Mitigating agricultural nitrogen load with constructed ponds in northern latitudes: A field study on sedimental denitrification rates

Uusheimo, Sari[1]
sari.uusheimo@helsinki.fi
Phone: +358 50 4620555

Tulonen, Tiina[1] tiina.tulonen@helsinki.fi
Aalto, Sanni L.[2] sanni.aalto@uef.fi
Arvola, Lauri[1] lauri.arvola@helsinki.fi

[1]Lammi Biological Station, University of Helsinki, Pääjärventie 320, 16900 Lammi, Finland
[2]Department of Environmental and Biological Sciences, University of Eastern Finland, P.O. Box 1627, 70211 Kuopio, Finland

Abstract

Constructed agricultural ponds and wetlands can reduce nitrogen loading from agriculture especially in areas where warm climate predominates. However, in cold climate temperature-dependency of microbiological processes have raised the question about the applicability of constructed wetlands in N removal. We measured *in situ* denitrification rates in a constructed agricultural pond using ^{15}N-isotope pairing technique at ambient light and temperature throughout a year as well as diurnally. The field IPT measurements were combined with a wide set of potentially important explanatory data, including air temperature, photosynthetically active radiation, precipitation, discharge, nitrate plus other water quality variables, sediment temperature, oxygen concentration and penetration depth, diffusive oxygen uptake and sediment organic carbon. Denitrification varied, on average, diurnally between 12 and 314 μmol N m^{-2} h^{-1} and seasonally between 0 and 12409 μmol N m^{-2} d^{-1}. Light and oxygen regulated the diel variation of denitrification, but seasonally denitrification was governed by a combination of temperature, oxygen and turbidity. The results indicated that the real N removal rate might be 30–35 % higher than the measured daytime rates, suggesting that neglecting the diel variation of denitrification we may underestimate N removal capacity of shallow sediments. We conclude, that by following recommended wetland:catchment -size ratios, boreal agricultural ponds can efficiently remove nitrogen by denitrification in summer and in autumn, while in winter and in spring the contribution of denitrification might be negligible relative to the loading, especially with short residence time.

Keywords

Denitrification, agriculture, *in situ*, sediment, nitrate, boreal

1. **Introduction**

Humans have transgressed the planetary boundaries in the fixation of N_2 (Rockström et al. 2009) by doubling the global amount of reactive nitrogen (N_r) (Fowler et al. 2013; Gruber and Galloway, 2008; Sutton et al. 2011). In Europe, the amount of Nr has been tripled and it is estimated that 40–70% of the fertilizer Nr applied for cereal production is lost to the environment (Sutton et al. 2011). Excess N leaching in receiving waterways has resulted in eutrophication and reduced water quality for drinking, agricultural, recreational, and other purposes (Galloway et al. 2013; Robertson and Vitousek, 2009). Only about half of the European surface waters met the Water Framework Directive (WFD) objective of good ecological status in 2015 and 25% of the ground waters investigated suffered from excess nitrate-N (NO_3^-) mainly caused by agriculture (EEA, 2015). Moreover, at the same time with the increased amount of Nr, the globe has lost more than half of its natural wetlands (Davidson 2014).

Constructed wetlands (CWs) have been successfully applied to remove excess nitrogen (N) from agricultural runoffs (e.g. O´Green et al. 2010; Strand and Weissner, 2013; Vymazal 2017). CWs have been shown to remove N in warm climates, but whether they work in cold conditions, has still been called into question (Arheimer and Pers, 2017; Wang et al. 2018). In Finland, some non-remunerative investments, e.g. agricultural CWs, are economically supported by the EU and national legislation, and over a thousand wetlands have been built since 1995. Furthermore, the number of smaller pond systems, e.g. individual or chains of sedimentation ponds is likely to be even greater. Agricultural wetlands and ponds are often reasonably non-vegetated, due to highly turbid agricultural waters (e.g. Tikkanen et al. 1985) limiting light penetration, and routine management practices like mechanical excavation. Boreal sedimentation ponds are considered to exhibit insignificant and/or highly variable N–retention, being more efficient in removing solids and phosphorus (P) (e.g. Häikiö 1998; Vuollekoski et al. 2015). However, study reports from Sweden conclude that CWs can have a high N removal, although it varies considerably (Strand and Weissner, 2013). Whether the reports of low N–retention results from insufficient retention time, or negligible N removal processes in cold climate, is poorly understood.

Three natural processes contribute to overall N retention in freshwater wetlands: denitrification, sedimentation, and assimilation by aquatic biota. However, denitrification is the only pathway that removes N entirely from the aquatic ecosystems. Denitrification is an anaerobic microbial process, where nitrate nitrogen (NO_3^-) is reduced into gaseous form, either into nitrous oxide (N_2O) or nitrogen gas (N_2) (Mitsch and Gosselink 2015). These gases are transferred into the atmosphere, balancing the natural and anthropogenic N input. Denitrifiers utilize organic carbon (C) (heterotrophic denitrification) or reduced inorganic compounds (e.g. sulfides; autotrophic denitrification) as energy sources. Autotrophic denitrification is typical to marine environments (Shao et al. 2010), while heterotrophic denitrification is considered to be the dominating process in freshwater ecosystems (Mullholland et al. 2008).

Denitrification can be based on the NO_3^- from the water above the sediment (D_w), and/or from the coupled nitrification-denitrification process (D_n), occurring in the oxic layers of the upper sediment. In shallow sites with benthic primary production, higher sediment oxygen concentration followed by increased photosynthetically active radiation (PAR) can promote D_n (An and Joye, 2001; Lorenzen et al. 1998; Risgaard-Petersen et al. 1994), while D_w can be lower during light hours (Christensen et al. 1990; Risgaard-Petersen et al. 1994). In boreal environments, where PAR amount changes significantly between seasons (Lakkala et al. 2016), light-induced changes in the N removal of shallow wetlands may be highly important. Besides changes in PAR and accompanying oxygen conditions, temperature has been found to govern denitrification activity, explaining variable denitrification rates in boreal lakes (Holmroos et al. 2012; Rissanen et al. 2011), temperate wetlands (Bastviken et al. 2007; Hernandez and Mitch, 2007) and temperate stream sediments (de Klein 2008; Veraart et al. 2014).

