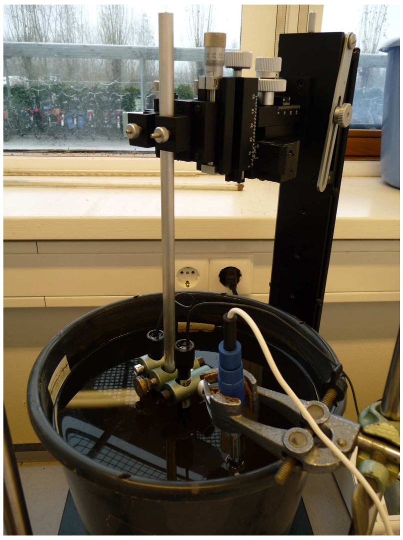

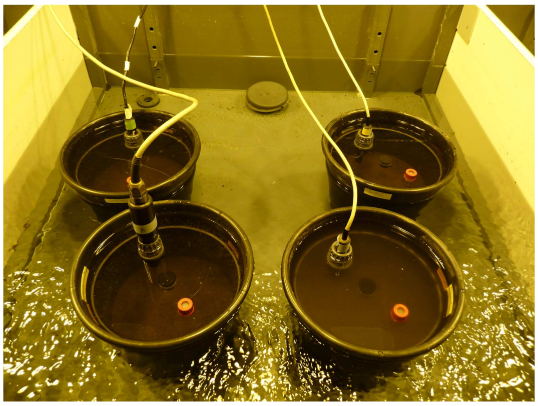

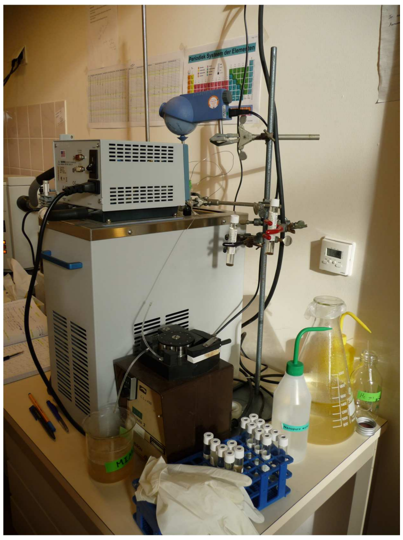

Effect of water temperature and light regime on denitrification rates and associated variables

Ciska Overbeek
Wageningen, June 2011

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Photographs on cover page from top left clockwise: Unisense microsensor, denitrification measurement, membrane inlet mass spectrometer (MIMS), buckets filled with sediment (all photographs in this thesis are of own making)

Abstract

Eutrophication in ditches has increased substantially in the past decades. Denitrification is the main removal process of bioavailable nitrogen in freshwater ecosystems, thereby counteracting eutrophication. Climate change is predicted to result in increased water temperatures. The aim of this study was to investigate the effect of temperature on denitrification rates and associated variables, in order to increase understanding of the processes and interactions involved. Since denitrification is an anoxic process, both dark and light conditions are investigated in this study because of the resulting difference in oxygen dynamics. Methane and hydrogen sulphide can act as electron donors for denitrification, and sediment phosphorus release can be influenced by hydrogen sulphide dynamics and can increase eutrophication. Because those variables might influence the direction and magnitude of the effect of warming on denitrification, they are investigated as well.

Microcosms with ditch sediment were placed in water baths in the laboratory at four different temperatures, measures were taken under both dark and light conditions using a full-factorial design. Sediment oxygen microprofiles were measured with a Unisense microsensor, denitrification rates with the isotope pairing technique (IPT) analysed on a membrane inlet mass spectrometer (MIMS). Sediment methane and hydrogen sulphide release rates were analysed on the MIMS as well. Sediment phosphorus release was determined by comparing water column phosphate concentrations at the start and end of the experiment.

An increase in denitrification rates (DR) with temperature was found (values ranged from 13.8 ± 6.4 and 2.7 ± 2.2 µmolN m^{-2} h^{-1} at 10 °C to 62.8 ± 15.6 and 81.0 ± 15.6 µmolN m^{-2} h^{-1} at 25 °C under light and dark conditions, respectively), comparing well to values found in literature. A higher influence of temperature on DR was found under dark conditions (θ_{dark} = 1.13, θ_{light} = 1.10), probably caused by the stronger temperature effect on respiration than photosynthesis as was also indicated by sediment and water oxygen measurements. Sediment methane release rates increased with warming and were positively correlated to DR (values ranged from -0.007 ± 0.002 and 0.003 ± 0.005 µmolCH_4 m^{-2} h^{-1} at 10 °C and 0.010 ± 0.013 and 0.021 ± 0.004 µmolCH_4 m^{-2} h^{-1} at 25 °C under light and dark conditions, respectively). The positive correlation might indicate use of methane as an electron donor for denitrification, although both variables might also be influenced by the same factor. Sediment hydrogen sulphide release was higher in the light and negatively correlated to DR in the dark (values ranged from -1.82 ± 4.26 and -5.35 ± 6.12 µmolH_2S m^{-2} h^{-1} at 10 °C to -0.85 ± 1.43 and -7.23 ± 2.93 µmolH_2S m^{-2} h^{-1} at 25 °C under light and dark conditions, respectively). A negative correlation with sediment phosphorus release was present in the light. Due to the very complex sulphur cycle it is hard to draw conclusions on the correlations found, it is recommended to measure sulphate in future research to increase understanding of the processes involved. Sediment phosphorus release rates were higher at lower oxygen concentrations in the dark (values ranged from -0.007 ± 0.049 and 0.064 ± 0.087 mgP m^{-2} d^{-1} at 10 °C to 0.043 ± 0.047 and 0.942 ± 1.392 mgP m^{-2} d^{-1} at 25 °C under light and dark conditions, respectively).

This study only considered short term effects of warming, even though climate change will result in long term warming. The short time between relocation of the microcosms to the experimental temperature and measurements may have prevented processes in the sediment from reaching steady state before the start of the measurements. Due to some complex interactions not all influences showed the expected result. Future research on individual processes and their combinations can help increase understanding of the functioning of the system. Nonetheless, the findings of this study provide interesting insights in the short term effects of warming and light on denitrification rates and associated variables, which can help water managers counteract eutrophication.

Keywords: denitrification, ditch, nitrogen, warming, membrane inlet mass spectrometer (MIMS), sediment oxygen microprofile, methane, hydrogen sulphide, sediment phosphorus release

Preface

It took quite some time studying to realise which field of research I really liked. I started off with a BSc Biology with specialisations in Ecology and Theoretical biology, combined this with a minor in Soil and water, and continued with an MSc Climate Studies. I liked all those studies, but when I took a field course in aquatic ecology I realised this was really my kind of thing. It brought together all the aspects that attracted my attention: water systems, ecology, modelling, nutrient cycling, and influence of climate change. Therefore I decided to start a second MSc in Hydrology and Water Quality, with a specialisation in Aquatic ecology and water quality management.

During discussions with Marc Schallenberg, my internship supervisor in New Zealand, my attention was attracted to nitrogen cycling and the processes of denitrification and anammox. During a summer holiday I had already been helping Annelies Veraart with fieldwork on denitrification in lakes, while Jeroen de Klein had been my internship supervisor at WUR. Therefore I contacted them about the possibilities to conduct an MSc thesis on one of those processes. Fortunately, I was welcome to participate in an experiment commissioned by Waternet, a Dutch water board, investigating the effect of dredging in ditches on denitrification rates. Adjacent to this research I could conduct my own experiment in the laboratory, which is presented in this report. The results of the dredging experiment are not included in this report, since they are already presented in a separate one (Veraart *et al.*, 2010). However, the dredging project is part of my MSc thesis.

I really enjoyed working on both projects, even when I was working in the rain, submersing my arms in the cold water for four hours to take denitrification measurements. Sometimes there was machinery malfunctioning, but these setbacks lead to inclusion of other interesting measurements in the research which otherwise would not have been included. Overall I have learned a lot while working on this thesis. I hope I can transfer some of my enthusiasm about denitrification measurements to you while reading this report. I wish you an interesting time reading it.

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1. Introduction

Large parts of the world have open drainage. Almost half of the drained land in the Netherlands, including most of the agricultural land, has open drainage to ditches (1.4 million hectare) (Herzon and Helenius, 2008). Agriculture is a major source of nitrogen (N) and phosphorus (P) for these ditches (Oenema et al., 2005), with a substantial increase in the nitrogen load during the past decades (Vitousek et al., 1997). On a yearly basis, most of the N leaves the system again (Kröger et al., 2007) while part of the P remains (Vermaat and Hellmann, 2010). Denitrification, the microbial conversion of nitrate (NO_3^-) to dinitrogen gas (N_2), is the main removal process of bioavailable nitrogen in freshwater ecosystems (Seitzinger, 1988); it can remove more than half of the nitrogen inputs (de Klein, 2008). Since ditches are at the basis of waters receiving nutrient-rich agricultural input, denitrification in ditches presents a very important ecosystem service diminishing eutrophication of downstream areas.

