




Effect of Temperature on Oxygen Profiles and Denitrification Rates in Freshwater Sediments

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Abstract Vegetated ditches and wetlands are important sites for nutrient removal in agricultural catchments. About half of the influx of inorganic nitrogen can be removed from these ecosystems by denitrification. Previous studies have shown that denitrification in aquatic ecosystems is strongly temperature dependent, resulting from temperature-dependent oxygen availability. Here, we study short-term temperature effects on sediment oxygen demand (SOD) and the maximum depth of oxygen penetration into the sediment (Z), in relation to overall denitrification rates. We set up sixteen wetland microcosms at four different temperatures (11–25 °C), in which we determined SOD and Z from sediment oxygen microprofiles. Denitrification rates were measured using ¹⁵N-labeling, analysed by membrane inlet mass

spectrometry. Temperature strongly affected sediment oxygen dynamics. SOD exponentially rose with temperature, ranging from 0.37 to 1.53 g m⁻² d⁻¹ ($Q_{10} = 2.4$). Correspondingly, warming led to shallower oxygen penetration into the sediment, ranging from 4.12 to 2.08 mm. Denitrification rates increased with warming ($Q_{10} = 2.6$), ranging from 8.4 to 86 μmol N m⁻² h⁻¹. The results of this short-term experiment confirm the potential increase of denitrification with rising temperature, promoted by lower oxygen availability in the top layer of the sediment, which supports the understanding of denitrification variability in freshwaters.

Keywords Agricultural wetland · Biogeochemistry · Climate change · Denitrification · Membrane inlet mass spectrometry (MIMS) · Nitrogen cycling

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Introduction

Climate change is projected to lead to an increase in the average global temperature of more than 2 degrees Celsius by the end of the twenty-first century (IPCC 2014). At the same time, nitrogen pollution is expected to increase, due to intensification of agricultural and industrial activities. This can amplify eutrophication effects in freshwater and coastal habitats, and increase the emission of the potent greenhouse gas N₂O (Erisman et al. 2013; Stein and Klotz 2016). Currently, the altered flow of reactive nitrogen into ecosystems is already one of the most pressing global environmental issues (Steffen et al. 2015). Freshwater ecosystems, such as wetlands and shallow lakes, can buffer effects of nitrogen pollution by transforming nitrogen in their sediments, water column, and biota (Saunders and Kalff 2001). Vegetated ditches

in wetland areas act as nitrogen filters in the agricultural landscape, removing a large proportion of nitrogen before they drain into connected rivers and lakes (de Klein 2008; Veraart et al. 2016). These ditches are common freshwater ecosystems in low-lying agricultural areas and peatlands, constituting natural sediment and bank vegetation similar to wetlands (Herzon and Helenius 2008). In the Netherlands, ditches are an important ecosystem type, covering an area similar to that of shallow lakes or rivers. Their tight terrestrial-aquatic coupling makes them biogeochemical hotspots. In these ecosystems, denitrification - the microbial conversion of nitrate (NO_3^-) or nitrite (NO_2^-) via nitric oxide (NO) and nitrous oxide (N_2O) to dinitrogen gas (N_2) - can remove more than half of the nitrogen influxes, making it a crucial process counteracting eutrophication (de Klein and Koelmans 2011; Veraart 2012). However, because denitrification on average produces 1–5% of its gaseous end product in the form of N_2O , enhanced denitrification rates can also pose a strong, yet understudied, positive feedback on global warming (Veraart et al. 2011; Hanke et al. 2015).

Denitrification is a major sink for inorganic nitrogen in freshwater ecosystems, however, several studies in the past two decades have demonstrated that anaerobic ammonium oxidation (anammox) can also significantly contribute to N removal (Risgaard-Petersen et al. 2003; Seitzinger et al. 2006). Denitrification is carried out by a wide range of microorganisms, most of which are facultative anaerobes, which will only denitrify once oxygen becomes limiting (Knowles 1982). Denitrifiers use organic carbon (canonical heterotrophic denitrification), sulphide, or hydrogen (chemolithoautotrophic denitrification) as an electron donor (Burgin et al. 2012). Or, in the special case of methane-oxidation dependent denitrification, methane reduction and oxidation are coupled to nitrite dismutation (Ettwig et al. 2010; Stein and Klotz 2016). Because of the prevailing nitrate and oxygen concentrations, freshwater denitrification activities are often highest at the oxic-anoxic boundary layer in the sediment, in particular at sites where the diffusion path of nitrate from the overlying water to the anoxic denitrification zone is small, i.e. when oxygen penetration into the sediment is shallow (Risgaard-Pedersen et al. 1994). Here, denitrification can be tightly coupled to nitrification, the aerobic oxidation of ammonium to nitrate, which takes place in the oxygenated top layer of the sediment (Seitzinger et al. 2006). The strength of this coupling depends on nitrogen availability and presence of oxic-anoxic interface. Thus, sediment oxygen concentrations, sediment oxygen demand and associated oxygen penetration depth will be important drivers of denitrification.