Current estimates of denitrification rates in agricultural wetland and stream sediments (e.g. Castaldelli et al. 2015; Pinardi et al. 2009; Roach and Grimm 2011) are based on laboratory incubations conducted in dark at constant temperature. Because of this the results do not necessarily reflect the real, *in situ* denitrification rates. Moreover, sampling has been targeted only on certain seasons like summer and on daytime. This paper reports *in situ* denitrification rates at ambient light and temperature conditions in a constructed agricultural pond of northern Europe, Finland. The field measurements were performed throughout the year, as well as diurnally. Using the direct ^{15}N-isotope pairing technique (IPT, Nielsen 1992) simultaneously with sediment oxygen profiling, allowed us to study the role of different environmental factors controlling denitrification rates at different temporal and spatial scales. We expected ambient light and temperature regime being important drivers of denitrification on annual basis in boreal agricultural ponds, which typically have high nutrient concentrations throughout the year. Furthermore, we hypothesized that denitrification rates may also show similar variation diurnally. On the basis of diel results, we recalculated the measured seasonal N removal in the sediment of a boreal agricultural pond.

2. **Materials and Methods**

2.1 The catchment and the study site

The study was conducted in an agricultural watershed in southern Finland (61°04´97’’N, 25°02´89´´E) (Fig. 1). Koiransuolenoja is an approximately 4 km long brook flowing through typical agricultural catchment (6.8 km^2) into Lake Pääjärvi. The brook is approximately 1–2 m wide with an average depth less than 0.5 m. The stream is heavily impacted by farming, as agricultural land covers up to 24% of the drainage area (Arvola et al. 2015). Nearly half of the catchment surface area soil is easily erodible material e.g. sand and silt (Tikkanen et al. 1985). During the study period (July 10th 2014–June 25th 2015), the average NO_x-N (indicating the NO_2^- plus NO_3^-) concentration in the brook was 194 µmol l^{-1} (SD ±58 µmol l^{-1}, n=48).

An agricultural sedimentation pond had been built one year earlier in March 2013. Aquatic vegetation had not yet developed into the littoral, and no shading was provided by trees or shrubs. Surface area of the pond was 320 m^2 and volume 226 m^3 (mean depth 0.7 m., max. depth 1.6 m). Average discharge in Koiransuolenoja was 0.058 m^3 s^{-1} resulting in theoretical residence time approximately one hour in the pond. The real residence time varied from 15 minutes to 4.5 hours depending on the discharge. Water flow in different parts of the pond was measured with a flow meter (MiniAir2, Schiltknecht) and the flow rate in the pond littoral was 0 m s^{-1} on each experiment date throughout the study. We investigated the grain size of the homogenized and dried (48h, 60 °C) top sediment (0–3 cm) with a vibratory sieve shaker (Analysette 3, Fritsch, Germany). The shallow littoral where denitrification was measured (Fig. 1) consisted of accumulation sediment having the highest amounts (91%) of fine materials, fine sand (grain size 0.063–0.125 mm) and silt (0.002–0.062 mm). In the deepest part of the pond, 64% of the top sediment was fine materials. Two species of non-nitrogen-fixing benthic algae *Spirogyra* and *Planktothrix* sp. was almost every time observed on the sediment surface.

2.2 Sampling, field and laboratory analyses

Sediment samples and water NO_x-N samples for denitrification measurements were taken manually from the littoral zone of the constructed pond (Fig. 1). Quality of the stream water (turbidity, dissolved organic carbon (DOC), NO_x-N, ammonium (NH_4^+-N) and total N (TN)) was investigated weekly from water samples taken at site K1 (Fig. 1), representing the water quality at catchment scale. Discharge was calculated from the discharge curve based on the measured water level and flow rate. Data for air temperature and precipitation measured at Lammi biological Station (Fig. 1), 800 m NE from the study site, were obtained from The Finnish Meteorological Institute. Photosynthetically active radiation (PAR) was measured with a quantum sensor (PQS1, Kipp & Zonen) at 10-min intervals, located 600 m NE from the study site (Fig. 1). In laboratory, NO_x-N, NH_4^+-N, and TN concentrations were analyzed using standard laboratory methods (see Arvola et al. 2015). Samples for DOC were filtered through pre-rinsed cellulose ester filters (pore size 0.45 μm, Millex-HA, Merck Millipore) and analyzed using a carbon analyzer (Ordior TOC-V, Shimadzu). Organic C content of the study littoral sediment (0–3 cm) was calculated from the loss on ignition (LOI%) of oven dried material (550 °C, 2 h). Temperature was measured from the top of the sediment using the flow meter. Oxygen (O_2) penetration depth in the sediment (OPD) and O_2 concentration on top of the sediment were measured with clark-type microelectrode (tip Ø 100 μm) in the laboratory from three replicate intact sediment-water cores within one hour of sampling (OX100-sensor, PA-2000, Unisense). Sediment diffusive O_2 uptake (DOU) was calculated from the flux through the diffusive boundary layer above the sediment from the oxygen profiles (Jørgensen and Revsbech, 1985; Revsbech et al. 1980).