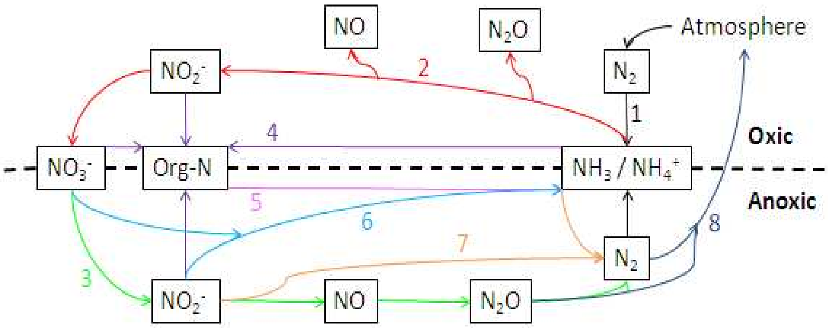

Figure 1: Schematic overview of N-pathways.
1: N-fixation, 2: nitrification, 3: denitrification, 4: assimilation,
5: mineralisation, 6: DNRA, 7: anammox, 8: volatilisation

Apart from denitrification there are several other processes that can play an important role in nitrate removal and N_2-production, e.g. dissimilatory reduction of nitrate to ammonium (DNRA), anaerobic oxidation of ammonium (NH_4^+) with nitrite (NO_2^-) (anammox), and DNRA coupled to anammox (Burgin and Hamilton, 2007, Kartal *et al.*, 2007) (Figure 1). DNRA is expected only to outcompete denitrification under low nitrate and oxygen conditions (van de Leemput et al., 2011), and anammox only occurs at very low rates at the oxic-anoxic boundary (Jetten *et al.*, 1998, Thamdrup and Dalsgaard, 2002). Unlike denitrification DNRA does not remove bioavailable N from the ecosystem. In addition to N_2, denitrification can result in the production of low concentrations of nitrous oxide (N_2O), an important greenhouse gas (Seitzinger *et al.*, 2006, Burgin and Hamilton, 2007).

Denitrification is a complex process which is, among others, influenced by temperature, light availability, presence of nitrate and organic matter, oxygen penetration depth, and the presence of macrophytes (Seitzinger, 1988, Christensen *et al.*, 1990, Bachand and Horne, 2000, Piña-Ochoa and Álvarez-Cobelas, 2006, Veraart *et al.*, 2011a), although the direction of the influence differs among studies. Climate change is projected to lead to an increase in the average global temperature of 1.1 to 6.4 degrees Celsius by the end of the 21st century, depending on the scenario used (IPCC, 2007). Warming decreases the solubility of oxygen in water and increases decomposition of organic matter, resulting in decreased oxygen levels. Furthermore, warming leads to a higher increase in respiration than photosynthesis (Allen et al., 2005), further stimulating the decrease in oxygen concentrations (Veraart *et al.*, 2011b). Since denitrification is an anoxic process it requires oxygen levels below 0.2

$mgO_2\ l^{-1}$ (Seitzinger, 1988, Nielsen *et al.*, 1990). The increase in temperature itself and the consequently decreasing oxygen levels are expected to result in increased denitrification rates (Seitzinger, 1988, Christensen *et al.*, 1990, Bachand and Horne, 2000, de Klein, 2008, Veraart *et al.*, 2011a, Veraart *et al.*, 2011b).

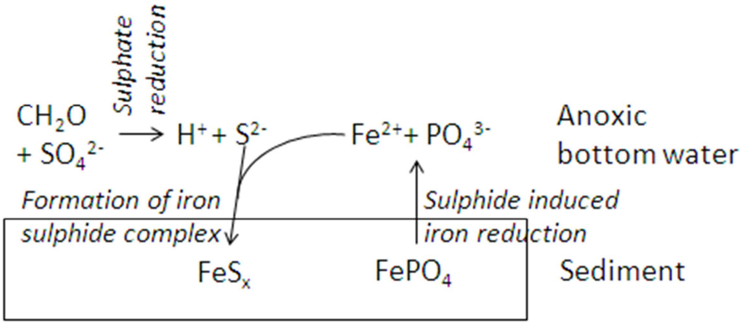

Figure 2: Schematic overview of the effect of S on iron cycling and sediment phosphate release.
Modified from Caraco *et al.* (1993)

Freshwaters that receive agricultural runoff often have sediments that are saturated with methane (CH_4) (Raghoebarsing et al., 2006). When they receive river water they can also be enriched with sulphate (SO_4^{2-}) (Smolders and Roelofs, 1993). In addition to organic matter, methane and hydrogen sulphide can be used as electron donors for denitrification as well (Costa *et al.*, 2000, Raghoebarsing *et al.*, 2006, Burgin and Hamilton, 2007, Haaijer *et al.*, 2007, Burgin and Hamilton, 2008), resulting in the production of carbon dioxide (CO_2) and SO_4^{2-}, respectively. Under anoxic conditions sulphate can also be reduced to hydrogen sulphide (H_2S) (Mulsow *et al.*, 2006, Haaijer *et al.*, 2007), which can dissociate into hydrogen ions and sulphide. Free sulphide inhibits the final two denitrification steps, thereby increasing levels of N_2O emission (Burgin and Hamilton, 2007, Burgin and Hamilton, 2008). Sulphide can be immobilised by binding to metal ions. When all free metal ions are occupied, the ferric iron from iron phosphate complexes will be reduced, resulting in the formation of iron sulphide complexes and the release of phosphate (PO_4^{3-}) from the sediment (Figure 2) (Sperber, 1958, Boström *et al.*, 1988, Caraco *et al.*, 1993, Smolders and Roelofs, 1993, Haaijer *et al.*, 2007, Smolders *et al.*, 2010). Phosphate can also be released from the sediment when iron is reduced under anoxic conditions (Holdren and Armstrong, 1980, Jensen and Andersen, 1992). Phosphorus, together with nitrogen, contributes to eutrophication of ditches.

In this study we aim to investigate the effect of temperature on denitrification rates and associated variables, in order to increase understanding of the processes and interactions involved. Since denitrification is an anoxic process, both dark and light conditions are used in this study because of the resulting difference in oxygen dynamics. Since methane and hydrogen sulphide can act as electron donors for denitrification and hydrogen sulphide can influence sediment phosphorus release rates, the effect of warming and light on those variables is investigated as well. Increased understanding of the processes and interactions involved can help water managers counteract eutrophication.

The study is conducted under laboratory conditions using buckets filled with ditch sediment and overlying water which are placed in water baths. Measurements are done at four different water temperatures, ranging from 10 to 25 °C. Four replicates per treatment are used to be able to account for variability between buckets. Since time between the start of the experiment and measurements is very short, only short term effects of warming are investigated.

2. Materials and methods

2.1. Experimental setup

Buckets (V: 9 l, h: 26 cm, top Ø 24.7 cm, bottom Ø 18.5 cm) were filled with 2 l sieved (mesh size 2 mm), homogenised sediment and 6 l Smart and Barko growth medium (Smart and Barko, 1985), containing 1 mg l^{-1} nitrogen ($NaNO_3$-N) and 0.19 mg l^{-1} phosphorus (K_2HPO_4-P). Phosphorus levels were kept low to prevent excessive algal growth (van Liere *et al.*, 2007). The sediment originated from a ditch in Wageningen (51°59'16.10"N, 5°39'02.80"E) with approximately 8.1 percent organic matter. The microcosms were incubated for seven weeks in a water bath at 17.5 °C, at a 12h/12h light/dark regime. The water in the microcosms was refilled to initial levels on a weekly basis by adding deionised water. At the same time nitrate was resupplied to the buckets, with 10 percent of the initial levels, to ensure adequate nutrient supply for biofilm development. After the incubation period the buckets were placed at four different experimental temperatures (10, 15, 20 and 25 °C). Measurements were taken under both light and dark conditions, using a full-factorial design with four replicates per treatment, resulting in a total number of 32 microcosms.

2.2. Oxygen in the sediment and water column

One day after relocation of the microcosms to the experimental water temperature sediment oxygen microprofiles were taken. Measurements took place after a period of 4-8 hours of light or dark conditions with a Unisense microelectrode using a high-precision micromanipulator (Unisense, Aarhus, Denmark). The profiles were taken outside the water bath while maintaining temperature and light regime, using a one to one-tenth of a millimetre interval, taken over a maximum total depth of 38 mm.

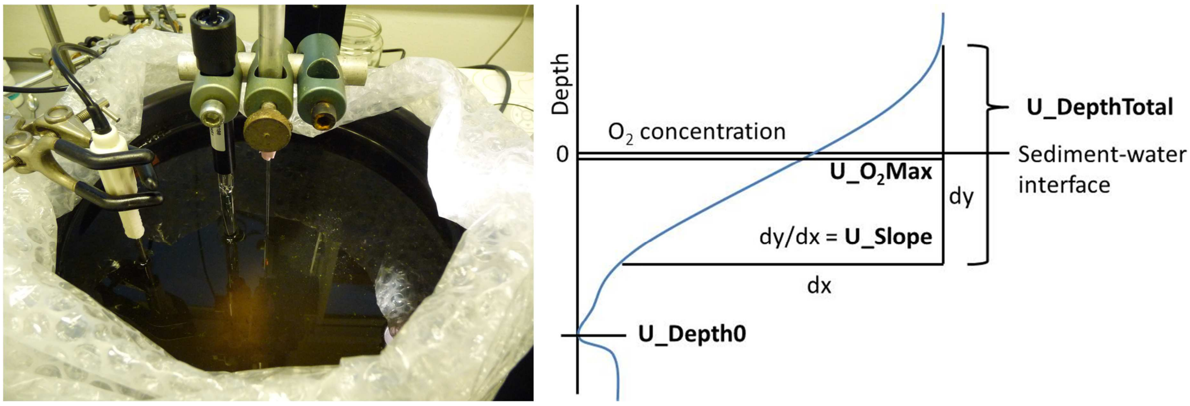

Figure 3: Unisense microsensor (left) and sediment oxygen microprofile (right).
Bold terms indicate the variables derived from the microprofile measurements, with U_Depth0 representing the oxygen penetration depth. For the part with linearly decreasing oxygen concentration with depth U_O_2Max represents the maximum oxygen concentration, U_DepthTotal the total depth, and U_Slope the inverse slope.

All measurements were corrected for instrument drift and characterised with four variables. The depth of the sediment-water interface was determined visually and set to zero. Three variables were determined from the part of the microprofile that tends to show a linear decrease in oxygen concentration with depth: the maximum oxygen concentration (U_O_2Max), total depth (U_DepthTotal) and inverse slope (U_Slope). The oxygen penetration depth (U_Depth0) was determined as the depth at which oxygen concentrations first reach zero (Figure 3).

