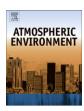
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# Transforming meadows into free surface water wetlands: Impact of increased nitrate and carbon loading on greenhouse gas production

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#### ABSTRACT

In a laboratory study we investigated 1) the potential production of nitrous oxide (N2O), methane (CH4) and carbon dioxide (CO<sub>2</sub>) and 2) the effect of nitrate (NO<sub>3</sub>) and anaerobic N<sub>2</sub>O development on CH<sub>4</sub> production in sediment from a recently recreated free surface water wetland (FSWW) and in soil from an adjacent meadow. We designed an experiment where production of greenhouse gases was registered at the time of maximum net development of N<sub>2</sub>O. We made additions of biodegradable carbon (glucose) and/or NO3 to sediment and soil slurries and incubated them at four temperatures (4, 13, 20, 28 °C). Gas production from both substrates was positively correlated with temperature. We also found that the sediment produced more N2O than the soil. N2O production in sediment was NO3 limited, whereas in soil carbon availability was lower and only combined additions of NO<sub>3</sub> and glucose supported increased N<sub>2</sub>O development, CH<sub>4</sub> production was generally low and did not differ between soil and sediment, Nor did glucose addition increase CH4 rates. The results suggest that neither soil nor sediment environment did support development of methanogenic populations. There were no clear effects of NO3 on CH4 production. However, the highest records of CH<sub>4</sub> were found in incubations with low N<sub>2</sub>O production. which indicates that N2O might be toxic to methanogens. In summary, our study showed that transforming meadows into FSWWs implies a risk of increased N2O emissions. This does not seem to be valid for CH<sub>4</sub>. However, since N<sub>2</sub>O is almost always produced wherever NO<sub>3</sub> is denitrified, increased N<sub>2</sub>O production in wetlands leads to reduced rates in downstream environments. Hence, we conclude that when balancing NO3 retention and global warming aspects, we find no reason to discourage future creation or restoration of wetlands.

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#### 1. Introduction

Three of the most abundant greenhouse gases in the atmosphere, i.e. carbon dioxide ( $CO_2$ ), methane ( $CH_4$ ) and nitrous oxide ( $N_2O$ ), are both anthropogenically and naturally produced. Although the share of greenhouse gases in the total mass of atmospheric gases is less than 1%, their importance for the climate of our planet is immense. Due to rapidly increasing emissions of greenhouse gases during the latest century, large interest has developed to reveal the anthropogenic influences on global warming by e.g. use of fossil fuels, changing land use and eutrophication. Besides, measures taken to abate diffuse N and P leakage from agricultural land can lead to increased production and emission of greenhouse gases. In many countries, including Sweden, strategies to lower the effects on marine coastal ecosystems of nutrient leakage include the creation and restoration of lakes and wetlands. One key aim of creating these environments is to remove nitrogen from run-off waters in the

denitrification process. There is a risk, however, that anaerobic conditions resulting from extensive water logging and heavy nutrient load lead to enhanced  $N_2O$  and  $CH_4$  emission. The warming potential of  $N_2O$  and  $CH_4$  per mass unit is much higher than that of  $CO_2$ . Hence, in a 100-year perspective  $N_2O$  has a 296-fold higher global warming potential (GWP) per mass unit than  $CO_2$ , and  $CH_4$  has 23 times higher effect than  $CO_2$  (IPCC, 2001).

In aquatic and terrestrial ecosystems CO<sub>2</sub> is produced in both aerobic and anaerobic decomposition processes. However, due to intense gross consumption of CO<sub>2</sub> by photosynthesis under light conditions, many ecosystems are net sinks of CO<sub>2</sub> at least during the growing season. Under anaerobic conditions organic carbon is transformed not only to CO<sub>2</sub> but also to CH<sub>4</sub> (Wetzel, 1983). A large proportion of the global CH<sub>4</sub> budget emanates from wetlands, reservoirs and lakes (Rotmans et al., 1992; St. Louis et al., 2000; IPCC, 2001). Electron acceptors like NO<sub>3</sub>, ferric iron and sulphate, if available in anaerobic environments, enable microbial oxidation of organic carbon to CO<sub>2</sub> (Mortimer, 1941; Kelly et al., 1982). NO<sub>3</sub> is used as electron acceptor in denitrification, which is a potential source of N<sub>2</sub>O (Conrad, 1996). This trace gas is also produced in aerobic environments in the nitrification process (Conrad, 1996).