2.3 Sediment core incubations and isotope analysis for denitrification

Before the field incubations, the validity of the method at the study site (independency of denitrification of ambient NO_x-N and positive dependency of denitrification of $^{15}N^-$labeled nitrate on the added tracer amount) and the possible presence of anammox (Nielsen 1992, Risgaard-Petersen et al. 2003), was investigated in a pre-experiment with a concentration series in the laboratory using $^{15}N^-$labeled potassium nitrate (50, 100, 200, 400, and 600 µmol of $K^{15}NO_3$, 98 atom %, Sigma-Aldrich).

Denitrification experiments were performed at the pond littoral at water depth of 30–40 cm. Sediment cores were incubated *in situ* under ambient light and temperature conditions, using ^{15}N-isotope pairing technique (IPT) by Nielsen (1992). Diel denitrification rates were measured twice, on the 28th and 25th of August in 2014 and 2015, respectively. Three undisturbed replicate sediment samples were collected in transparent plastic cores (height 16 cm, diam. 2.6 cm) so that each core contained 1/3 of sediment and 2/3 water. Both 24 h denitrification experiments were divided into eight 3-hour incubation periods. Seasonal denitrification was measured during daytime, between 10:00 and 14:00, 24 times between July 7th 2014 and June 25th 2015 (except not in January and February 2015) using three replicate sediment cores (height 34.2 cm, diam. 4.1 cm). For each 3 h period in the 24 h experiments and in the seasonal experiment, OPD and O_2 concentration on the top of the sediment was measured with the microprofiler, and DOU was calculated. Since we were not able to use stirring during the field incubations, there might have been some oxygen stratification, decreasing OPD and affecting the ratio between D_w and D_n. The average NO_x-N in the pond littoral water was based on two samples during each 24 h experiment, due to the limited accuracy of the NO_x-N analysis (±10 %

in concentrations >21 µmol l^{-1}). To test the immediate effect of PAR on daytime denitrification, three replicate all-covered, dark cores were incubated simultaneously on two occasions in the seasonal study: May 13th under high turbidity (36.3 ftu) and May 26th under low turbidity (7.0 ftu).

Based on the pre-experiment in the laboratory, concentration of 200 µmol $K^{15}NO_3$ was used in labeling in the field experiments. After labeling, the water column was gently mixed with a glass rod to ensure complete mixing of the label to the water phase, not disturbing the sediment. The three labeled cores were sealed tightly, pushed back into the original sediment depth, and incubated for 3 h. To avoid changes in sediment oxygen conditions, metal foil cover was adjusted to the height of the sediment surface to prevent the deeper layers of the sediment being exposed to sunlight during labeling in the seasonal study. One unlabeled core was always used as a control for ambient N_2 concentration. After the incubation, the sediment and water were mixed into a slurry in the core and let settle for five minutes, before transferring a 12-ml subsample with a syringe and a gas-tight Tygon-tube into a glass vial (Exetainer 12 ml 738W, Labco Limited). One hundred µl of formaldehyde solution (37 wt%, Sigma-Aldrich) was added to terminate all microbial activity. Subsamples were stored upside down (+4 °C, in dark) until N isotope analysis. A helium headspace was added to each sample before the isotope analysis, following Tiirola et al. (2011). The isotope mass-area -ratios (*m/z* 28, 29, and 30) and N_2 concentration of the samples were analyzed with Isoprime IRMS connected to a Tracegas preconcentrator unit, using a modified N_2O project with no cryotrapping, and valves in CO_2 mode. Total denitrification, D_w and D_n were calculated as in Rissanen et al. (2013).

2.4 Data analysis

For diel data, we used paired t-test and Wilcoxon rank test to identify potential differences in denitrification and environmental factors during light and dark. General linear model (GLM) was used to explore the relationship of the denitrification rates to environmental variables, including temperature, O_2 concentration, DOU, OPD, and PAR. Due to data skewness, denitrification rates were LN-transformed in GLM. To control the non-independency of observations arising from the similarity of the sampling site, year was added to the models as a categorical variable. Akaike information criterion (AIC) was used for selecting the minimum adequate model. Model selection was carried out using the step AIC function from R package "MASS" (Venables and Ripley, 2002). To test whether the main effect of the categorical variable 'year' was significant, GLM models with and without categorical variables were compared with log-likelihood tests.

For seasonal data, we used a model describing the interactions between environment and denitrification. First, we performed correlation and head component analysis between the catchment scale factors (average air temperature, 2-day temperature sum, 2-day rain sum and daily PAR sum as well as discharge, turbidity, pH, DOC, TN, NO_x-N, NH_4^+-N from K1), process scale factors (sediment temperature, DOU, O_2 concentration on the sediment surface, O_2 penetration depth, PAR sum during incubation, NO_x-N in the water above the sediment, top sediment LOI%) and denitrification. Secondly, we performed structural equation modeling (SEM, e.g. Sutton-Grier et al. 2010) using the R package 'lavaan'. A chi-square test of model fit (P-value >0.05) was used for indicating that the model and data structure do not differ significantly. In addition, comparative fit index (CFI) >0.95 and root mean square error of approximation (RMSEA) <0.05 were used in model estimation. Significance for individual path coefficients was accepted below 0.05. Path coefficients in the SEM models were standardized against their standard deviation and express the approximate change of observed range. Thus, the approximate strength of various paths should be cautiously compared. It should be noted, that the three replicate denitrification samples might not be independent from each other, due to the same sampling site, thus violating the statistical requirements of true replicates. Consequently, the P-values should be considered with caution. Statistical analyses were performed with SPSS 24 (IBM) and R (R Development Core Team 2016).

Finally, we estimated the influence of the length of daylight through oxygenation on denitrification. We calculated the weighted average on the basis of measured denitrification rates in dark and in light combining them to the light/dark hours from the study days based on PAR data.