Dissolved oxygen concentrations in the water column were measured with a HQ multiprobe with a luminescent dissolved oxygen sensor (HACH Company, Loveland, CO, USA) four or five days after measurement of the sediment microprofiles. Dissolved oxygen saturation concentration can be calculated with the formula provided by Weiss (1970):

$$DO = 1.42903 \cdot e^{-177.7888+255.5970\cdot\frac{100}{T}+146.4813\cdot ln\frac{T}{100}-22.204\cdot\frac{T}{100}} \tag{1}$$

With DO the dissolved oxygen concentration (mg l^{-1}) and T being temperature (K).

2.3. Denitrification, methane and hydrogen sulphide

Two days after relocation of the microcosms to the experimental temperature denitrification rates and sediment release rates of methane and hydrogen sulphide were measured in the water column. For these measurements four buckets of one treatment at a time were closed with airtight lids placed under the water surface, containing a septum. The water under the lids was enriched with 6 ml ^{15}N ($NaNO_3$, 6 g l^{-1}) through the septum and gently mixed using an airtight glass syringe. Water samples were taken in quadruplo through the septum 15 minutes and 3 hours after addition of the ^{15}N labelled nitrate. The samples were stored until analysis in 12 ml exetainers (Labco, High Wycombe, United Kingdom), containing 240 µl (50% w:v) $ZnCl_2$ solution to stop biological processes (Dalsgaard *et al.*, 2000). Storing took place under water to prevent exchange of gasses with the atmosphere, at room temperature under dark conditions. Denitrification rates were calculated using the isotope pairing technique (IPT) (Nielsen, 1992) by analysing the production of $^{29}N_2$ and $^{30}N_2$ gas with a membrane inlet mass spectrometer (MIMS) containing a cryogenic trap to remove water vapour and other gasses that could interfere with measurement of N_2 gas (Kana *et al.*, 1994, An *et al.*, 2001). Sediment release rates of CH_4 and H_2S were analysed with the MIMS without using a cryogenic trap. According to mass cracking patterns, it was assumed that a mass of 13 indicated 8 percent of methane and a mass of 34 indicated 100 percent hydrogen sulphide. Ratios between measured gasses and argon were used to correct for noise in the measurements (Eyre *et al.*, 2002), assuming argon concentrations would remain constant during a measurement.

2.4. Sediment phosphorus release

Nutrient samples were taken from the microcosms just before measuring the microprofiles and four or five days later, after which they were frozen until analysis. Samples were analysed for nitrate + nitrite (hereafter nitrate), ammonium, phosphate, total nitrogen and total phosphorus as described in Veraart *et al.* (2011a).

To determine sediment phosphorus release (Rel_PO_4^{3-}, in mg m^{-2} d^{-1}) the following equation was used:

$$Rel\_PO_4^{3-} = \frac{C_e - C_s}{t} \cdot \frac{V}{A} \cdot 1000 \tag{2}$$

With C_e and C_s the end and start concentration (mg l^{-1}), respectively, t the time in days between the two measurements, V the water volume (m^3) and A the sediment area (m^2).

2.5. Data analysis

If necessary, data were transformed ($\log_{10}(|x|+1)\cdot\frac{x+1}{|x+1|}$ or $\text{sqrt}(|x|+1)\cdot\frac{x+1}{|x+1|}$) to obtain normal distribution and homogeneity of variances. A full-factorial univariate General Linear Model (GLM) with a Tukey post-hoc test was used to test for influence of temperature, light, and their interaction on measured variables. When data transformations did not result in normal distribution and homogeneity of variance, a non-parametric Kruskal-Wallis test was performed with Mann-Whitney U as a post-hoc test.

Pearsons product-moment correlation and non-parametric Spearmans rank were calculated to evaluate the correlation between all variables. Even though temperature was a control variable in this study it was not excluded from correlation analysis, since investigation of the correlations with other variables can still provide new insights.

To determine which combination of predictors explained the most variation in denitrification rates, a stepwise multiple linear regression analysis was carried out. Correlated variables could not be simultaneously included in this analysis. Sediment release rates of CH_4, H_2S and PO_4^{3-} were dependent variables in the study and as such were excluded from linear regression. Sediment release rates for NO_3^-, NH_4^+, TN and TP were excluded as well. For each predictor variable that is included in a regression model about ten data points are required (Nunally, 1978). Since the data set used in this study contains only 32 measurements, interactions between predictors were ignored. Differences between corrected Akaike's Information Criterion (ΔAICc) were used to evaluate the models, with the lowest AICc value indicating the preferred model. As a rule of thumb all models with ΔAICc <2 are considered as being almost equal, with ΔAICc between four and seven clearly distinguishable, and with ΔAICc > 10 definitely different (Bolker, 2008).

All statistical analyses were performed using PASW Statistics 17 (IBM, New York, NY, USA).

3. Results

3.1. Oxygen

The dissolved oxygen concentrations (DO) under both dark and light conditions are presented in Figure 4, with the solid line representing dissolved oxygen saturation concentration (Equation 1). Temperature, light and the interaction between the two all had a significant influence on DO levels (Table 1). Dissolved oxygen levels decreased steadily with temperature in the dark, with significantly lower levels at 20 and 25 °C and a negative correlation between DO and temperature (Appendix 1). In the light DO remained equal across the whole temperature range. At 20 and 25 °C DO levels were significantly higher under light than dark conditions.

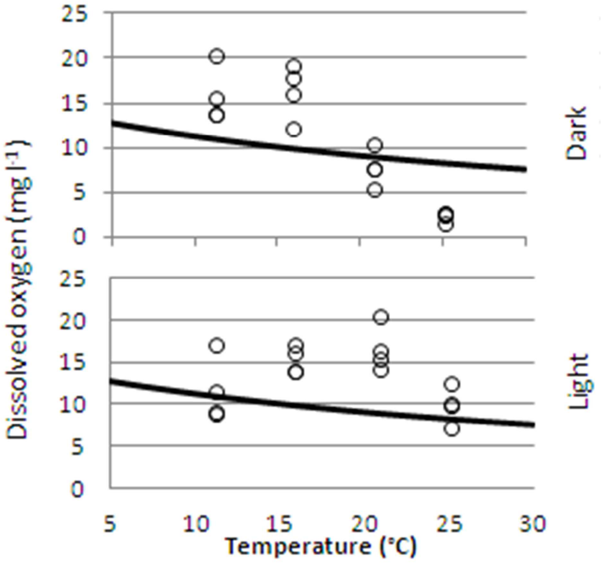

Figure 4: Dissolved oxygen concentrations under both dark and light conditions.
Open circles represent measured values, solid lines represent dissolved oxygen saturation concentration.

Full-factorial univariate GLM analysis indicated a significant effect of temperature, light and the interaction between the two on the maximum oxygen concentration as measured in the part of the sediment oxygen microprofile with linearly decreasing oxygen concentrations with depth (U_O_2Max) (Table 1). Light conditions gave higher values for all temperatures except 10 °C, with an optimum at 20 °C. Under dark conditions a steady decrease with warming was found (Figure 5A). U_O_2Max was positively correlated with DO under both dark and light conditions and had a negative correlation with temperature under dark conditions (Appendix 1 and 2). U_Slope, the change in oxygen concentration with depth for the linearly decreasing part of the microprofile, was also significantly influenced by temperature, light and the interaction between the two (Table 1). For all temperatures except 10 °C U_Slope was higher under light conditions. U_Slope increased with warming in the light while it remained equal across the whole temperature range in the dark (Figure 5B). In the light a positive correlation between U_Slope and temperature was found, while no correlation with DO was found for both light conditions (Appendix 2). Non-parametric test results for U_DepthTotal, the total

Table 1: Results of full-factorial univariate General Linear Model

	Temperature			Light			Interaction		
	df	F	p	df	F	p	df	F	p
Dissolved oxygen	3,24	12.061	**<0.001****	1,24	9.728	**0.005****	3,24	12.428	**<0.001****
U_O_2Max	3,24	2.981	**0.051***	1,24	55.362	**<0.001****	3,24	11.015	**<0.001****
U_Slope	3,24	3.171	**0.043****	1,24	43.925	**<0.001****	3,24	236.114	**<0.001****
U_Depth0	3,24	3.380	**0.035****	1,24	1.058	0.314	3,24	1.451	0.253
Denitrification	3,24	30.830	**<0.001****	1,24	5.133	**0.033****	3,24	7.340	**0.001****
Hydrogen sulphide production	3,24	3.255	**0.039****	1,24	44.091	**<0.001****	3,24	4.550	**0.012****

Bold values indicate significant differences, with ** p-value is significant at the 0.05 value, with * p-value is significant at the 0.10 value.

