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Sediment diffusion method improves wastewater nitrogen removal in the receiving lake sediments

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ABSTRACT

Sediment microbes have a great potential to transform reactive N to harmless N_2, thus decreasing wastewater nitrogen load into aquatic ecosystems. Here, we examined if spatial allocation of the wastewater discharge by a specially constructed sediment diffuser pipe system enhanced the microbial nitrate reduction processes. Full-scale experiments were set on two Finnish lake sites, Keuruu and Petäjävesi, and effects on the nitrate removal processes were studied using the stable isotope pairing technique. All nitrate reduction rates followed nitrate concentrations, being highest at the wastewater-influenced sampling points. Complete denitrification with N_2 as an end-product was the main nitrate reduction process, indicating that the high nitrate and organic matter concentrations of wastewater did not promote nitrous oxide (N_2O) production (truncated denitrification) or ammonification (dissimilatory nitrate reduction to ammonium; DNRA). Using 3D simulation, we demonstrated that the sediment diffusion method enhanced the contact time and amount of wastewater near the sediment surface especially in spring and in autumn, altering organic matter concentration and oxygen levels, and increasing the denitrification capacity of the sediment. We estimated that natural denitrification potentially removed 3–10% of discharged wastewater nitrate in the 33 ha study area of Keuruu, and the sediment diffusion method increased this areal denitrification capacity on average 45%. Overall, our results indicate that sediment diffusion method can supplement wastewater treatment plant (WWTP) nitrate removal without enhancing alternative harmful processes.

1. Introduction

Wastewater effluents are important point sources of reactive nitrogen (N), significantly altering the biogeochemistry of the receiving aquatic ecosystems (Carey and Migliaccio, 2009). Recent studies highlight the importance of efficient N removal in wastewater treatment plants (WWTPs; Lofton et al., 2007; Lee et al., 2016). Currently, the most common wastewater treatment standard in Europe, North America and Australia is the secondary treatment (Morris et al., 2017), where activated sludge is used to remove organic material and convert incoming ammonium (NH_4^+) to nitrate (NO_3^-; Carey and Migliaccio, 2009). To protect the ecological condition of the receiving waterbodies, national and international protection acts and regulations (e.g. EU Urban Waste Water Directive, US Clean Water Act; Morris et al., 2017) have established N removal limits for WWTPs. In order to achieve these limits also in future, WWTPs have to acquire more sophisticated treatment methods (e.g. tertiary treatment), meaning high investing costs especially for small WWTPs (population equivalent $\leq$ 80 000), which are usually the most common (Hautakangas et al., 2014). In addition, a more efficient N removal at the WWTPs may promote emissions of greenhouse gases, e.g. nitrous oxide (N_2O; Hauck et al., 2016).

Lakes, wetlands and other freshwater ecosystems are large global sinks for reactive N, removing incoming N through denitrification (Seitzinger et al., 2006; Finlay et al., 2013). During denitrification, NO_3^- is sequentially converted into nitrite (NO_2^-), nitric

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oxide (NO) and N_2O, and, in optimal conditions, into biologically inert nitrogen gas (N_2) (Seitzinger et al., 2006). As increasing nitrogen loading enhances nitrogen removal (Finlay et al., 2013) and denitrification (Seitzinger et al., 2006), denitrification can potentially diminish the detrimental effect of wastewater on the receiving ecosystem, and act as a supplemental N removal for treated wastewater. However, high NO_3^- concentrations together with a lack of carbon (C) can enhance higher production of greenhouse gas N_2O relative to N_2 through incomplete denitrification (Zhao et al., 2014). Thus, wastewater-induced denitrification, although removing reactive N, could also be "an ecosystem disservice" (Burgin et al., 2013). However, wastewater typically contains high amounts of organic C as compared to receiving waterbodies (DeBruyn and Rasmussen, 2002), which may facilitate complete heterotrophic denitrification with the N_2 as the end-product in the receiving sediments (Weymann et al., 2010). Moreover, high C:N and high C loading have been demonstrated to promote another "ecosystem disservice", DNRA (dissimilatory reduction of nitrate to ammonium), over denitrification (Kraft et al., 2014; Hardison et al., 2015). DNRA converts wastewater NO_3^- into biologically more reactive ammonium (NH_4^+), retaining N in the ecosystem. Previous results from sediments influenced by aquaculture waste (Christensen et al., 2000) suggest that wastewater effluent with both high C and N concentrations could support DNRA, or even favor it over denitrification, in the receiving sediments.