3. **Results**

3.1 Environmental conditions during seasonal sampling

Pre-experiment with a concentration series of $^{15}NO_3$-N showed that denitrification was limited by NO_x-N (ambient NO_x-N 163 µmol l^{-1}), as the denitrification of labeled NO_x-N (D15) increased relative to the concentration of labeled NO_x-N (Fig. 3). Also, denitrification was the only process producing N_2, because denitrification based on the natural NO_x-N (D14) was stable in spite of the increased labeled NO_x-N (Fig. 3), and the conclusion was that anammox was not present. The presence of anammox would have resulted in increasing D14 relative $^{15}NO_3$-N amendments due to the production of $^{28}N_2$ ja $^{29}N_2$ gases.

During the study days, the temperature on the sediment surface was above the freezing point. The minimum was measured in late October (+0.1 °C) and the maximum (+17.2 °C) in July (Fig. 2A). During winter the sediment temperature was higher than the air temperature (Fig. 2A). The lowest OPD and O_2 concentration were measured in summer on the sediment surface in the first year (Fig. 2B). In this agricultural watershed, the N consisted mostly of NO_x-N (Fig. 2D). The average NO_x-N concentration in the pond littoral water was 167 µmol l^{-1} following the concentrations in the incoming water at K1 (87–388 µmol l^{-1}). The maximum NH_4^+-N concentration (36 µmol l^{-1}; data not shown) was measured on July 30th 2014 and was most likely connected to the spread manure in the fields and the heavy rain that occurred the next day. The highest stream discharge was measured after spring melt 2015 simultaneously with heavy rain (Fig. 2C). In the spring, average pH was 6.9 and during other seasons 7.3. Average discharge of Koiransuolenoja was 58 l s^{-1}, varying between 5–200 l s^{-1}. Highest turbidity and DOC concentrations occurred after the rain. Average LOI of the upper sediment in the pond varied between 7.3–9.8%.

3.2 Diel denitrification

In field incubations, O_2 concentration on the sediment surface was lower during dark (PAR <1 µmol s^{-1} m^{-2}) than during light (Table 1, Fig. 4). The maximum temperature on the sediment surface was measured at 18:00 and the minimum between 03:00–06:00 at night. Denitrification rates were driven by light conditions and varied between the two study occasions (Table 2). Average denitrification rates during dark were significantly higher (180 µmol N m^{-2} h^{-1}) than during light (67 µmol N m^{-2} h^{-1} Table 1, Fig. 4). In 2015, average denitrification was nearly two-fold (142 µmol N m^{-2} h^{-1}) as compared to the previous year (77 µmol N m^{-2} h^{-1}). Also, in 2015 the water above the sediment had higher NO_x-N concentration and sediment temperature (138 µmol l^{-1}, 14.3 °C) than the previous year (112 µmol l^{-1}, 12.6 °C).

3.3 Seasonal denitrification

Maximum denitrification rate (12409 µmol N m^{-2} d^{-1}) took place during the summer (July 30th; Fig. 5). At the time, sediment surface O_2 and littoral water NO_x-N concentration were the lowest (Fig. 2B and D) after a heavy rain and sediment temperature was the second highest measured (+15.8 °C). Daytime *in situ* denitrification rates clearly decreased in sediment temperatures below +10 ºC, and no activity was detected during the minimum sediment temperature +0.1 °C in October (Fig. 6). Annual average denitrification was 2441 µmol N m^{-2} d^{-1}, consisting mainly of the denitrification of the water column NO_x-N (66%). Fall was the only season when D_n (nitrification-denitrification) slightly dominated over D_w by 53% (Table 3).

Turbidity level affected the daytime denitrification rates, because under high turbidity (36.3 ftu), denitrification was 19% higher in the covered cores than in the transparent ones (Fig. 7), but during clear water (7.0 ftu), denitrification was 68% higher in the covered cores than in the transparent ones. The seasonal model explained 40% of the denitrification variability, 4% of the sediment surface temperature variability and 60% of the sediment surface O_2 variability (Fig. 8). Season (represented by the 2-day temperature sum) regulated sediment surface temperature and higher turbidity and temperature led to lower O_2 concentration on the sediment surface.

3.4 Recalculated denitrification rates

On the basis of light/dark correction of denitrification rate, average annual estimate of denitrification was 3415 μmol N m^{-2} d^{-1}, being 40% higher than the measured daytime denitrification (Fig. 5). Compared with measured seasonal averages in Table 3, recalculated denitrification rates were 35% higher in summer, 71% higher in fall, 137% higher in winter and 31% higher in spring.

4. **Discussion**

Following our hypothesis, the diel measurements in August 2014 and 2015 showed that denitrification rates varied significantly between night and day. Our result corroborates with previous findings from freshwater systems, where highest denitrification rates were measured in dark conditions (Christensen et al. 1990; de Klein 2008; Soana et al. 2017). Higher PAR during the day promotes O_2 production on the sediment surface by the benthic algae, pushing the denitrification zone deeper (e.g. Christensen et al. 1990; Laursen and Carlton, 1999; Nielsen et al. 1990). Consequently, nitrate in the above water needs to diffuse longer path to reach the denitrifying bacteria in the anoxic sediment layers. Indeed, we found D_w being lower when OPD was higher, which is in line with earlier results (Andersen et al. 1984; Christensen et al. 1990; Risgaard-Petersen et al. 1994). Compared to sediment surface O_2 concentration, sediment temperature remained more stable throughout the 24h experiments. Higher denitrification rate observed in 2015 than 2014 in the diel studies is probably linked to higher NO_x-N availability, but also to other factors combined such as higher sediment temperature, thinner oxygenated sediment layer, and thus, higher D_w.