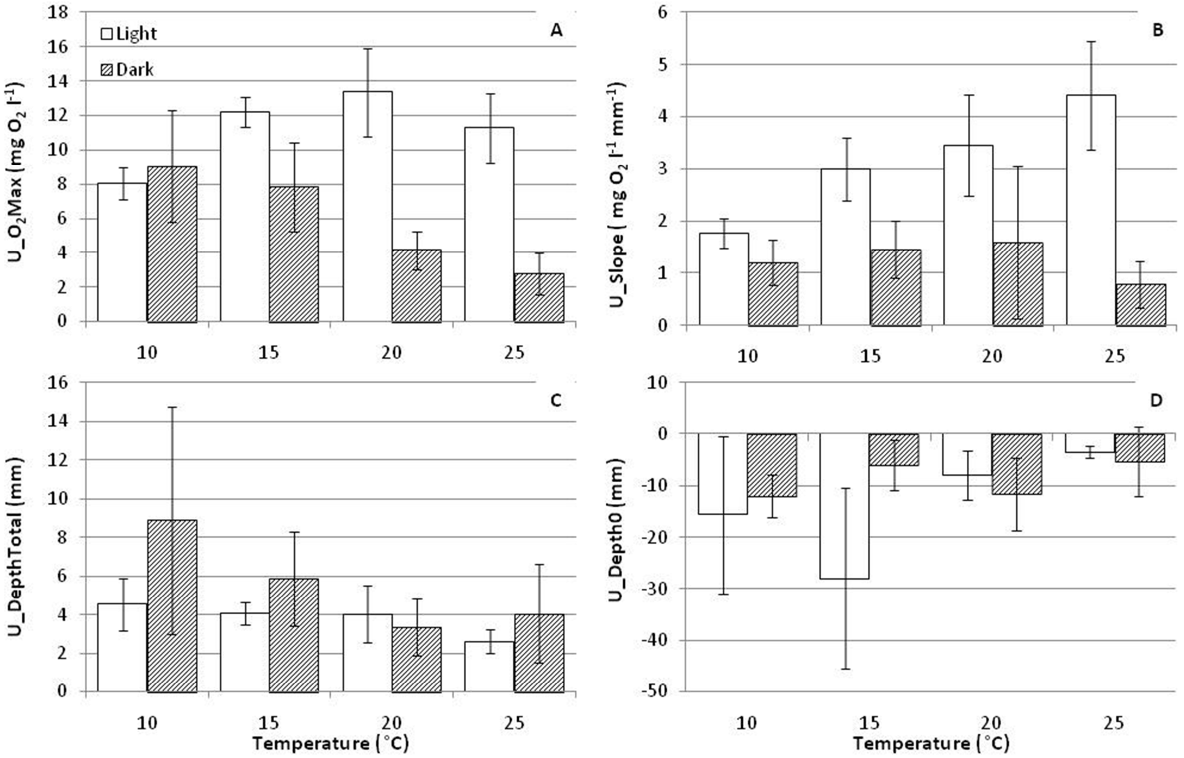

Figure 5: Variables from the sediment oxygen microprofiles: U_O_2Max (A), U_Slope (B), U_DepthTotal (C), and U_Depth0 (D) (mean ± SD, n = 4) under light and dark conditions.

depth of the linearly decreasing part of the microprofile, are presented in Appendix 3. Only under light conditions there was a significant difference between treatments, with 25 °C being lower than the other ones (Figure 5C). Under dark conditions a positive correlation between U_DepthTotal and DO was found, while under light conditions a negative correlation with temperature was found (Appendix 1 and 2). The oxygen penetration depth (U_Depth0) was significantly influenced by temperature (Table 1), although significant differences between treatments were absent. Under light conditions U_Depth0 was positively correlated with temperature (Appendix 2). Values for the oxygen penetration depth ranged from 15.6 ± 15.3 and 12.1 ± 4.2 mm depth at 10 °C to 3.5 ± 1.2 and 5.3 ± 6.6 mm depth at 25 °C under light and dark conditions, respectively (Figure 5D).

3.2. Denitrification

Temperature, light, and the interaction between the two all had a significant effect on denitrification rates (Table 1). Denitrification rates increased with increasing temperatures under both light and dark conditions, with values ranging from 13.8 ± 6.4 and 2.7 ± 2.2 µmolN m^{-2} h^{-1} at 10 °C to 62.8 ± 15.6 and 81.0 ± 15.6 µmolN m^{-2} h^{-1} at 25 °C, respectively (Figure 6A). Under dark conditions a negative correlation with temperature, DO, U_O_2Max and U_DepthTotal was found, while under light conditions a positive correlation with U_Slope and a negative correlation with U_DepthTotal was found (Appendix 1 and 2).

Temperature dependence of denitrification can be represented by a modified Arrhenius equation:

$$DR_T = DR_{20} \cdot \theta^{T-20} \tag{3}$$

With DR_T and DR_{20} the denitrification rate (µmolN m^{-2} h^{-1}) at temperature T and 20 °C, respectively, and θ a dimensionless temperature coefficient (Kadlec and Reddy, 2001). Under dark conditions a linear model fitted the data better than a modified Arrhenius equation (R^2 = 0.541 vs.

0.449, resp., θ = 1.13), while under light conditions there was no difference between the two (R^2 = 0.703 vs. 0.705, resp., θ = 1.10). When θ = 1.13 reaction rates will increase 13 percent with a one degree temperature increase.

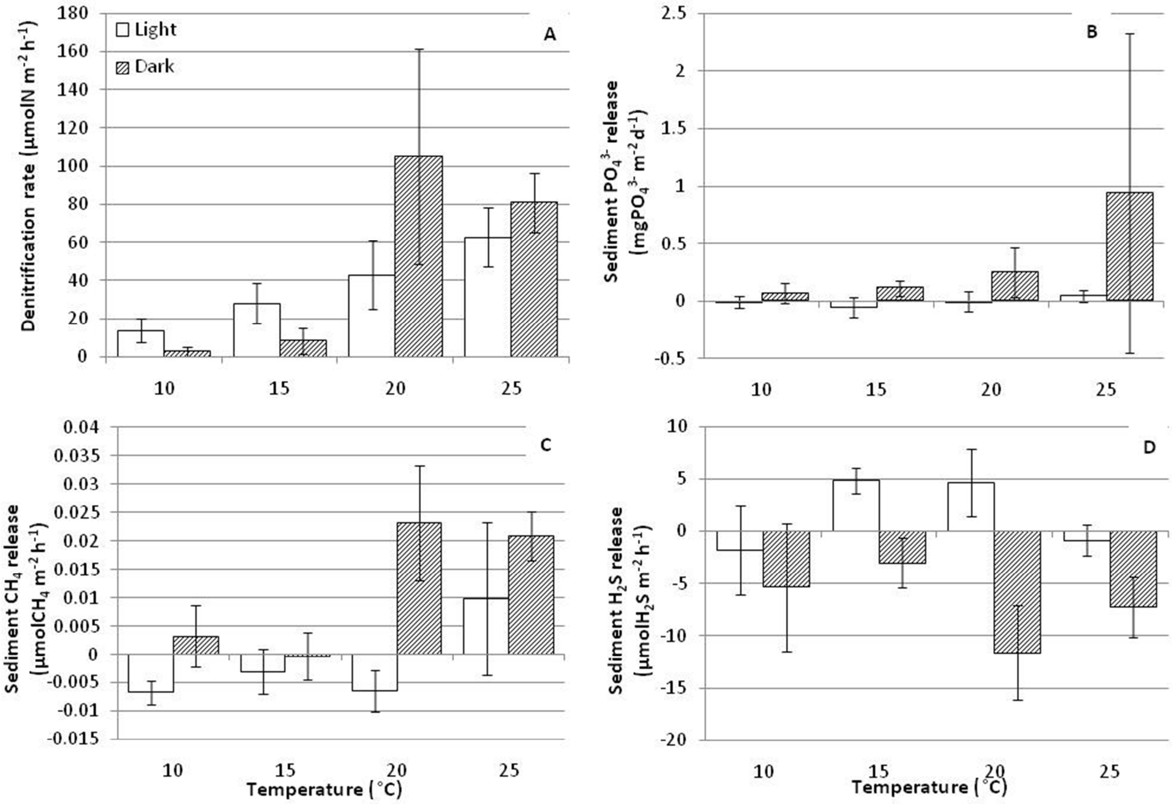

Figure 6: Denitrification rates (A), sediment phosphorus release (B), sediment methane release (C), and sediment hydrogen sulphide release (D) (mean ± SD, n = 4) under light and dark conditions.

3.3. Methane and hydrogen sulphide

Sediment methane release rates tended to be lower in the light (-0.007 ± 0.002 and 0.003 ± 0.005 µmolCH$_4$ m^{-2} h^{-1} at 10 °C and 0.010 ± 0.013 and 0.021 ± 0.004 µmolCH$_4$ m^{-2} h^{-1} at 25 °C under light and dark conditions, respectively), although this was only significant for the treatments at 10 and 20 °C. Increased temperatures tended to show higher release rates for both light regimes (Figure 6C, Appendix 3), with a positive correlation with temperature under dark conditions. Under these conditions a negative correlation with DO and U_O$_2$Max was present, while a positive correlation with DR was present under both light regimes (Appendix 1 and 2).

Temperature, light and the interaction between the two all had a significant effect on sediment hydrogen sulphide release rates (Table 1). Light conditions resulted in release or low values of uptake (-1.82 ± 4.26 and -0.85 ± 1.43 µmolH$_2$S m^{-2} h^{-1} at 10 and 25 °C, respectively), while under dark conditions uptake was more pronounced (-5.35 ± 6.12 and -7.23 ± 2.93 µmolH$_2$S m^{-2} h^{-1} at 10 and 25 °C, respectively) (Figure 6D). Release of hydrogen sulphide from the sediment was positively correlated with DO and negatively with DR in the dark, while in the light a positive correlation with U_O$_2$Max and a negative correlation with sediment PO$_4^{3-}$ was found (Appendix 1 and 2).

3.4. Sediment phosphorus release

Sediment phosphorus release rates were higher under dark than light conditions except for the 10 °C treatment. No significant differences between temperatures were found for both light regimes

(Appendix 1). Values ranged from -0.007 ± 0.049 and 0.064 ± 0.087 mgP m^{-2} d^{-1} at 10 °C to 0.043 ± 0.047 and 0.942 ± 1.392 mgP m^{-2} d^{-1} at 25 °C under light and dark conditions, respectively (Figure 6B).

3.5. Stepwise multiple linear regression analysis

Under dark conditions logarithmically transformed temperature together with oxygen penetration depth was the strongest predictor of denitrification rates, explaining 67 percent of variation in the response variable (Appendix 4). Temperature alone was present in the second best model, explaining 52 percent of the variation, with the two models being almost indistinguishable (ΔAIC_c = 2.6). When separately evaluating all other variables with the Enter-method, none were considered to be good predictors.