All enzymatic reactions are affected by warming, both as a direct effect on enzyme activity and by indirect temperature

effects on substrate availability. Recent studies indicate that within freshwater sediments and phototrophic biofilms, warming can strongly increase denitrification rates (Bonnett et al. 2013), due to temperature-regulated oxygen concentrations (Veraart et al. 2011; Bouletreau et al. 2012). However, in these studies oxygen concentrations were measured in the water column, whereas most denitrification takes place in the sediment.

The zonation of respiratory organic matter degradation and denitrification in a sediment profile is strongly linked to the oxygen concentration at a given depth (Rysgaard et al. 1994; Seitzinger 1994). Sediment oxygen demand arises from respiration, most notably aerobic degradation of organic material. Warming can affect sediment oxygen demand and oxygen penetration depth in different ways. On one hand, it can lower oxygen concentrations by reducing oxygen solubility and by more strongly enhancing respiration rates relative to photosynthesis (Hancke and Glud 2004; Allen et al. 2005; Yvon-Durocher et al. 2010; Bouletreau et al. 2012). On the other hand, effective diffusion rates of oxygen into the sediment will also increase with rising temperature (Elberling and Damgaard 2001). Therefore, temperature effects on sediment oxygen dynamics are expected to vary between sediments, depending on carbon substrate and electron availability and physical sediment structure.

Because sediment oxygen dynamics are driven by several biotic and abiotic processes which each have their own temperature response, effects of increased temperatures on sediment oxygen demand, oxygen penetration depth, and denitrification are not straightforward. To further unravel the complexity of these interacting processes we determine the short-term effects of temperature on oxygen availability and uptake in ditch sediments. To this end, we measured oxygen microprofiles in the top layer of the sediment and denitrification rates in laboratory microcosms at 11, 16, 21 and 25 °C. From the oxygen microprofiles we determine the oxygen penetration depth (Z) and the areal respiration rate, expressed as Sediment Oxygen Demand (SOD), by fitting an oxygen flux model to the data. The results of this analysis can contribute to a better understanding of the carbon and nitrogen cycle in ditches, which are important ecosystems in agricultural environments.

Materials and Methods

Experimental Setup

We used a microcosm set-up to create an intermediate system between core incubations and in-situ measurements, as larger and less disturbed systems may better

the oxygen penetration depth Z and SOD can be derived from Eq. 3:

$$Z = \sqrt{\frac{2C_0D}{R}} \quad (4)$$

$$SOD = R \cdot Z = \sqrt{2C_0DR} \quad (5)$$

The molecular diffusion coefficient for oxygen in water (D_m) is $1.18 \cdot 10^{-4}$, $1.36 \cdot 10^{-4}$, $1.55 \cdot 10^{-4}$ and $1.72 \cdot 10^{-4}$ $\text{m}^2 \text{d}^{-1}$ at 11, 16, 21 and 25 °C, respectively (Han and Bartels 1996). Porosity of the cores was estimated from dry matter and organic matter content (Avnimelech et al. 2001). Average porosity showed to be 0.84 ± 0.09 (–).

For all individual cores the values for R , Z and the relative position of the sediment-water interface ($z = 0$) were derived by fitting Eq. 3 to the measured oxygen profiles. Parameter calibration was done by the solver in MS Excel, by minimising the sum of squares. For the model fitting we used the condition, that:

$$\begin{aligned} \text{above sediment-water interface : } & C = C_0 (= Z^2 R / 2D) \\ \text{from } z = 0 \text{ to } z = Z : & C = R / 2D \cdot (Z - z)^2 \\ \text{if } z > Z : & C = 0 \end{aligned}$$

Finally, SOD was calculated from R and Z (Eq. 5).