Denitrification rates showed clear seasonal variation, which was driven by temperature, oxygen and turbidity. The denitrification rates were clearly accelerated in sediment temperatures above +10 °C, but the summer was the only season when the average sediment temperature reached this high. Seasonal denitrification was more related to air temperature (2-day air temp. sum) than to PAR. The seasonal study measurements were conducted on daytime and we used PAR data merely from the incubation time (3h), which may be the reason why air temperature explained more about the variation in denitrification than PAR. The 2-day air temperature reflects longer term thermal conditions in the watershed, and apparently also reflects sediment temperature slightly better than PAR. However, air temperature explained only 4% of the sediment temperature variability of the study site. This can be explained by the on-stream nature of the pond, where different discharge situations are reflected into temperature of the sediment.

Temperature and turbidity mediated denitrification indirectly by modifying sediment O_2 concentrations. However, turbidity also directly controlled denitrification in seasonal results, which can refer to higher C availability or lower O_2 concentration (Liu et al. 2013) of turbid waters. Turbidity is typical in agricultural streams and reduces light reaching the stream bottom. The highest denitrification rates coincided with heavy rains washing out the liquid manure spread on the fields to the stream increasing turbidity and C:N–ratio in the stream water. Carbon in the organic manure was likely more labile to the denitrifiers as compared to the terrestrial C typically leaching from the catchment area. The importance of C quantity and lability for denitrifiers has been addressed in several studies (e.g. Asmala et al. 2013; Grebliunas and Perry, 2016; Stelzer et al. 2014). Denitrification based on water NO_x-N was high (81%) at the time, indicating the denitrification was not based on the NO_x-N produced in the sediment. In this study, NO_x-N concentrations did not regulate the denitrification rates, which was presumably a result of the high NO_x-N level in the stream. Considering spatial variability in the pond sediment denitrification (Uusheimo, unpublished), we observed 1.7 times higher rates in the deepest part of the pond compared to the littoral. These laboratory experiments were conducted six times during the year in dark at constant temperature with the same method resulting mean denitrification rates of ca. 5400 μmol N m^{-2} d^{-1}, while in the littoral sediment the rate was ca. 3100 μmol N m^{-2} d^{-1}. This difference may be explained by the higher C content and lower oxygen conditions of the deep sediment. The observed patchiness also indicates that even our recalculated rates from the littoral sediment underestimate N removal and should be regarded as the minimum in these conditions.

It is challenging to compare our measured seasonal daytime denitrification results (0–12409 μmol N m^{-2} d^{-1}) to other freshwater studies in the boreal region due to the scarcity of actual denitrification rate measurements and especially because of the differences in the methods applied. Isotope pairing technique has been applied for measuring denitrification rates only in a few boreal lakes in Finland (Holmroos et al. 2012; Rissanen et al. 2013) and in Sweden (Ahlgren et al. 1994), but not in boreal streams or constructed agricultural ponds. The rates measured in our study were significantly higher as compared to the results from boreal lakes (0–2070 μmol N m^{-2} d^{-1}). One reason may be that even the most eutrophic lakes have lower NO_x-N concentration and temperature than agricultural wetlands. Similar or higher denitrification rates using IPT have been reported in studies conducted in temperate nitrate-rich aquatic systems (e.g. de Klein 2008; Racchetti et al. 2011; Soana et al. 2017).

Recalculated denitrification rates, based on the diel study, indicated that daytime denitrification measurements can lead to substantial underestimations of denitrification rates. For example, the measured denitrification was 35 % smaller than the light-corrected rate in summer. This can be explained with day-time top sediment oxygenation decreasing the denitrification based on D_w, which is usually the dominant denitrification pathway in systems with high nitrate level. In fall, our corrected estimate was 71% higher than the measured one. We acknowledge that this estimate may be too high, since denitrification was mostly based on coupled nitrification-denitrification fed by active mineralization during fall. Furthermore, during the one study date at air temperature below zero in winter, the darkness lasted for 17 hours which explains the large proportional difference between the measured and the recalculated denitrification. We emphasize that this kind of correction can be applied in sites where denitrification is based mostly on water nitrate, to form a proxy of the influence of light reaching the sediment primary producers and thus, denitrification.

In the agricultural pond sediment, the highest denitrification rates were measured during the growing season. During other seasons, denitrification rate was slower, but detectable still at sediment temperature +0.4 °C, at the time the air temperature had fell to -6.2 °C. This indicates some microbial activity in the sediment throughout the winter. Denitrification rates might have been underestimated in winter, since higher oxygen penetration depth could have led to inhomogenous mixing of natural and labeled nitrate or slow nitrate diffusion rate into the sediment denitrification zone (Nielsen 1992). Low denitrification rates can be compensated to some extent by longer retention time (e.g. Addy et al. 2016). In a literature review by Leonardson (1994), retention time of 3–5 days was recommended for N removal at times of high discharge. One of the main reasons sedimentation ponds are not considered to be applicable to N removal is typically their small surface area relative to the catchment size, and small volume relative to discharge, and thus, a short retention time counted in hours (e.g. Puustinen et al. 2007).

The seasonal nitrogen removal of small Koiransuolenoja pond was not capable of corresponding to the high N loading from the catchment, although the actual denitrification rates in the sediment were high, because the average seasonal residence time was relatively low varying from 0.5 hours to 2.3 hours. The mean nitrate load in the stream was 4.4 kg d^{-1} in summer, 5.6 kg d^{-1} in fall, 11 kg d^{-1} in winter, and 40 kg d^{-1} in spring, meaning denitrification could remove only 0.8% of incoming nitrate in summer and 0.08% in fall. Recommendations stating a sufficient wetland:catchment -size ratios for efficient nutrient removal vary from 0.5% (Puustinen et al. 2007) to 2% (Hammer 1992). In our study pond, this ratio was only 0.005%, meaning that a notable increase in the wetland area is needed, if we want to remove a major proportion of the annual nitrate loading by microbial processes. With the seasonal recalculated denitrification rates in the shallow littoral, representing the lower end in the spatial variation in denitrification rates, and wetland:catchment -size ratio of 2% suggested by Hammer (1992), nitrate removal by denitrification in Koiransuolenoja pond would be 100% in summer, 32% in fall, 4.7% in winter and 5.2% in spring, respectively. Furthermore, with the wetland:catchment -size ratio of 6%, nearly all (95%) of the nitrate load could be removed also during the fall, but the efficiency during winter and spring would be only 15%. Thus, not only the denitrification rates, but also residence time should be taken into account when defining the sufficient surface area and volume for the wetland to remove nitrate efficiently also in winter and spring.