Under light conditions logarithmically transformed temperature together with phosphate concentration was the strongest predictor, explaining 84 percent of the variation. The second best model only contained temperature and explained 70 percent variability. However, the best model was clearly distinguishable from the second (ΔAIC_c = 6.3). Separate evaluation of all variables with the Enter-method did not result in any other good predictors.

4. Discussion

In this study the effect of temperature and light on denitrification rates, sediment and water oxygen dynamics, and sediment methane, hydrogen sulphide and phosphorus release rates were investigated. In the following sections the results for each of these variables and the interactions between them are discussed.

4.1. Oxygen

Increased temperatures lead to lower oxygen solubility. Dissolved oxygen concentrations decreased with increasing temperature in the dark, while DO remained equal across the whole temperature range in the light. The maximum oxygen concentration for the part of the microprofile with a linear decrease in oxygen concentrations with depth (U_O_2Max) showed dynamics that resembled dissolved oxygen dynamics. Both results indicate a stronger temperature effect on respiration than photosynthesis, as earlier shown by Allen *et al.* (2005).

The change in oxygen concentration with depth for the linearly decreasing part of the microprofile (U_Slope) showed higher values in the light which increased with warming. The increase with warming further supports the stronger temperature effect on respiration than photosynthesis, because oxygen concentrations will decrease faster with depth when the magnitude of respiration relative to photosynthesis increases, resulting in increased values for U_Slope. Values in the dark remained equal across the whole temperature range and were lower than values measured in the light. We expected to find an increase in U_Slope with warming in the dark, since respiration is expected to increase with temperature, but results contradicted with our expectations. Possibly, decreased oxygen concentrations in the dark prevented respiration rates from reaching their potential values, leading to equal slopes across the whole temperature range. We also expected to find higher values for U_Slope under dark than light conditions because of the absence of photosynthesis; with equal respiration rates the decrease in oxygen with depth will increase. However, results contradicted with the expectations, showing higher values in the light. It might be that respiration rates in the dark were lower than in the light if low oxygen levels prevented potential respiration rates to be reached. When the difference in respiration rates between light and dark conditions is large enough, this can result in higher values for U_Slope in the light. Another explanation is that the total depth of the linearly decreasing part of the microprofile (U_DepthTotal) was higher under dark conditions; lower oxygen concentrations that decrease linearly over a larger depth interval automatically result in lower values for the slope. U_DepthTotal was indeed higher in the dark, although differences were not significant. However, U_DepthTotal was not expected to be higher under dark conditions, as a consequence the reason for this result is not clear. Therefore it is more likely that potential respiration rates were not reached in the dark.

Oxygen penetration depth (U_Depth0) was expected to be higher in the light because of the presence of photosynthesis and higher oxygen levels in the water column, as reported in literature (Christensen *et al.*, 1990, Risgaard-Petersen *et al.*, 1994). However, no significant differences were found between treatments, just like for U_DepthTotal. Probably the same mechanisms are responsible for both results.

The high turbidity of the water column made it difficult to estimate the depth of the sediment-water interface, influencing oxygen penetration depth measurements. Sediment oxygen microprofile measurements were not always stable, in this case indicating instrument instability. Because of this instability, there is some uncertainty in the microprofile variables, although uncertainty is assumed to be small because measured standard deviations were small. A pilot study showed equal ranges of standard deviations measured within and between buckets for all microprofile variables, therefore the sediment is heterogeneous on a microscale even though it was homogenised before addition to the buckets. Sediment heterogeneity can increase variation in microprofile measurements, resulting in increased standard deviations. However, for most of the microprofile measurements standard deviations were small compared to average values.

4.2. Denitrification

Presence of anammox can overestimate denitrification rates as measured by IPT (Risgaard-Petersen *et al.*, 2003), but in this study anammox activity was assumed to be negligible. Underestimation of DR can be caused by the presence of nitrogen fixation (An *et al.*, 2001), oxygen passing freely through the cryogenic trap lowering N_2 values in the MIMS (Eyre *et al.*, 2002), and coupled nitrification-denitrification. In this study nitrogen fixation is assumed to be absent. Coupled nitrification-denitrification can cause underestimation of denitrification rates because the $^{15}NO_3^-$ is required to mix uniformly with endogenous sources of $^{14}NO_3^-$ for the IPT to work properly, which will not happen in places with high rates of nitrification (Nielsen, 1992, Steingruber *et al.*, 2001). In our experiment nitrate concentrations of 1 mg l^{-1} were added at the start of the measurements. Under these conditions nitrate from the water just above the sediment is the dominant source of nitrate for denitrification (Seitzinger *et al.*, 2006), therefore underestimation caused by coupled nitrification-denitrification is assumed to be unlikely.

In our study denitrification rates increased with warming, values found compare well to those presented in literature (Table 2). Values for the Arrhenius temperature coefficient are lower than those presented in literature for other drainage ditches, but comparable to values measured in wetlands (Table 3).

Table 2: Literature values for denitrification rates

Reference	System type	Conditions	DR (µmolN m^{-2} h^{-1})
De Klein (2008)	Drainage ditch	Summer	200 – 350
		Fall	50 – 150
		Dark, unvegetated	85 – 250
		Light, unvegetated	75 – 170
Veraart *et al.* (2011a)	Drainage ditch	Unvegetated	3 – 9
		Vegetated	2 – 28
Veraart *et al.* (2010)	Drainage ditch	Cold (~7 °C)	0 – 9
		Warm (~16 °C)	0 – 120
Veraart *et al.* (2011b)	Microcosms	Vegetated	0 – 320
	Ditch enclosures	Vegetated	10 – 360
Christensen *et al.* (1990)	Nutrient-rich lowland stream	Dark	100 – 1400
		Light	100 – 750
Seitzinger (1988)	Streams and rivers		54 – 345
Mulholland *et al.* (2008)	Streams and rivers		40 – 10000
Piña-Ochoa and Álvarez-Cobelas (2006)	Rivers	Annual	126 – 400
		Highest temperature	0 – 3277
	Lakes	Annual	11 – 264
		Highest temperature	1 – 312
Risgaard_Petersen *et al.* (1994)	Freshwater lake	Dark	48 – 103
		Light	18 – 36

Table 3: Literature values for Arrhenius temperature coefficient

Reference	System type	Conditions	Θ (-)
De Klein (2008)	Drainage ditch	Vegetated	1.28
Veraart *et al.* (2011b)	Microcosms	Vegetated	1.24
Bachand and Horne (2000)	Constructed wetlands		1.15 – 1.22
Kadlec and Reddy (2001)	Waste-water treatment wetlands	10 – 25 °C	1.07 – 1.14
Stanford *et al.* (1975)	Water-logged soil		1.07

Apart from being influenced by temperature, denitrification rates can, among others, be influenced by light availability, presence of nitrate and organic matter, and oxygen levels (Seitzinger, 1988, Christensen *et al.*, 1990, Bachand and Horne, 2000, Piña-Ochoa and Álvarez-Cobelas, 2006, Veraart *et al.*, 2011a).

Denitrification is an anoxic process. With increasing temperature and consequently decreasing oxygen levels the oxygen penetration depth is expected to decrease (Revsbech *et al.*, 1980, Jensen and Andersen, 1992), leading to a decrease in the diffusion path of nitrate to the anoxic sediment layer. This results in a decrease in the relative importance of coupled nitrification-denitrification (Christensen et al., 1990, Jensen et al., 1994, Rysgaard et al., 1994), further decreasing the likelihood for underestimation of DR. Correlations between DR and oxygen variables found in this study indeed show increased denitrification rates with decreasing oxygen levels (Appendix 1 and 2).

Under dark conditions oxygen penetration depth together with temperature is the best predictor for denitrification, confirming the important influence of temperature and oxygen. In the light temperature together with phosphate concentrations is the best predictor, again emphasising the important influence of temperature on denitrification.

According to a meta-analysis performed by Piña-Ochoa and Álvarez-Cobelas (2006), increased nitrate concentrations, as can be caused by eutrophication, can lead to higher denitrification rates. However, the efficiency of denitrification can decline with increasing nitrate concentrations, resulting in a bigger proportion of nitrate remaining in the system (Mulholland et al., 2008). This study did not investigate the effect of varying levels of nitrate on denitrification rates directly, but since temperature changes and associated changes in other variables can influence nitrification rates and therefore coupled nitrification-denitrification, it is important to consider this in future research.

Although no source of easily oxidisable energy was added in the experiments, carbon limitation of microorganisms (Bachand and Horne, 2000) is unlikely because the sediment had a high organic matter content.

4.3. Methane, hydrogen sulphide, and sediment phosphorus release

Comparison of sediment methane release rates with literature shows good agreement with the values found in a dredging experiment in ditches from Veraart *et al.* (2010), who used the same type of analysis, while compared with values for drainage ditches and wetlands as presented in Schrier-Uijl *et al.* (2011) and Kayranli *et al.* (2010), respectively, values are very low.

Methane is expected to be converted to carbon dioxide under oxic conditions, resulting in a negative correlation between sediment CH_4 release rates and dissolved oxygen concentrations, which is indeed found under dark conditions. Results mainly showed sediment CH_4 release under dark conditions and low values of uptake in the light. Since lower oxygen concentrations are found in the dark, the results agree with the expectations.

In addition to organic carbon, methane can act as electron donor for denitrification (Costa *et al.*, 2000, Raghoebarsing *et al.*, 2006, Burgin and Hamilton, 2007). DR is positively correlated to sediment CH_4 release rates under both light regimes, which might indicate that methane is used as an electron donor. However, the correlation does not have to indicate a causal relationship; it might also be that both variables are influenced by the same factors.

Sediment hydrogen sulphide was taken up under dark conditions and both release and uptake were found in the light. Measured values compare well to the ones presented by Veraart *et al.* (2010). As far as we know, this is one of the first experiments in which sediment H_2S release in a drainage ditch was measured on the MIMS. Since this technique has not been validated yet, it is important to interpret the results with caution.