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These two processes correspond most of the global N<sub>2</sub>O emissions (65%) from soils and sediments to the atmosphere (Smith, 1997).

Earlier studies have shown that increased loading of NO<sub>3</sub> (Azam et al., 2002; Liikanen et al., 2002a, 2003a) and degradable carbon (Swerts et al., 1996a,b; Garcia-Montiel et al., 2003) can increase the N<sub>2</sub>O emission from lakes and terrestrial environments. On the other hand, NO<sub>3</sub>, a prerequisite for denitrification, is considered to prevent methanogens from being active. Competition can affect methanogens since denitrifying bacteria that occur at higher redox potential exploit substrates to lower concentrations than can be used in processes requiring lower redox potential, i.e. making substrates unavailable to methanogenesis (Achtnich et al., 1995). In addition, it has been shown that presence of the denitrification intermediate N<sub>2</sub>O can suppress both acetoclastic and hydrogenotrophic methanogeneses (Clarens et al., 1998; Klüber and Conrad, 1998). In denitrification the N<sub>2</sub>O:N<sub>2</sub> ratio usually is between 0.1 and 6% (Seitzinger, 1988; Groffman et al., 2000). This means that most leached inorganic nitrogen is released to the atmosphere as  $N_2$ . The ratio is important if one wants to evaluate the impact on the atmosphere since areas with low N2O:N2 ratios result in lower greenhouse effect in comparison with the nitrogen removal effect.

Here we investigate how the availability of NO<sub>3</sub> and organic carbon influences the production of N2O, CH4 and CO2. We hypothesize that NO<sub>3</sub> would have a positive impact on N<sub>2</sub>O and CO<sub>2</sub> production due to an increased amount of electron acceptors available for microbial degradation, but a negative impact on CH<sub>4</sub> production. On the other hand, high carbon availability would support denitrification, CH<sub>4</sub> and CO<sub>2</sub> production. By comparing wet soil and sediment under anaerobic conditions, our intention is to illustrate the differences in microorganism metabolism and gas production in a periodically wet meadow that could be transformed into a permanently inundated free surface water wetland (FSWW). We also hypothesize that high carbon quality shortens the time period until maximum N2O concentration. Furthermore, we assume that higher temperature (as during summer and in a future climate perspective) will increase heterotrophic respiration and result in higher production of greenhouse gases.

#### 2. Material and methods

#### 2.1. Study site

The recently (August 2003-May 2004) constructed FSWW (55° 29' N,  $13^{\circ} 30' \text{ E}$ ) has a surface area of  $0.45 \text{ km}^2$ , making up about 10%of the former Lake Näsbyholmssjön, and a maximum depth of 1.6 m. It is situated in an open landscape and has high wind exposure. The catchment area consists of 60% arable land, of which most is drained to the main influent of the wetland. Before the lake was drained (1780, 1866-1869), it was described as shallow, nutrient rich and productive. The drained area, including both the new FSWW and the investigated meadow, has thereafter been used for grass production and cattle grazing. The entire area has been influenced by groundwater fluctuations and water had to be pumped from the area that is now an FSWW to make it suitable for grazing. The concentrations of  $NO_3^-$ ,  $PO_4^{3-}$ , total nitrogen and total phosphorus as well as the conductivity are high in the wetland (Table 1). On the sampling occasion nitrogen and carbon contents were higher in sediment than in soil, while the TC/TN ratio was slightly higher in soil than in sediment (Table 2). Soil had higher density than sediment, while water content was higher in sediment than in soil. Both substrates had neutral pH (Table 2).

#### 2.2. Sampling and pre-treatment

Soil, sediment and water samples were collected in September 2004. The vegetation and uppermost root zone were removed from

**Table 1**Water characteristics of the FSWW in September 2004, on the date of sediment and soil collection, and minimum–maximum values of monthly sampling in the in- and outlet of the wetland during June 2004–December 2007.