5. **Conclusions**

Nitrogen removal capacity of shallow wetlands can be underestimated due to day-time top sediment oxygenation, if the potential is solely based on day-time denitrification measurements. Seasonally, denitrification was controlled by temperature, which makes N removal challenging in boreal latitudes during cold seasons. In the warming climate, efficiency and importance of constructed wetlands will increase, as precipitation and nitrogen load have been estimated to rise. Mitigating agricultural nitrogen load in northern latitudes requires wetlands large enough, relative to their catchment size, and elaborated plans for restoring watersheds to utilize and accelerate their ecosystem services, including denitrification.

Acknowledgements

This study was funded by Maj and Tor Nessling Foundation, Maa- ja vesitekniikan tuki ry and the funding of Academy of Finland project 310302 for SLA. The authors would like to thank Dr. Antti Rissanen for his advice and the staff of Lammi Biological Station.

References

Addy K., Gold, A.J., Christianson L.E., David, M.B. Schipper, L.A., Ratigan N.A. 2016. Denitrifying bioreactors for nitrate removal: a meta-analysis. J. Environ. Qual. 45, 873–881.

Ahlgren I., Sörensson F., Waara T., Vrede K. 1994. Nitrogen budgets in relation to microbial transformations in lakes. Ambio 23(6), 367–377.

An S., Joye S.B. 2001. Enhancement of coupled nitrification-denitrification by benthic photosynthesis in shallow estuarine sediments. Limnol. Oceanogr. 46(1), 62–74.

Andersen, T.K., Jensen, M.H., Sørensen, J. 1984. Diurnal variation of nitrogen cycling in coastal marine sediments. J. Mar. Biol. 83, 171–176.

Arheimer B., Pers B.C. 2017. Lessons learned? Effects of nutrient reductions from constructing wetlands in 1996–2006 across Sweden. Ecol. Eng. 103, 404–414.

Arvola L., Einola E., Järvinen M. 2015. Landscape properties and precipitation as determinants for high summer nitrogen load from boreal catchments. Landsc. Ecol. 30, 429–442.

Asmala E., Autio R., Kaartokallio H., Pitkänen L., Stedmon C.A., Thomas D.N. 2013. Bioavailability of riverine dissolved organic matter in three Baltic Sea estuaries and the effect of catchment land use. Biogeosci. 10, 6969–6986.

Bastviken, S.K., Eriksson, P.G., Ekström, A., Tonderski K. 2007. Seasonal denitrification potential in wetland sediments with organic matter from different plant species. Water Air Soil Pollut. 183, 25-35.

Castaldelli G., Soana E., Racchetti E., Vincenzi F., Fano E.A., Bartoli M. 2015. Vegetated canals mitigate nitrogen surplus in agricultural watersheds. Agriculture, Ecosyst. Environ. 212, 253–262.

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

Davidson N.C. 2014. How much wetland has the world lost? Long-term and recent trends in global wetland area. Mar. Freshw. Res. 65(10), 934–941.

EEA, 2015. The European environment — state and outlook 2015: synthesis report, European Environment Agency, Copenhagen. https://www.eea.europa.eu/soer-2015/synthesis (accessed 5 January 2018).

Fowler D., Coyle M., Skiba U., Sutton M.A., Cape J.N., Reis S., Sheppard L.J., Jenkins A., Grizzetti B., Galloway J.N., Vitousek P., Leach A., Bouwman A.F., Butterbach-Bahl K., Dentener F., Stevenson D., Amann M., Voss M. 2013. The global nitrogen cycle in the twenty-first century. Phil. Trans. R. Soc. B 368(1621), 1–13.

Galloway J.N., Leach A.M., Bleeker A., Erisman J.W. 2013. A chronology of human understanding of the nitrogen cycle. Phil. Trans. R. Soc. B 368(1621), 1–11.

Grebliunas B.D., Perry, W.L. 2016. Carbon limitation of sediment bacterial production and denitrification in high nitrate low carbon systems. Environ. Earth Sci. 75(8), 1–9.

Gruber N., Galloway J.N. 2008. An earth-system perspective of the global nitrogen cycle. Nature 451, 293–296.

Hammer, D.A. 1992. Designing constructed wetlands to treat agricultural nonpoint source pollution. Ecol. Eng. 1, 49–82.

Hernandez M.E., Mitch W.J. 2007. Denitrification in created riverine wetlands: Influence of hydrology and season. Ecol. Eng. 30, 78–88.

Holmroos H., Hietanen S., Niemistö J., Horppila J. 2012. Sediment resuspension and denitrification affect the nitrogen to phosphorus ratio of shallow lake waters. Fundam. Appl. Limnol. 180, 193–205.

Häikiö, M. 1998. Laskeutusaltaan toimivuus maatalouden vesiensuojelussa. SYKEn moniste nro 110. 90 s. Oy Edita Ab. Helsinki.

Jørgensen B.B., Revsbech N.P. 1985. Diffusive boundary layers and the oxygen uptake of sediments and detritus. Limnol. Oceanogr. 30(1), 111–122.

de Klein J.J.M. 2008. From Ditch to Delta: Nutrient retention in running waters. PhD-thesis. Wageningen University. The Netherlands. ISBN: 978-90-8504-930-2.