Sulphur dynamics are very complex. Under anoxic conditions several conversions are possible; H_2S can be used as an electron donor for denitrification, resulting in the production of sulphate (Burgin and Hamilton, 2007, Haaijer *et al.*, 2007, Burgin and Hamilton, 2008), sulphate can be reduced to H_2S, and H_2S can be dissociated to hydrogen ions and sulphide, which can form iron sulphide complexes, leading to sulphide induced iron reduction and consequently sediment phosphorus release (Sperber, 1958, Boström *et al.*, 1988, Caraco *et al.*, 1993, Smolders and Roelofs, 1993, Haaijer *et al.*, 2007, Smolders *et al.*, 2010).

In the dark a negative correlation between sediment H_2S release and DR was found. Due to the complex sulphur cycle it is hard to draw conclusions on the correlation between sediment H_2S release and DR, since significant correlations do not have to indicate a causal relationship. In the light a negative correlation between sediment hydrogen sulphide and sediment phosphorus release was found. We expected a positive correlation, because increased sediment H_2S release is expected to lead to increased sulphide levels, which can lead to increased sulphide induced iron reduction. However, the complex sulphur cycle might have caused hydrogen sulphide levels to behave differently. It is recommended to measure sulphate concentrations in future research to increase understanding of the interactions.

Values for sediment phosphorus release compare well with literature (Holdren and Armstrong, 1980, Jensen and Andersen, 1992). Increased release rates are expected under anoxic conditions, especially when the sediment iron content is low or the sediment P content is high (Smolders *et al.*, 2010). No correlation between DO and sediment phosphorus release was present under both light regimes, but sediment total phosphorus release showed a weak positive correlation with U_O_2Max, partly confirming the expectations.

4.4. Short term vs. long term effects

The microcosms were relocated to the experimental temperature only one day before sediment microprofiles and two days before denitrification rates were measured. As a result it is possible that processes in the sediments did not reach steady state yet before being measured, therefore the conclusions from this study are only valid for short term temperature influence. Climate change will result in long term temperature influence; to investigate this effect the time between relocation of the microcosms and measurements needs to be increased. However, with an increase in this time the complexity of the study will also increase, because variability between treatments will increase. For example organic matter content and thus availability of dissolved organic carbon as an electron donor for denitrification may be influenced, and there can be a difference in algae densities or biofilm development. This increased variability leads to higher uncertainty when drawing conclusions, because more processes may interact. Therefore in this study only short term temperature effects were considered. Since this study was conducted in the laboratory and not in the field, it is also important to be cautious when upscaling the results. However, the findings still provide interesting insights in the dynamics and interactions that are present.

5. Conclusion

A schematic overview of all the direct and indirect effects of temperature and light on dissolved oxygen concentrations, sediment oxygen microprofiles, denitrification rates, and sediment methane, hydrogen sulphide, and phosphorus release rates is presented in Figure 7.

In this study dissolved oxygen concentrations were found to decrease with warming under dark conditions, while the oxygen penetration depth did not differ between treatments. Even though other processes may influence the effect of warming on denitrification rates, denitrification will likely increase with warming and consequently decreasing oxygen levels, especially under dark conditions.

Methane and hydrogen sulphide show a positive and negative correlation to DR, respectively. They are both possibly used as electron donors for denitrification. To be able to draw sound conclusions on their role as electron donors, more research and measurement of sulphate concentrations are necessary.

This study did not show a clear influence of sediment hydrogen sulphide release and sediment oxygen levels on sediment phosphorus release, although an increase in phosphorus release rates was found with warming under dark conditions.

Due to some complex interactions not all influences showed the expected result, future research on individual processes and their combinations can help increase understanding of the functioning of the system. Nonetheless, the findings of this study provide interesting insights in the short term effects of warming and light on denitrification rates and associated variables, which can help water managers counteract eutrophication.

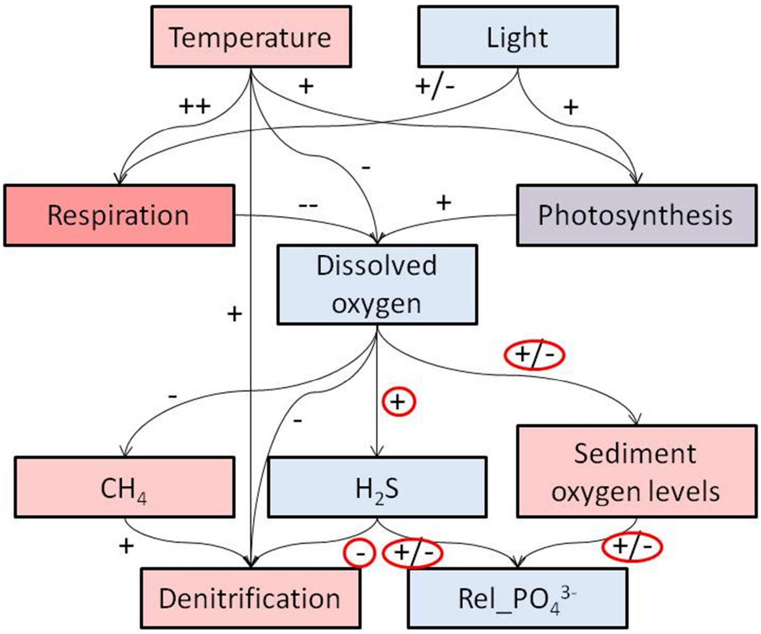

Figure 7: Schematic overview of direct and indirect influences of temperature and light on measured variables.
Arrows show interactions between variables, with + indicating positive influence and – negative influence. Red coloured boxes indicate increase, blue coloured boxes decrease, and purple coloured boxes no change in the value for the variable present within the box. Red circles indicate observed effects that differed from expectations. Modified from Veraart *et al.* (2011b)

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7. References

Allen, A.P., Gillooly, J.F. and Brown, J.H. (2005). Linking the global carbon cycle to individual metabolism. *Functional Ecology,* **19** (2), 202-213.

An, S., Gardner, W.S. and Kana, T. (2001). Simultaneous measurement of denitrification and nitrogen fixation using isotope pairing with membrane inlet mass spectrometry analysis. *Applied Environmental Microbiology,* **67** (3), 1171-1178.

Bachand, P.A.M. and Horne, A.J. (2000). Denitrification in constructed free-water surface wetlands: II. Effects of vegetation and temperature. *Ecological Engineering,* **14**, 17-32.

Bolker, B.M. (2008). *Ecological models and data in R,* Princeton University Press, Princeton and Oxford, United Kingdom.

Boström, B., Andersen, J.M., Fleischer, S. and Jansson, M. (1988). Exchange of phosphorus across the sediment-water interface. *Hydrobiologia,* **170** (1), 229-244.

Burgin, A. and Hamilton, S. (2008). NO_3^- driven SO_4^{2-} production in freshwater ecosystems: implications for N and S cycling. *Ecosystems,* **11** (6), 908-922.

Burgin, A.J. and Hamilton, S.K. (2007). Have we overemphasized the role of denitrification in aquatic ecosystems? A review of nitrate removal pathways. *Frontiers in Ecology and the Environment,* **5** (2), 89-96.

Caraco, N.F., Cole, J.J. and Likens, G.E. (1993). Sulfate control of phosphorus availability in lakes. *Hydrobiologia,* **253** (1), 275-280.

Christensen, P.B., Nielsen, L.P., Sorensen, J. and Revsbech, N.P. (1990). Denitrification in nitrate-rich streams: diurnal and seasonal variation related to benthic oxygen metabolism. *Limnology and Oceanography,* **35** (3), 640-651.

Costa, C., Dijkema, C., Friedrich, M., García-Encina, P., Fernández-Polanco, F. and Stams, A.J.M. (2000). Denitrification with methane as electron donor in oxygen-limited bioreactors. *Applied Microbiology and Biotechnology,* **53** (6), 754-762.

Dalsgaard, T., Nielsen, L.P., Brotas, V., Viaroli, P., Underwood, G.J.C., Nedwell, D.B., Sundbäck, K., Rysgaard, S., Miles, A., Bartoli, M., Dong, L., Thornton, D.C.O., Ottosen, L.D.M., Castaldelli, G. and Risgaard-Petersen, N. (2000). *Protocol handbook for NICE - Nitrogen Cycling in Estuaries: a project under the EU research programme: Marine Science and Technology (MAST III),* National Environmental Research Institute, Silkeborg, Denmark.

de Klein, J.J.M. (2008). *From ditch to delta, nutrient retention in running waters.* PhD thesis, Wageningen University, Wageningen, the Netherlands.

Eyre, B., Rysgaard, S., Dalsgaard, T. and Christensen, P. (2002). Comparison of isotope pairing and N2:Ar methods for measuring sediment denitrification - Assumption, modifications, and implications. *Estuaries and Coasts,* **25** (6), 1077-1087.

Haaijer, S.C.M., Lamers, L.P.M., Smolders, A.J.P., Jetten, M.S.M. and Op den Camp, H.J.M. (2007). Iron sulfide and pyrite as potential electron donors for microbial nitrate reduction in freshwater wetlands. *Geomicrobiology Journal,* **24** (5), 391 - 401.

Herzon, I. and Helenius, J. (2008). Agricultural drainage ditches, their biological importance and functioning. *Biological Conservation,* **141** (5), 1171-1183.

Holdren, G.C. and Armstrong, D.E. (1980). Factors affecting phosphorus release from intact lake sediment cores. *Environmental Science & Technology,* **14** (1), 79-87.

IPCC (2007). Climate change 2007: synthesis report. Contribution of working groups I, II and III of the fourth assessment report of the Intergovernmental Panel on Climate Change. Core Writing Team and R.K. Pachauri and A. Reisinger), p. 104. IPCC, Geneva, Switzerland.

Jensen, H.S. and Andersen, F.Ø. (1992). Importance of temperature, nitrate, and pH for phosphate release from aerobic sediments of four shallow, eutrophic lakes. *Limnological Oceanography,* **37** (3), 577-589.