Denitrification

Denitrification rates were measured after 48 h of incubation at the experimental temperature (11, 16, 21 or 25 °C), after 4 h of light irradiance. For these measurements the microcosms were closed with airtight lids under the water surface. The water under the lids was enriched with ^{15}N (NaNO_3 , 98 atom %) to a concentration of $1 \text{ mg } ^{15}\text{N l}^{-1}$, through an airtight septum and gently mixed using an airtight glass syringe. Water samples were taken in duplicate through the septum, 15 min and 3 h after addition of the ^{15}N labelled nitrate. The samples were stored in 12 ml exetainers until analysis (Labco, High Wycombe, United Kingdom). Exetainers contained $240 \mu\text{l}$ (50% w:v) ZnCl_2 solution to stop biological processes (Dalsgaard 2000). Samples were stored in the dark at room temperature, and were kept under water to prevent exchange of gasses with the atmosphere. Isotopic ratios of the produced N_2 were measured by membrane inlet mass spectrometry (MIMS), using an OmniStar Gas Analysis System (Pfeiffer Vacuum, Asslar, Germany), equipped with a quadrupole QMS 200 mass spectrometer with Channeltron detector (Burle Industries), with inlet described by Schrier-Uijl et al. (2011). Additionally, a cryogenic trap ($-196 \text{ }^\circ\text{C}$) was installed before the MS inlet to remove water vapour and other gasses that could interfere with measurement of N_2 gas (Kana et al. 1994;

An et al. 2001). Ratios between measured gasses and argon in air-saturated water were used to standardise isotopic ratios and correct for measurement variability, assuming argon concentrations would remain at saturation level during the measurements. Next, microcosm denitrification rates were calculated from the increase in $^{29}\text{N}_2$ and $^{30}\text{N}_2$ gas concentration over time, measured in the overlying water. The production of unlabelled $^{28}\text{N}_2$ is calculated from the ratio between $^{29}\text{N}_2$ and $^{30}\text{N}_2$ production (Nielsen 1992). The relative contribution of nitrification-denitrification and anammox to the N_2 -production was evaluated from the ratio of the gasses (Risgaard-Petersen et al. 2003). Note that the nitrogen addition needed for this type of denitrification measurements restricts its validity to non-nitrogen limited systems. Additionally, the addition of labelled nitrate as the main nitrogen source for denitrification restricts our conclusions to effects on denitrification, reducing potential temperature effects on coupled nitrification-denitrification.

Temperature dependence of denitrification and SOD was represented by a modified Arrhenius equation:

$$R_T = R_{20} \cdot \theta^{T-20} \quad (6)$$

With R_T and R_{20} representing the SOD or denitrification rate at temperature T and 20 °C, respectively, and θ a dimensionless temperature coefficient (Kadlec and Reddy 2001) which was determined by fitting the model to the experimental data.

Data Analysis

One-way Analysis of Variance with Tukey HSD post-hoc tests were used to test for the effect of temperature on measured variables ($\alpha = 0.05$) after testing for normal distributions. Statistical analyses were performed using PASW Statistics 20 (IBM, New York, NY, USA).

Results

Oxygen Microprofiles

Measured oxygen microprofiles showed similar patterns for most microcosms (Fig. 2). Oxygen concentrations remained more or less constant in the upper part of the profile, indicating a mixed water column. This was followed by a sharp decrease of oxygen across the diffusive boundary layer (DBL) and in the top layer of the sediment, until anoxic conditions were reached. The oxygen penetration depth (Z) varied between 1.4 and 5.0 mm (Table S1), and decreased with increasing temperature,

