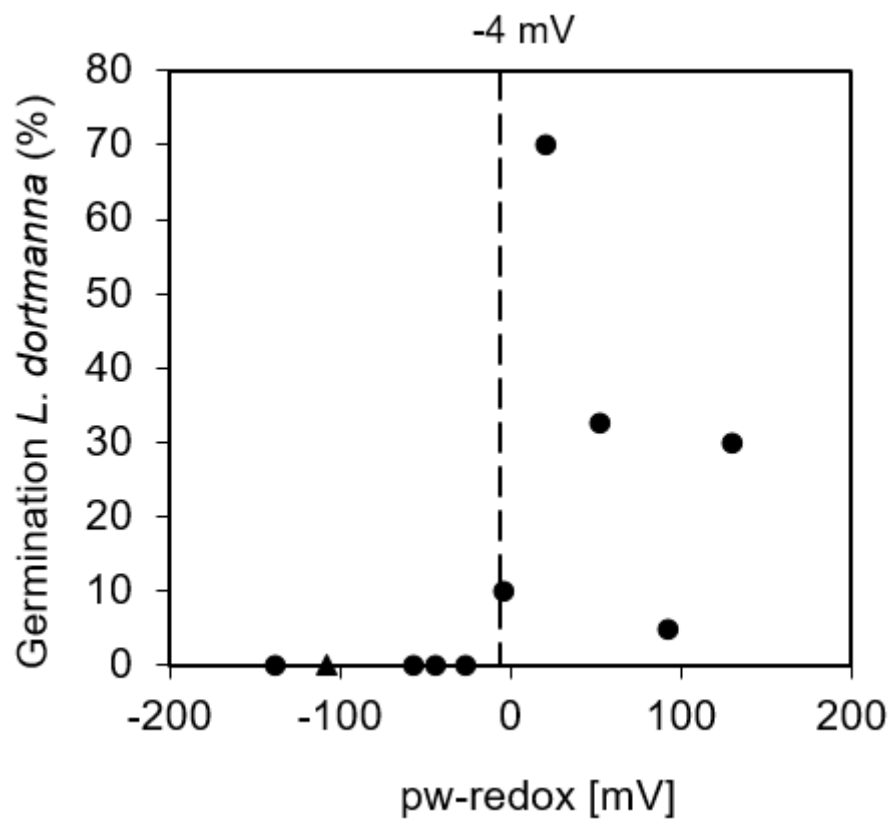


Figure 5

Correlations between germination (%) of *Lobelia dortmanna* and pore water (pw) redox potential of the bare sediments. The triangle represents sediment from the lake with massive uprooting in Figure 1

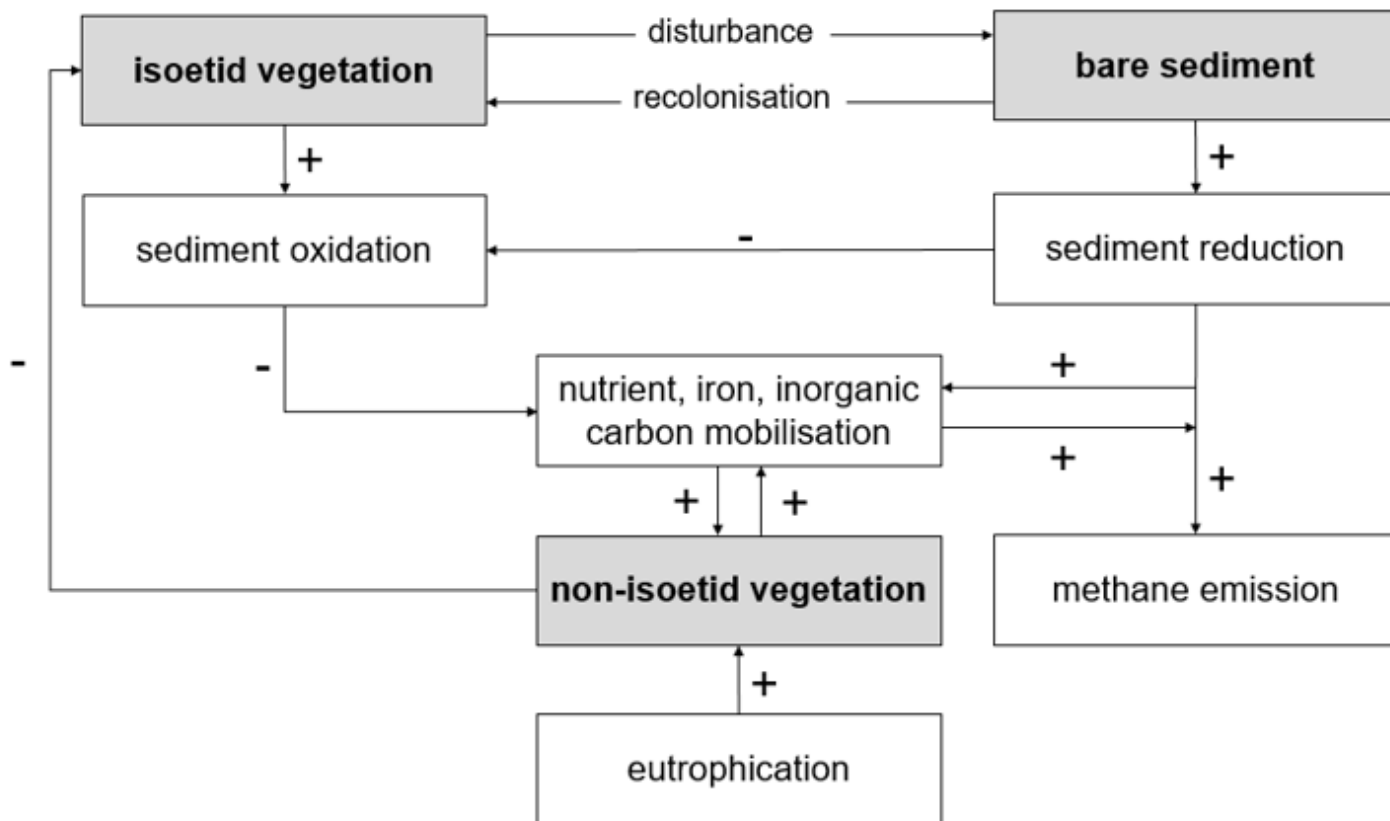


Figure 6

The results of our experiment showing the effects of isoetid vegetation (removal) on biogeochemical carbon (including methane) and nutrient cycling in organically rich sediments. Arrows with “+” indicate stimulation, arrows with “-” indicate suppression.