Jensen, K., Sloth, N.P., Risgaard-Petersen, N., Rysgaard, S. and Revsbech, N.P. (1994). Estimation of nitrification and denitrification from microprofiles of oxygen and nitrate in model sediment systems. *Applied Environmental Microbiology,* **60** (6), 2094-2100.

Jetten, M.S., Strous, M., van de Pas-Schoonen, K.T., Schalk, J., van Dongen, U.G., van de Graaf, A.A., Logemann, S., Muyzer, G., van Loosdrecht, M.C. and Kuenen, J. (1998). The anaerobic oxidation of ammonium. *FEMS Microbiology Reviews,* **22** (5), 421-437.

Kadlec, R.H. and Reddy, K.R. (2001). Temperature effects in treatment wetlands. *Water Environment Research,* **73** (5), 543-557.

Kana, T.M., Darkangelo, C., Hunt, M.D., Oldham, J.B., Bennett, G.E. and Cornwell, J.C. (1994). Membrane inlet mass spectrometer for rapid high-precision determination of N2, O2, and Ar in environmental water samples. *Analytical Chemistry,* **66** (23), 4166-4170.

Kartal, B., Kuypers, M.M.M., Lavik, G., Schalk, J., Op den Camp, H.J.M., Jetten, M.S.M. and Strous, M. (2007). Anammox bacteria disguised as denitrifiers: nitrate reduction to dinitrogen gas via nitrite and ammonium. *Environmental Microbiology,* **9** (3), 635-642.

Kayranli, B., Scholz, M., Mustafa, A. and Hedmark, Å. (2010). Carbon storage and fluxes within freshwater wetlands: a critical review. *Wetlands,* **30** (1), 111-124.

Kröger, R., Holland, M.M., Moore, M.T., Cooper, C.M. and USDA, A. (2007). Hydrological variability and agricultural drainage ditch inorganic nitrogen reduction capacity. *Journal of environmental quality,* **36** (6), 1646-1652.

Mulholland, P.J., Helton, A.M., Poole, G.C., Hall, R.O., Hamilton, S.K., Peterson, B.J., Tank, J.L., Ashkenas, L.R., Cooper, L.W., Dahm, C.N., Dodds, W.K., Findlay, S.E.G., Gregory, S.V., Grimm, N.B., Johnson, S.L., McDowell, W.H., Meyer, J.L., Valett, H.M., Webster, J.R., Arango, C.P., Beaulieu, J.J., Bernot, M.J., Burgin, A.J., Crenshaw, C.L., Johnson, L.T., Niederlehner, B.R., O/'Brien, J.M., Potter, J.D., Sheibley, R.W., Sobota, D.J. and Thomas, S.M. (2008). Stream denitrification across biomes and its response to anthropogenic nitrate loading. *Nature,* **452** (7184), 202-205.

Mulsow, S., Krieger, Y. and Kennedy, R. (2006). Sediment profile imaging (SPI) and micro-electrode technologies in impact assessment studies: Example from two fjords in Southern Chile used for fish farming. *Journal of Marine Systems,* **62** (3-4), 152-163.

Nielsen, L.P., Christensen, P.B., Revsbech, N.P. and Sørensen, J. (1990). Denitrification and oxygen respiration in biofilms studied with a microsensor for nitrous oxide and oxygen. *Microbial Ecology,* **19** (1), 63-72.

Nielsen, L.P. (1992). Denitrification in sediment determined from nitrogen isotope pairing. *FEMS Microbiology Letters,* **86** (4), 357-362.

Nunally, J.C. (1978). *Psychometric theory,* McGraw-Hill, New York, NY, USA.

Oenema, O., van Liere, L. and Schoumans, O. (2005). Effects of lowering nitrogen and phosphorus surpluses in agriculture on the quality of groundwater and surface water in the Netherlands. *Journal of Hydrology,* **304** (1-4), 289-301.

Piña-Ochoa, E. and Álvarez-Cobelas, M. (2006). Denitrification in aquatic environments: A cross-system analysis. *Biogeochemistry,* **81** (1), 111-130.

Raghoebarsing, A.A., Pol, A., van de Pas-Schoonen, K.T., Smolders, A.J.P., Ettwig, K.F., Rijpstra, W.I.C., Schouten, S., Damste, J.S.S., Op den Camp, H.J.M., Jetten, M.S.M. and Strous, M. (2006). A microbial consortium couples anaerobic methane oxidation to denitrification. *Nature,* **440** (7086), 918-921.

Revsbech, N.P., Jørgensen, B.B. and Blackburn, T.H. (1980). Oxygen in the sea bottom measured with a microelectrode. *Science,* **207** (4437), 1355-1356.

Risgaard-Petersen, N., Rysgaard, S., Nielsen, L.P. and Revsbech, N.P. (1994). Diurnal variation of denitrification and nitrification in sediments colonized by benthic microphytes. *Limnology and Oceanography,* **39** (3), 573-579.

Risgaard-Petersen, N., Nielsen, L.P., Rysgaard, S., Dalsgaard, T. and Meyer, R.L. (2003). Application of the isotope pairing technique in sediments where anammox and denitrification coexist. *Limnology and Oceanography: Methods,* **1**, 63-73.

Rysgaard, S., Risgaard-Petersen, N., Sloth, N.P., Jensen, K. and Nielsen, L.P. (1994). Oxygen regulation of nitrification and denitrification in sediments. *Limnology and Oceanography,* **39** (7), 1643-1652.

Schrier-Uijl, A., Veraart, A., Leffelaar, P., Berendse, F. and Veenendaal, E. (2011). Release of CO_2 and CH_4 from lakes and drainage ditches in temperate wetlands. *Biogeochemistry,* **102** (1), 265-279.

Seitzinger, S.P. (1988). Denitrification in freshwater and coastal marine ecosystems: ecological and geochemical significance. *Limnology and Oceanography,* **33** (4), 702-724.

Seitzinger, S.P., Harrison, J.A., Böhlke, J.K., Bouwman, A.F., Lowrance, R., Peterson, B., Tobias, C. and van Drecht, G. (2006). Denitrification across landscapes and waterscapes: a synthesis. *Ecological Applications,* **16** (6), 2064-2090.

Smart, R.M. and Barko, J.W. (1985). Laboratory culture of submersed freshwater macrophytes on natural sediments. *Aquatic Botany,* **21** (3), 251-263.

Smolders, A. and Roelofs, J.G.M. (1993). Sulphate-mediated iron limitation and eutrophication in aquatic ecosystems. *Aquatic Botany,* **46** (3-4), 247-253.

Smolders, A., Lucassen, E., Bobbink, R., Roelofs, J. and Lamers, L. (2010). How nitrate leaching from agricultural lands provokes phosphate eutrophication in groundwater fed wetlands: the sulphur bridge. *Biogeochemistry,* **98** (1), 1-7.

Sperber, J.I. (1958). Release of phosphate from soil minerals by hydrogen sulphide. **181** (4613), 934-934.

Stanford, G., Dzienia, S. and Vander Pol, R.A. (1975). Effect of temperature on denitrification rate in soils. *Soil Science Society of America Journal,* **39** (5), 867-870.

Steingruber, S.M., Friedrich, J., Gachter, R. and Wehrli, B. (2001). Measurement of denitrification in sediments with the 15N isotope pairing technique. *Applied Environmental Microbiology,* **67** (9), 3771-3778.

Thamdrup, B. and Dalsgaard, T. (2002). Production of N2 through anaerobic ammonium oxidation coupled to nitrate reduction in marine sediments. *Applied Environmental Microbiology,* **68** (3), 1312-1318.

van de Leemput, I.A., Veraart, A.J., Dakos, V., de Klein, J.J.M., Strous, M. and Scheffer, M. (2011). Predicting microbial nitrogen pathways from basic principles. *Environmental Microbiology*, no-no.

van Liere, L., Janse, J. and Arts, G. (2007). Setting critical nutrient values for ditches using the eutrophication model PCDitch. *Aquatic Ecology,* **41** (3), 443-449.

Veraart, A.J., Overbeek, C.C., Gillissen, F. and de Klein, J.J.M. (2010). Effect van baggeren op de stikstofhuishouding van sloten. Chair group Aquatic Ecology and Water Quality Management, Centre for Water and Climate, Wageningen University, Wageningen, the Netherlands.

Veraart, A.J., de Bruijne, W.J.J., de Klein, J.J.M., Peeters, E.T.H.M. and Scheffer, M. (2011a). Effects of aquatic vegetation type on denitrification. *Biogeochemistry,* **104** (1-3), 267-274.

Veraart, A.J., de Klein, J.J.M. and Scheffer, M. (2011b). Warming can boost denitrification disproportionately due to altered oxygen dynamics. *PLoS ONE,* **6** (3), e18508.

Vermaat, J. and Hellmann, F. (2010). Covariance in water- and nutrient budgets of Dutch peat polders: what governs nutrient retention? *Biogeochemistry,* **99** (1), 109-126.

Vitousek, P.M., Aber, J.D., Howarth, R.W., Likens, G.E., Matson, P.A., Schindler, D.W., Schlesinger, W.H. and Tilman, D.G. (1997). Human alteration of the global nitrogen cycle: sources and consequences. *Ecological Applications,* **7** (3), 737-750.

Weiss, R.F. (1970). The solubility of nitrogen, oxygen and argon in water and seawater. *Deep Sea Research and Oceanographic Abstracts,* **17** (4), 721-735.