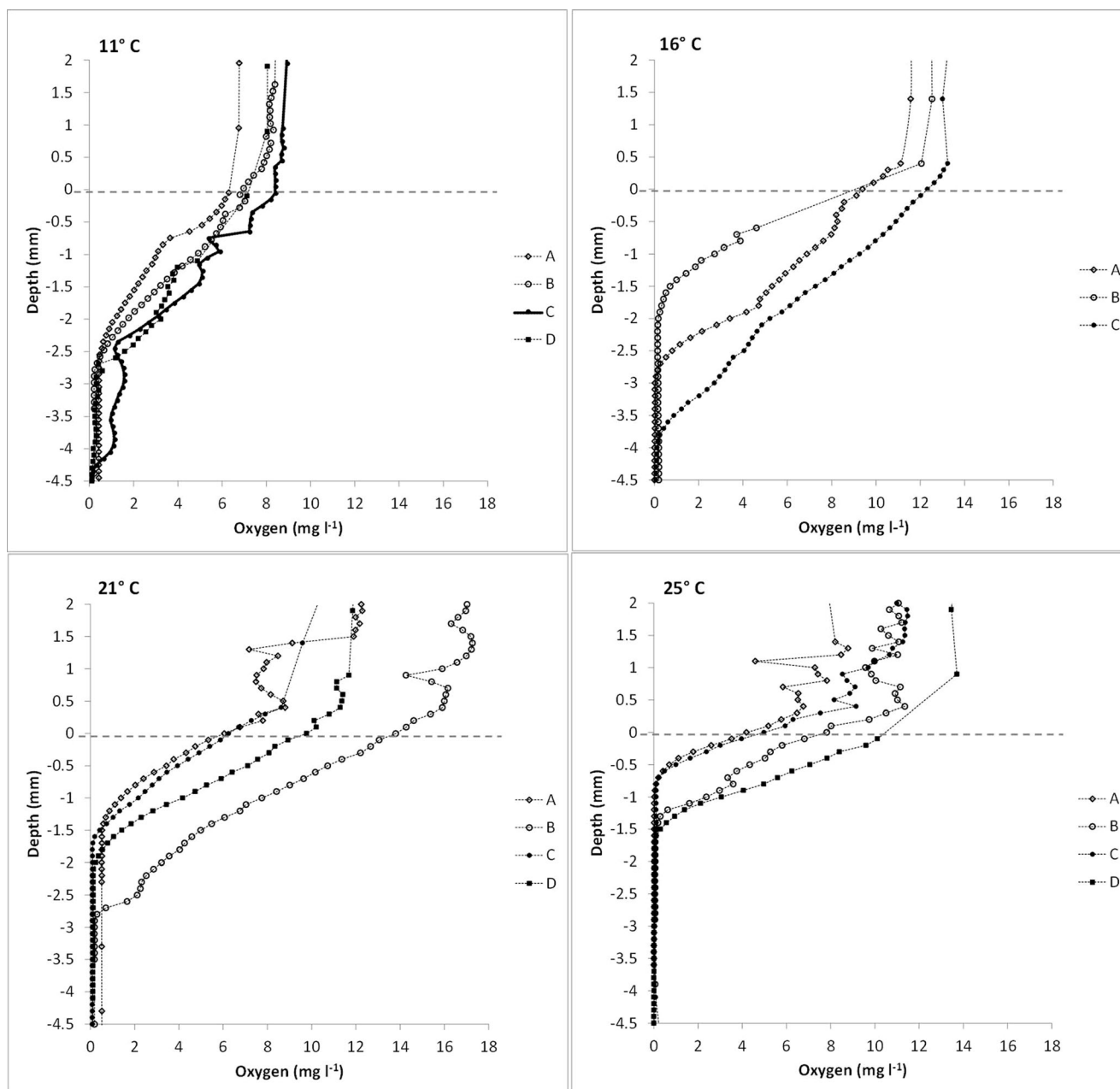


Fig. 2 Oxygen microprofiles measured in the microcosms at different temperatures

ranging from 4.12 ± 0.66 mm at 11 °C, to 2.08 ± 0.39 mm at 25 °C (Fig. 3c). There was a significant difference in Z between 11, 16, 21 °C and 25 °C.

The slopes of the oxygen profiles generally increased with temperature (Fig. 2), resulting in significantly different sediment oxygen demand (SOD) (Fig. 3B). Average SOD ($n = 4$) were 0.37, 0.76, 0.94 and $1.53 \text{ g m}^{-2} \text{ d}^{-1}$ at 11, 16, 21 and 25 °C, respectively. Relating the SOD to the actual temperatures in the microcosms by fitting Eq. 2 resulted in a temperature coefficient θ of 1.09 ($\text{SE} = 0.017$, $R^2 = 0.818$) corresponding with a Q_{10} -value of 2.4.