	September 2004, middle of the FSWW <sup>c</sup>	Min-Max (2004-2007)	
		Inlet	Outlet
TN (mg l <sup>-1</sup> ) <sup>a</sup>	5.2	0.93-11.0	1.5-9.1
$(NO_3^- + NO_2^-)$ -N $(mg l^{-1})^b$	0.83	0.005-9.1	0.005-6.7
TP $(mg l^{-1})^a$	0.57	0.026-1.1	0.041-1.1
$PO_4^{3-}$ -P (mg l <sup>-1</sup> ) <sup>b</sup>	0.35	0.012-1.1	0.003-1.0
Conductivity (mS <sub>20°C</sub> m <sup>-1</sup> )	57.9		
pН	7.66		

- <sup>a</sup> Unfiltered.
- <sup>b</sup> GF/F-filtered.
- <sup>c</sup> Free surface water wetland.

the soil to simulate the gas production potential of the groundwater influenced soil. Soil samples were then taken from the top 10 cm. Sediment samples (uppermost 5 cm) were taken at 1 m water depth. Soil and sediment samples were then brought to the laboratory and stored in darkness at 4 °C. Water samples were collected near the bottom in the central part of the lake. Conductivity and pH were measured in the laboratory on the same day. Water for  $NO_3^- + NO_2^- - N$  and  $PO_4^{3-} - P$  analyses was filtered through GF/F (Whatman), whereas water for TN and TP analyses was unfiltered. All water samples were stored frozen until analysis.

#### 2.3. Incubations

Twenty grams wet weight of homogenized sediment and soil, respectively, were placed in 60 ml glass bottles. To establish similar conditions regarding water content and to simulate soil flooding artificial lake water (Lehman, 1980), which resembles the lake's water in composition, was added to a final total water content in all bottles of 20 ml. Nitrate  $(Ca(NO_3)_2)$  and carbon (glucose) were added to the sediment and soil samples. Hence, pre-incubation concentrations of the  $NO_3^-$  (N), glucose (G) and  $NO_3^-$  plus glucose (NG) treatments amounted to 30 mg N  $I^{-1}$  and 20 mg C  $I^{-1}$ , respectively, and were in the same range as e.g. in the denitrification experiments of Lalisse-Grundmann et al. (1988).

To establish different temperature regimes (4, 13, 20 and 28 °C), sediment and soil were kept in constant-temperature laboratories during 2 h prior to the addition of water and N, G or NG, and during the incubation. After the additions the bottles were flushed with  $N_2$  during 5 min to attain anaerobic condition, and immediately sealed with gas tight butyl rubber stoppers (diameter 14 mm, 10 mm thick, Apodan Nordic, Denmark) and aluminium lids. To avoid low pressure during gas sampling 20 ml extra  $N_2$  was added through the rubber stopper.

To determine the time period required for development of maximum N<sub>2</sub>O concentration, i.e. when we expected the highest negative effect of N<sub>2</sub>O on CH<sub>4</sub> production, three replicates of control (C) and each treatment (N, G, NG) at each temperature (4, 13, 20, 28 °C) and substrate (soil and sediment) were incubated, in total 96 bottles. In a second experiment potential N<sub>2</sub>O, CH<sub>4</sub> and CO<sub>2</sub>

**Table 2** Soil and sediment characteristics (mean  $\pm$  standard deviation, n = 2 or mean, n = 3).

	Soil	Sediment
DW/WW (%)	86	27
pH	$7.19\pm0$	$6.95\pm0.05$
Carbon content (%C g <sup>-1</sup> DW)	$2.6\pm0$	$16.7\pm0.9$
Nitrogen content (%N g <sup>-1</sup> DW)	$0.2\pm0.004$	$1.52\pm0.07$
TC/TN (mass basis)	$13.0\pm0.3$	$11.1 \pm 0.1$
Density (g cm <sup>-3</sup> )	1.9	1.3

DW = dry weight; WW = wet weight; TC = total carbon; TN = total nitrogen.

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