Lakkala K., Jaros A., Aurela M., Tuovinen J-P., Kivi R., Suokanerva H., Karhu J.M., Laurila T. 2016. Radiation measurements at the Pallas-Sodankylä Global Atmosphere Watch station — diurnal and seasonal cycles of ultraviolet, global and photosynthetically-active radiation. Bor. Environ. Res. 21, 427–444.

Laursen A.E., Carlton R.G. 1999. Responses to atrazine of respiration, nitrification, and denitrification in stream sediments measured with oxygen and nitrate microelectrodes. FEMS Microbiol. Ecol. 29, 229–240.

Leonardson, L. 1994. Våtmarker som kvävefällor — Svenska och internationella erfarenheter. Naturvårdsverket, rapport 4176. Stockholm.

Liu T., Xia X., Liu S., Mou X., Qiu Y. 2013. Acceleration of denitrification in turbid rivers due to denitrification occurring on suspended sediment in oxic waters. Environ. Sci. Technol. 47(9), 4053–4061.

Lorenzen J., Larsen L.H., Kjær T., Revsbech N–P. 1998. Biosensor determination by the microscale distribution of nitrate, nitrate assimilation, nitrification, and denitrification in a diatom-inhabited freshwater sediment. Appl. Environ. Microbiol. 64(9), 3264–3269.

Mitsch W.J., Gosselink J.G. 2015. Wetlands, 5th ed. John Wiley & Sons, Inc., New Jersey, USA. ISBN: 978-1-118-67682-0.

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., Thomas, S.M. 2008. Stream denitrification across biomes and its response to anthropogenic nitrate loading. Nature 452, 202–206.

Nielsen L.P., Christensen P.B., Revsbech N.P., Sørensen J. 1990. Denitrification and photosynthesis in stream sediment studied with microsensor and whole-core techniques. Limnol. Oceanogr. 35(5), 1135–1144.

Nielsen L.P. 1992. Denitrification in sediment determined from nitrogen isotope pairing. FEMS Microbiol. Ecol. 86, 357–362.

O´Green A.T., Budd R., Gan J., Maynard J.J., Parikh S.J., Dahlgren R.A. 2010. Mitigating nonpoint source pollution in agriculture with constructed and restored wetlands. Adv. Agron. 108, 1–76.

Pinardi M., Bartoli M., Longhi D., Marzocchi U., Laini A., Ribaudo C., Viaroli P. 2009. Benthic metabolism and denitrification in a river reach: a comparison between vegetated and bare sediments. J. Limnol. 68(1), 133‑145.

Puustinen M., Koskiaho J., Jormola J., Järvenpää L., Karhunen A., Mikkola-Roos M., Pitkänen J., Riihimäki J., Svensberg M., Vikberg P. 2007. Maatalouden monivaikutteisten kosteikkojen suunnittelu ja mitoitus. Suomen ympäristö 21. Vammala. ISBN 978-952-11-2720-5.

R Core Team. 2016. R: A language and environment for statistical computing. R Foundation for Statistical Computing. http://www.R-project.org (accessed 15 April 2017)

Racchetti E., Bartoli M., Soana E., Longhi D., Christian R.R., Pinardi M.,Viaroli P. 2011. Influence of hydrological connectivity of riverine wetlands on nitrogen removal via denitrification. Biogeochem. 103, 335–354.

Revsbech N.P., Sørensen J., Blackburn T.H., Lomholt J.P. 1980. Distribution of oxygen in marine sediments measured with microelectrodes. Limnol. Oceanogr. 25(3), 403–411.

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

Risgaard-Petersen N., Nielsen L.P., Rysgaard S., Dalsgaard T., Meyer R.L. 2003. Application of the isotope pairing technique in sediments where anammox and denitrification coexist. Limnol.

Oceanogr.: Methods 1, 63–73.

Rissanen A., Tiirola M., Ojala A. 2011. Spatial and temporal variation in denitrification and in the denitrifier community in a boreal lake. Aquat. Microb. Ecol. 64(1), 27–40.

Rissanen A.J., Tiirola M., Hietanen S., Ojala A. 2013. Interlake variation and environmental controls of denitrification across different geographical scales. Aquat. Microb. Ecol. 69, 1–16.

Roach W.J., Grimm N.B. 2011. Denitrification mitigates N flux through the stream–floodplain complex of a desert city. Ecol. Appl. 21(7), 2618–2636.

Robertson G.P., Vitousek P.M. 2009. Nitrogen in agriculture: Balancing the cost of an essential resource. Annu. Rev. Environ. Resour. 34, 97–125.

Rockstrom, J., Steffen, W. Noone, K. Persson, A., Chapin, F.S., Lambin, E., Lenton, T.M., Scheffer, M., Folke, C., Schellnhuber, H., Nykvist, B., De Wit, C.A., Hughes, T., van der Leeuw, S., Rodhe, H., Sorlin, S., Snyder, P.K., Costanza, R., Svedin, U., Falkenmark, M., Karlberg, L., Corell, R.W., Fabry, V.J., Hansen, J., Walker, B., Liverman, D., Richardson, K., Crutzen, P., Foley J. 2009. Planetary boundaries:exploring the safe operating space for humanity. Ecol. Soc. 14(2), 32. http://www.ecologyandsociety.org/vol14/iss2/art32/ (accessed 2 January 2018).

Shao, M-F., Zhang, T., Fang, H.H-P. 2010. Sulfur-driven autotrophic denitrification: diversity, biochemistry, and engineering applications. Appl. Microbiol. Biotechnol. 88, 1027–1042.

Soana E., Balestrini R., Vincenzi F., Bartoli M., Castaldelli G. 2017. Mitigation of nitrogen pollution in vegetated ditches fed by nitrate-rich spring waters. Agric. Ecosyst. Environ. 243, 74–82.