Denitrification

Apparent denitrification rates ranged from 8.4 to $86 \mu\text{mol N m}^{-2} \text{ h}^{-1}$ (Fig. 3, Table S1). A significant increase in denitrification was found with increasing temperature, ranging from $13.8 \pm 6.4 \mu\text{mol N m}^{-2} \text{ h}^{-1}$ at 11 °C to $62.8 \pm 15.6 \mu\text{mol N m}^{-2} \text{ h}^{-1}$ at 25 °C (Fig. 3A). Fitting Eq. 2 to the denitrification rates and the actual temperatures in the microcosms resulted in a temperature coefficient θ of 1.10 ($\text{SE} = 0.022$, $R^2 = 0.705$). This implies that with one degree warming denitrification rates increased 10%, corresponding to a Q_{10} -value of 2.6. Denitrification rates show a significant positive increase with

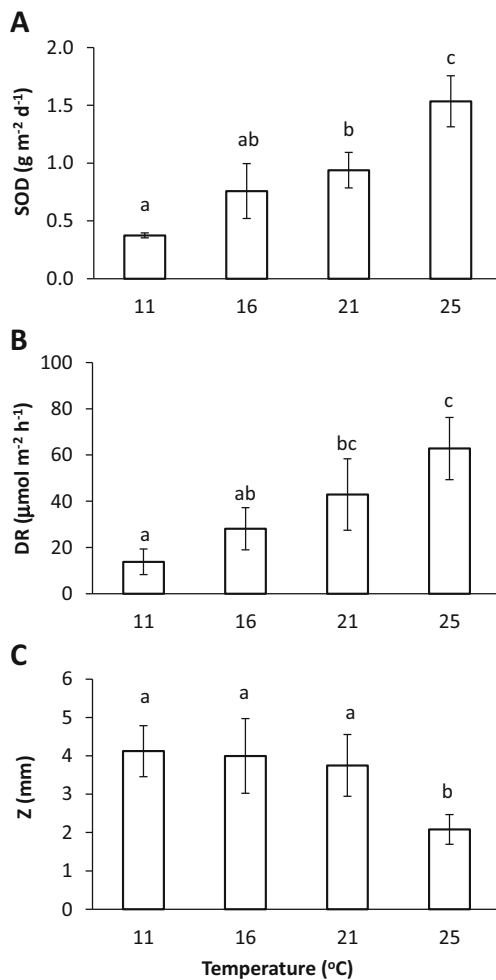


Fig. 3 Oxygen flux into the sediment (SOD, panel A), denitrification rate (DR, panel B) and oxygen penetration depth (Z, panel C) (mean \pm SD, $n = 4$; $n = 3$ for 15 °C). Letters indicate significant differences between treatments ($\alpha = .05$)

rising sediment oxygen demand ($R^2 = 0.70$, $P < 0.001$, Fig. 4a), and significant negative relationship with oxygen penetration depth (Z, $R^2 = 0.60$, $P < 0.01$, Fig. 4b).

When comparing the ratios of the different isotopic N_2 -gasses we found that most of the produced gas was $^{30}N_2$, between 88 and 100%, with a lower share at higher temperatures (fig S1), indicating that most of the N_2 was derived from the added $^{15}NO_3$ and not from nitrification or anammox of $^{14}NH_4/NO_2$. From these results we conclude that the relative contribution of coupled nitrification-denitrification and potentially anammox to total N_2 -production accounted only for 0 to 12%, and increased with warming.

Discussion

In this study we tested the effects of warming on sediment oxygen dynamics (SOD, Z) and denitrification rates in drainage ditch sediments. We found that oxygen penetration depth