8. Supplementary material

Appendix 1: Correlation matrix for dark conditions

	DO	DR	CH_4	H_2S	U_Slope	U_O_2Max	U_DepthTotal	U_Depth0	NO_3^-	logNH_4^+	logPO_4^{3-}	TN	TP	Rel_NO_3^-	logRel_NH_4^+	Rel_PO_4^{3-}	Rel_TN	Rel_TP
DR	**-.710****																	
CH_4	**-.754****	**.814****																
H_2S	**.515***	**-.681****	**-.607***															
U_Slope	**.099**	**.092**	**-.219**	**-.237**														
U_O_2Max	**.792****	**-.658****	**-.748****	**.355**	**.148**													
U_DepthTotal	**.565***	**-.503***	**-.392**	**.299**	**-.401**	**.742****												
U_Depth0	**-.192**	**-.156**	**-.149**	**.244**	**.143**	**-.253**	**-.172**											
NO_3^-	**.050**	**-.319**	**-.093**	**.206**	**-.115**	**-.201**	**-.171**	**.306**										
logNH_4^+	-.632**	.675**	.618*	-.286	-.418	-.725**	-.250	.404	.450*									
logPO_4^{3-}	-.315	.614*	.461	-.279	.064	-.468	-.533*	.204	.346	.645**								
TN	**.430***	**-.358**	**-.181**	**.444**	**-.477**	**.115**	**.254**	**.123**	**.147**	-.238	-.372*							
TP	**-.242**	**.051**	**.027**	**-.130**	**.067**	**.152**	**.309**	**.455**	**-.229**	.162	.271	**-.261**						
Rel_NO_3^-	**-.057**	**-.288**	**-.074**	**.183**	**-.105**	**-.239**	**-.195**	**.313**	**.993****	.382	-.129	**.059**	**-.294**					
logRel_NH_4^+	**-.913****	**.552***	**.525***	**-.219**	**-.290**	**-.661****	**-.292**	**.372**	**.056**	.975**	.582*	**-.013**	**.182**	**.069**				
Rel_PO_4^{3-}	**-.447**	**.278**	**.151**	**.059**	**.097**	**-.355**	**-.382**	**.468**	**.012**	.518*	.750**	**-.187**	**.494**	**.030**	**.451**			
Rel_TN	**-.490**	**.241**	**.618***	**-.162**	**-.563***	**-.492**	**.006**	**-.123**	**.100**	.314	.179	**.178**	**-.066**	**.144**	**.444**	**-.016**		
Rel_TP	**.415**	**-.748****	**-.716****	**.416**	**.109**	**.506***	**.413**	**.396**	**.238**	-.371	-.446	**.287**	**.173**	**.227**	**-.172**	**.024**	**-.204**	
logTemp	-,719**	.788**	.752**	-.388	-.412	-.837**	-.400	.303	.061	.623**	.444*	-.212	.060	.234	.910**	.470	.461	-.461

Pearson's product-moment correlations (bold data) and non-parametric Spearman rank test showing relationships between all variables (n=16, except for NO_3^-, NH_4^+, PO_4^{3-}, Rel_NO_3^-, Rel_NH_4^+, Rel_PO_4^{3-} where n=15). With ** correlation is significant at the 0.01 level (2-tailed), with * correlation is significant at the 0.05 level (2-tailed). Rel_ indicates sediment release rates.

Appendix 2: Correlation matrix for light conditions

	DO	DR	CH_4	H_2S	U_Slope	U_O$_2$Max	U_DepthTotal	U_Depth0	NO_3^-	logNH_4^+	logPO_4^{3-}	TN	TP	Rel_NO_3^-	logRel_NH_4^+	Rel_PO_4^{3-}	Rel_TN	Rel_TP
DR	**-.144**																	
CH_4	**-.459**	**.544***																
H_2S	**.484**	**-.060**	**-.283**															
U_Slope	**.072**	**.750****	**.297**	**.216**														
U_O$_2$Max	**.574***	**.207**	**.001**	**.655****	**.580***													
U_DepthTotal	**.187**	**-.718****	**-.376**	**.044**	**-.776****	**-.058**												
U_Depth0	**-.446**	**.375**	**.068**	**-.088**	**.250**	**.013**	**-.136**											
NO_3^-	.213	-.379	-.194	-.665**	-.444	-.806**	.031	.137										
logNH_4^+	-.364*	-.026	.021	-.503*	-.103	-.465	-.143	.469	.503**									
logPO_4^{3-}	**.174**	**.341**	**.238**	**.176**	**.467**	**.477**	**-.183**	**.112**	-.169	.084								
TN	**-.125**	**-.179**	**.067**	**-.651****	**-.298**	**-.756****	**-.155**	**-.061**	.551**	.210	**-.427***							
TP	**.234**	**.168**	**.202**	**.063**	**.278**	**.119**	**-.278**	**-.614***	.103	-.189	**.240**	**.162**						
Rel_NO_3^-	**-.665****	**-.304**	**-.101**	**-.616***	**-.465**	**-.822****	**.118**	**.285**	.950**	.612*	**-.581***	**.732****	**-.431**					
logRel_NH_4^+	**-.770****	**.058**	**.477**	**-.414**	**-.115**	**-.406**	**-.188**	**.394**	.635**	.932**	**-.042**	**.447**	**-.181**	**.466**				
Rel_PO_4^{3-}	**-.432**	**.261**	**.265**	**-.633****	**.023**	**-.267**	**-.080**	**.455**	.118	.294	**.416**	**.032**	**-.329**	**.309**	**.447**			
Rel_TN	**-.357**	**-.120**	**-.008**	**-.424**	**-.314**	**-.691****	**-.213**	**-.075**	.488	.297	**-.624****	**.859****	**-.086**	**.590***	**.376**	**-.112**		
Rel_TP	**-.069**	**.488**	**.511***	**.023**	**.407**	**.193**	**-.230**	**-.215**	-.194	-.091	**.239**	**-.232**	**.648****	**-.344**	**-.181**	**-.115**	**-.311**	
logTemp	-.057	.885**	.424	-.073	.812**	.412	-.637**	.622*	-.236	.148	.435*	-.157	.049	-.340	.158	.267	-.049	.473

Pearson's product-moment correlations (bold data) and non-parametric Spearman rank test showing relationships between all variables (n=16). With ** correlation is significant at the 0.01 level (2-tailed), with * correlation is significant at the 0.05 level (2-tailed). Rel_ indicates sediment release rates.

Appendix 3: Non-parametric test results

Variable	Treatment	df	X^2	p	Groups	Z	p (2-tailed)
U_DepthTotal	Dark	3	3.474	0.324	10 °C - 15 °C	-0.577	0.564
					10 °C - 20 °C	-1.597	0.110
					10 °C - 25 °C	-1.155	0.248
					15 °C - 20 °C	-1.155	0.248
					15 °C - 25 °C	-0.866	0.386
					20 °C - 25 °C	-0.577	0.564
	Light	3	7.008	**0.072***	10 °C - 15 °C	-0.289	0.773
					10 °C - 20 °C	-0.866	0.386
					10 °C - 25 °C	-2.191	**0.028****
					15 °C - 20 °C	-0.866	0.386
					15 °C - 25 °C	2.033	**0.042****
					20 °C - 25 °C	-1.742	**0.081***
	10 °C				Light-dark	-0.866	0.386
	15 °C				Light-dark	-0.577	0.564
	20 °C				Light-dark	0.000	1.000
	25 °C				Light-dark	-0.296	0.767
Methane production	Dark	3	11.515	**0.009****	10 °C - 15 °C	-0.866	0.386
					10 °C - 20 °C	-2.309	**0.021****
					10 °C - 25 °C	-2.309	**0.021****
					15 °C - 20 °C	-2.309	**0.021****
					15 °C - 25 °C	-2.309	**0.021****
					20 °C - 25 °C	-0.289	0.773
	Light	3	5.801	0.122	10 °C - 15 °C	-1.443	0.149
					10 °C - 20 °C	0.000	1.000
					10 °C - 25 °C	-1.732	**0.083***
					15 °C - 20 °C	-1.155	0.248
					15 °C - 25 °C	-1.443	0.149
					20 °C - 25 °C	-1.732	**0.083***
	10 °C				Light-dark	-2.309	**0.021****
	15 °C				Light-dark	-0.866	0.386
	20 °C				Light-dark	-2.309	**0.021****
	25 °C				Light-dark	-1.155	0.248
Sediment PO_4^{3-} release	Dark	3	3.113	0.375	10 °C - 15 °C	-0.707	0.480
					10 °C - 20 °C	-1.061	0.289
					10 °C - 25 °C	-1.414	0.157
					15 °C - 20 °C	-0.577	0.564
					15 °C - 25 °C	-1.155	0.248
					20 °C - 25 °C	-0.866	0.386
	Light	3	2.029	0.566	10 °C - 15 °C	-0.866	0.386
					10 °C - 20 °C	-0.577	0.564
					10 °C - 25 °C	-0.866	0.386
					15 °C - 20 °C	-0.577	0.564
					15 °C - 25 °C	-1.443	0.149
					20 °C - 25 °C	0.000	1.000

(Appendix 1 continued)

Variable	Treatment	df	X^2	p	Groups	Z	p (2-tailed)
Sediment PO_4^{3-} release	10 °C				Light-dark	-1.061	0.289
	15 °C				Light-dark	-2.021	**0.043****
	20 °C				Light-dark	-1.732	**0.083***
	25 °C				Light-dark	-2.021	**0.043****

Bold values indicate significant differences, with ** p-value is significant at the 0.05 value, with * p-value is significant at the 0.10 value.

Appendix 4: Stepwise multiple linear regression on denitrification rates

Conditions	Predictor	n	R^2	ΔAIC_c	Intercept		Slope	
					value	p	value	p
Dark	logTemp	16	0.519	2.632	-347.352	**0.006****	312.348	**0.002****
	logTemp &	16	0.672	0	-446.654	**0.001****	366.146	**<0.001****
	U_Depth0						-3.457	**0.036****
Light	logTemp	16	0.701	6.311	-147.904	**0.001****	146.417	**<0.001****
	logTemp &	15	0.842	0	-206.462	**<0.001****	214.890	**<0.001****
	PO_4^{3-}						-5267.139	**0.007****

Bold values indicate significant differences, with ** p-value is significant at the 0.05 value, with * p-value is significant at the 0.10 value.