Stelzer R.S., Scott J.H., Bartsch L.A., Parr T.B. 2014. Particulate organic matter quality influences nitrate retention and denitrification in stream sediments: evidence from a carbon burial experiment.

Biogeochem. 119, 387–402.

Strand J.A., Weissner S.E.B. 2013. Effects of wetland construction on nitrogen transport and species richness in the agricultural landscape – experiences from Sweden. Ecol. Eng. 56, 14–25.

Sutton, M.A., Howard, C.M., Erisman J.W., Billen G., Bleeker A., Grennfelt P., Grinsven H., Grizzetti B. 2011. The European nitrogen assessment. First ed. Cambridge University Press, New York. http://www.nine-esf.org/node/360/ENA-Book (accessed 2 January 2018).

Sutton-Grier, A.E., Kenney, M.A., Richardson, C.J. 2010. Examining the relationship between ecosystem structure and function using structural equation modelling: A case study examining denitrification potential in restored wetland soils. Ecol. Model. 221(5), 761–768.

Tiirola M., Rissanen A.J., Sarpakunnas M., Arvola L., Nykänen H. 2011. Stable isotope profiles of nitrogen gas indicate denitrification in oxygen-stratified humic lakes. Rapid Commun. Mass Spectrom. 25, 1497–1502.

Tikkanen M., Seppälä M., Heikkinen O. 1985. Environmental properties and material transport of two rivulets in Lammi, southern Finland. Fennia 163, 217–282.

Venables, W.N., Ripley, B.D. 2002. Modern Applied Statistics with S. 4th Edition. Springer, New York.

Veraart A.J., Audet J., Dimitrov M.R., Hoffmann C.C., Gillissen F., de Klein J.J.M. 2014. Denitrification in restored and unrestored Danish streams. Ecol. Eng. 66, 129–140.

Vuollekoski M., Joensuu S., Kauppila M. 2015. Tutkimuksia metsätalouden vesiensuojelusta 33, 2015. Luonnonvarakeskus. Helsinki.

Vymazal J. 2017. The use of constructed wetlands for nitrogen removal from agricultural drainage: A Review. Scient. Agric. Bohem. 48(2), 82–91.

Wang M., Zhang D., Dong J., Tan S.K. 2018. Application of constructed wetlands for treating agricultural runoff and agro-industrial wastewater: a review. Hydrobiol. 805, 1–31.

Weyhenmeyer, G.A., Jeppesen E. 2010. Nitrogen deposition induced changes in DOC:NO_3-N ratios determine the efficiency of nitrate removal from freshwaters. Global Change Biol. 16, 2358–2365.

Table 1 Pairwise comparisons of denitrification rate, oxygen (O_2) concentration on sediment surface, sediment diffusive O_2 uptake (DOU) and temperature, photosynthetically active radiation (PAR), O_2 sediment penetration depth and denitrification based on the water column nitrate (D_w%) between light (09:00–18:00) and dark conditions (21:00–06:00) in the diel studies. Degrees of freedom (df), test values (t) and significance (P-value) of the results.

	df	**t**	**P-value**
Denitrification rate	16	-7.639	*<0.001*
O_2 concentration	14	6969	*<0.001*
DOU	14	0.289	0.78
Temperature	17	0.14	0.99
PAR	18	0*	*<0.05**
O_2 penetration depth	15	35.5	0.165*
D_w%	11	32	0.928*

Statistically significant results (P<0.05) are shown in italics.

*) Wilcoxon signed rank test for data with non-normally distributed variance.

Table 2 General linear model (GLM) results for denitrification in relation to the environmental factors: temperature and oxygen (O_2) concentration on sediment surface, sediment diffusive O_2 uptake and photosynthetically active radiation (PAR) in the diurnal experiments. Estimates, standard error, test values (t) and significance (P-value) of the results.

		estimate	**std. error**	**t**	**P-value**
Denitrification	intercept	7.05	1.56	4.53	0.001
AIC 21.25	Year			0.59	*0.044*
	Temperature	0.21	0.13	1.66	0.127
	O_2 concentration	-0.23	0.12	-1.91	0.086
	DOU	-0.32	0.19	-1.74	0.112
	PAR	-0.001	0.0004	-2.97	*0.014*

Statistically significant ($P<0.05$) results are shown in italics.

Table 3 Seasonal averages of the data July 10th 2014–June 25th 2015: Measured and estimated denitrification, denitrification based on the water column NO_x-N (D_w%), sediment temperature, O_2 concentration, O_2 depth (OPD), diffusive O_2 uptake (DOU), loss on ignition (LOI%, 0-3 cm) and NO_x-N above the pond littoral sediment. NO_x-N, turbidity, dissolved organic carbon (DOC) and discharge at K1 as well as photosynthetically active radiation (PAR) and air temperature.

Seasons	**Summer**	**Fall**	**Winter**	**Spring**
Months	(VI–VIII)	(IX–XI)	(XII–II)	(III–V)
Study days included	9	8	1	6
Meas. denitrification (µmol N m^{-2} d^{-1})	5520	576	120	864
Est. denitrification (µmol N m^{-2} d^{-1})	7444	984	285	1135
D_w (%)	69	47	88	71
Sediment surface temp. (°C)	14	7	0	5
Sediment surface O_2 (µmol l^{-1})	182	298	218	265
Sediment OPD (mm)	5	7	11	7
Sediment DOU (mmol O_2 m^{-2} d^{-1})	5	4	2	5
LOI (%)	9	8	8	8
NO_x-N above sediment (µmol l^{-1})	131	135	190	256
NO_x-N at K1 (µmol l^{-1})	135	128	156	251
Turbidity (ftu)	16	10	15	16
Water DOC (mg C l^{-1})	10	10	8	11
Discharge (l s^{-1})	27	36	58	130
PAR (mmol m^{-2} s^{-1})	592	218	1	187
Air temp. (°C)	17	6	-6	5