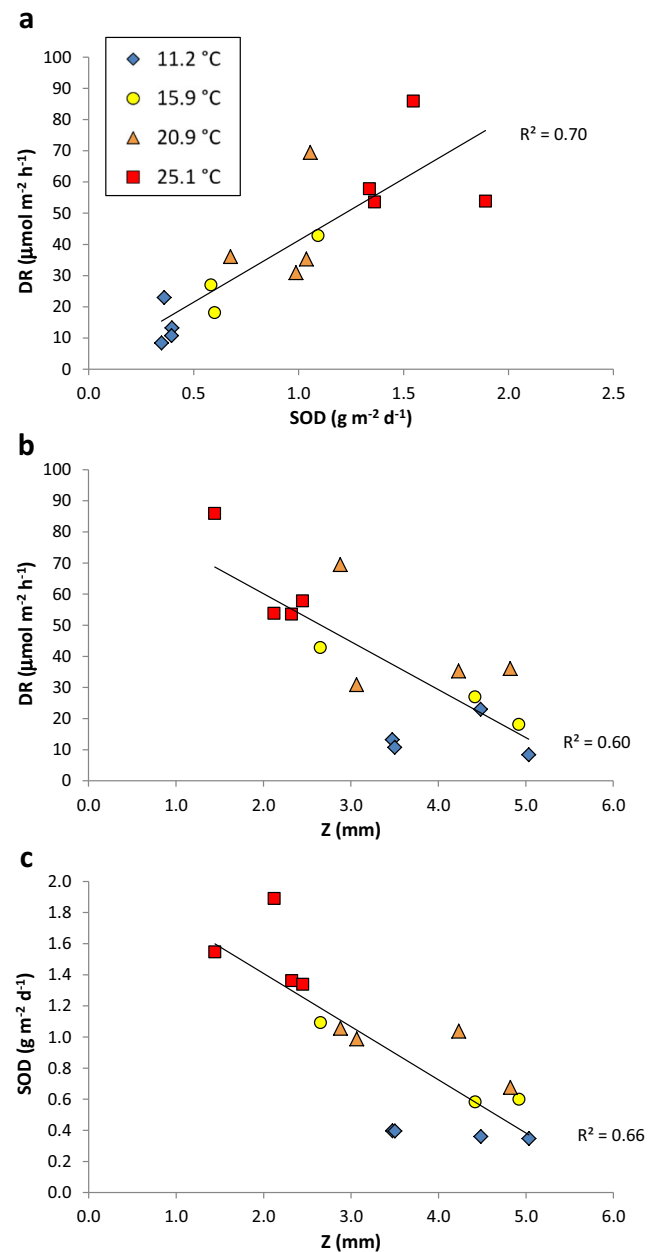


Fig. 4 Relations between sediment oxygen demand (SOD) and denitrification (panel a), oxygen penetration depth (Z) and denitrification (panel b) and oxygen penetration depth and sediment oxygen demand (panel c), each dot represents a unique microcosm, warmer colours indicate higher temperatures (blue = 11, yellow = 16, orange = 21, red = 25 °C)

decreases with warming, related to enhanced SOD, which is in agreement with previous findings in marine sediments (Hancke and Glud 2004), caused by increased respiration activity at higher temperatures (Hancke and Glud 2004; Allen et al. 2005). Oxygen fluxes into the sediment are higher than those reported by other studies using microprofiles (Kim and Kim 2007; Hartnett et al. 2008). This can be explained by the sediments' characteristics and incubation procedure. Our sediments originated from freshwater ditches and contained about

8% organic matter, whereas those tested in previous studies were marine sediments, and contained less than 1% OM, leading to different biogeochemical properties and less oxygen consumption due to organic matter degradation. On the other hand, our oxygen fluxes were lower than reported by Hancke and Glud (2004), which can be explained by their measurements being in the dark with a strong influence of benthic microphyte respiration, whereas our measurements were conducted in light conditions. The microprofiles measured in our study indicate some photosynthesis in the water column, but within the sediment light availability is low, and we did not find evidence of benthic photosynthesis. Temperature dependence of SOD ($Q_{10} = 2.4$) is well in range with reported temperature dependences of degradation and respiration (Allen et al. 2005; Bouletreau et al. 2012).

In lakes and coastal sediments, warming has been found to lead to shifts from phototrophic to heterotrophic communities and overall higher mineralization, resulting in higher CO_2 emission and less carbon burial (Hancke and Glud 2004; Gudasz et al. 2010). The increase of SOD and decrease of Z with warming in our study also point at increased mineralization relative to carbon sequestering photosynthesis, indicating potentially greater CO_2 fluxes from agricultural drainage ditches at higher temperatures. So far, when calculating regional greenhouse gas budgets, these water bodies have hardly been accounted for, although their total surface area within lowland catchment areas such as the Netherlands approximates that of shallow lakes or rivers.

Denitrification

The observed denitrification rates compare well to those previously reported, which are typically in the range of 10 to $500 \mu\text{mol N m}^{-2} \text{h}^{-1}$ (Seitzinger 1988; Christensen et al. 1990; Pina-Ochoa and Alvarez-Cobelas 2006; Mulholland et al. 2008; Veraart et al. 2011). However, comparisons between denitrification studies using different approaches should be treated with caution, for example, our methodology may underestimate rates compared to those using the Isotope Pairing Technique in sediment slurries. Still, we believe the set-up used in this study allows for investigating integrated temperature effects, as oxygen concentration and sediment nitrogen diffusion remained un-manipulated, and were left to respond to temperature as well. Denitrification rates increased with warming; the strength of the temperature response found in this study is lower ($\theta = 1.10$; $Q_{10} = 2.6$) than those observed for other drainage ditches, but comparable to values measured in wetlands (Kadlec and Reddy 2001). The weaker temperature effect indicates that other drivers besides temperature are of importance in controlling denitrification rates. This corroborates the findings of Brin et al. (2015) and Hanke et al. (2015), who both showed that availability of organic carbon

is another key determinant controlling denitrification and its response to warming.

In our experiment nitrate concentrations were set at 1 mg N l^{-1} at the start of the denitrification measurements. Under these conditions nitrate from the water just above the sediment is expected to be the dominant source of nitrate for denitrification (Seitzinger et al. 2006). Interestingly, although NH_4 concentrations were low in all microcosms ($< 0.06 \text{ mg NH}_4\text{-N l}^{-1}$), the results suggest a small contribution of coupled-nitrification and potential for N_2 produced by anammox, only in the 20 and 25 °C microcosms. Ammonium needed for these processes can be derived from mineralization of organic N (Seitzinger et al. 1993; Laursen and Seitzinger 2004), and may indicate higher nitrogen mineralization at higher temperatures.

Microbial populations change with warming. For example, in enrichment bioreactors which contained communities derived from marine sediments, warming resulted in changed denitrification activity and community shifts, from a community where fermenters and organisms capable of nitrous oxide reduction were the dominant organic matter degraders at 10 degrees, to a system dominated by denitrifying populations, performing the complete denitrification pathway, at 25 degrees (Hanke et al. 2015). Thus, Hanke et al. (2015) show that temperature effects on denitrification rates are not only controlled by oxygen, but also by organic carbon, driven by changed interactions between fermenting and denitrifying bacteria. Effects of warming may also depend on the successional stage of microbial communities, as was observed in freshwater biofilms (Rysgaard et al. 1994), in which young biofilms showed increased denitrification rates with warming, whereas denitrification rates in older biofilms decreased. Because our measurements were performed within 48 h of incubation at a certain temperature, our results only reflect short-term temperature effects. They do not capture long-term changes in microbial community composition and associated biogeochemical effects (Wallenstein et al. 2006), but will nonetheless reflect effects acting on fast physical-chemical and enzymatic processes. Thus, there remains a need for long-term studies in ditches and wetlands, to better understand effects of warming and altered N-influx on the interplay of oxygen and nitrogen dynamics, and the underlying changes in microbial community structure and function. Studies on effects of prolonged warming and altered N-influx on greenhouse gas emission will be especially relevant in this respect, to facilitate improvement of regional greenhouse gas budgets.

Conclusions

Temperature strongly affected sediment oxygen dynamics and denitrification rates in drainage ditch sediments. Warming led to higher sediment oxygen demand and shallower oxygen

penetration into the sediment. Denitrification rates increased exponentially with temperature, and were strongly related to both sediment oxygen demand and penetration depth. Our results show how different physical, chemical and microbial processes interact to change oxygen fluxes and denitrification under different warming regimes. Since many other processes in the sediment are regulated by oxygen availability the observed mechanisms are likely to affect other biogeochemical cycles (i.e. phosphorus, iron and sulphur) in a similar manner, which should be taken into account when predicting temperature effects on freshwater ecosystems.

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Author Contributions Conceived and designed the experiments: AV CO JdK. Performed the experiments: AV CO. Analyzed the data: JdK AV CO CJJ. Wrote the paper: JdK AV CO.

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