Currently, the knowledge on the influence of wastewater on nitrate reduction processes in natural systems is still rather poor, since previous studies have focused mainly on changes in the genetic N transformation potential (e.g. Rahm et al., 2016; Saarenheimo et al., 2017). They suggest that wastewater supports genetic denitrification potential by bringing more substrate and electron donors to the sediment microbes, but also by bringing new WWTP microbes and shaping the natural microbial community. In an urban stream study, Lofton et al. (2007) measured higher potential denitrification rates at the wastewater-influenced sites than at the pristine upstream sites, but there is no information on wastewater-driven changes in N_2O production. Furthermore, the effect of wastewater on DNRA rates has only been measured in Baltic Sea estuary, where it was found to increase DNRA (Bonaglia et al., 2014). This means that at the moment, it is impossible to estimate the ultimate fate of wastewater nitrate, and the true N removal potential of sediment microbes, at least in lakes and other freshwater ecosystems. In this study, we used stable isotope approach (IPT; Nielsen, 1992) to measure denitrification, truncated denitrification, and DNRA process rates in boreal lake sediments along a wastewater gradient. Wastewater could have a significant impact on N transformation processes especially in boreal lakes, where denitrification rates are lower as compared to temperate lakes (Rissanen et al., 2013).

One important factor regulating denitrification of wastewater effluents in water bodies is the water residence time (Seitzinger et al., 2006), which affects the contact time between water column nitrate and sediment. Wastewaters are commonly discharged several meters above the sediment surface, into lake water columns, meaning that in boreal area, the seasonal variation in stratification patterns could control the contact times between wastewater and sediment, e.g. wastewater would be near the sediment surface only in winter. This would further lead to seasonal differences in sediment denitrification capacity. In this study, we increased the wastewater contact time throughout the year by discharging the water on the lake sediment surface through a special diffuser pipe system. By this, we aimed to increase concentration of wastewater near the sediment surface to support natural denitrification. By using a full-scale experimental approach, we aimed to: 1) compare how spatial allocation of wastewater affects seasonal N transforming processes, and 2) estimate the applicability of the sediment diffuser method in wastewater N removal. We hypothesized that wastewater increases denitrification rate, but potentially also unfavorable N_2O production or DNRA. In addition, we hypothesized that sediment diffusion method reduces seasonal variation, leading to higher overall N removal capacity.

2. Material and methods

2.1. Study sites

The two WWTPs, Keuruu (8200 population equivalent; PE) and Petäjävesi (1800 PE), are located in Central Finland (Fig. 1). Both WWTPs have primary (clarification) and secondary treatment (activated sludge with nitrification), and discharge their treated effluents from a one-point outlet to the lake under the normal conditions. In Keuruu, WWTP nitrification process collapsed in winter 2015 due to cold weather. In Keuruu, the recipient lake, Lake Keurusselkä, is a large humic lake (117 km^2), belonging to Kokemäenjoki drainage area, with average depth of 6.4 m and a maximum depth of 40 m. During the experimental study, the diffuser pipe system, having 50 holes (30 mm wide) on both sides of the 30 m long and 60 cm diameter PE pipe (Suppl. Fig. 1), was attached to the end of the original WWTP discharge pipe at the depth of 9 m for one year (October 2014–November 2015). In Petäjävesi, the treated WWTP effluent is discharged to Lake Jämsänvesi, which is a medium-sized humic lake (0.9 km^2; Fig. 1), belonging to Kymijoki drainage area, with average depth of 4.2 m and maximum depth of 27 m. In August 2016, the original WWTP discharge pipe was extended with a similar diffuser pipe system as in Keuruu (except it had 100 holes (30 mm wide), and the total length was 10 and the diameter 20 cm), which directed the wastewater effluent to the sediment surface. The costs of the diffuser systems were ~35 000€ in Keuruu (including technical design, environmental permits, construction and installation conducted by private companies) and ~3000€ in Petäjävesi (conducted by the local authorities). See Saarenheimo et al. (2017) for further description of the study sites.

2.2. Sampling

Intact sediment cores for process measurements were collected using the Kajak sediment core sampler (KC Denmark A/S). In Keuruu, there were eight sampling trips between years 2014 and 2015 (11 Feb 2014, 6 May 2014, 4 Aug 2014, 14 Oct 2014, 20 Jan 2015, 19 May 2015, 11 Aug 2015 and 20 Oct 2015), and in Petäjävesi, seven trips in 2014 and 2016 (8 May 2014, 9 Jun 2014, 11 Aug 2014, 13 Aug 2014, 16 Oct 2014, 11 Aug 2016, 20 Oct 2016). During each sampling trip, three to fifteen sediment cores (sediment height ~25 cm and diameter 4 cm) were collected per each lake site. On both lake areas, three main sampling points were sampled each time, one located ~0.8 km upstream from the wastewater discharge point, one at the discharge point and one at approximately 200–300 m downstream from the discharge point (except in 11 Aug 2014 in Petäjävesi; see Supplemental Table 1). To study the effect of sediment diffusion system, additional 2–4 points were sampled between discharge and downstream sampling points (Supplemental Table 1). Temperature and dissolved oxygen concentrations in the water column were measured, and bottom water collected for measuring concentration of dissolved inorganic ammonium (NH_4^+) and nitrate + nitrite (NO_x^-) as in Saarenheimo et al. (2017).

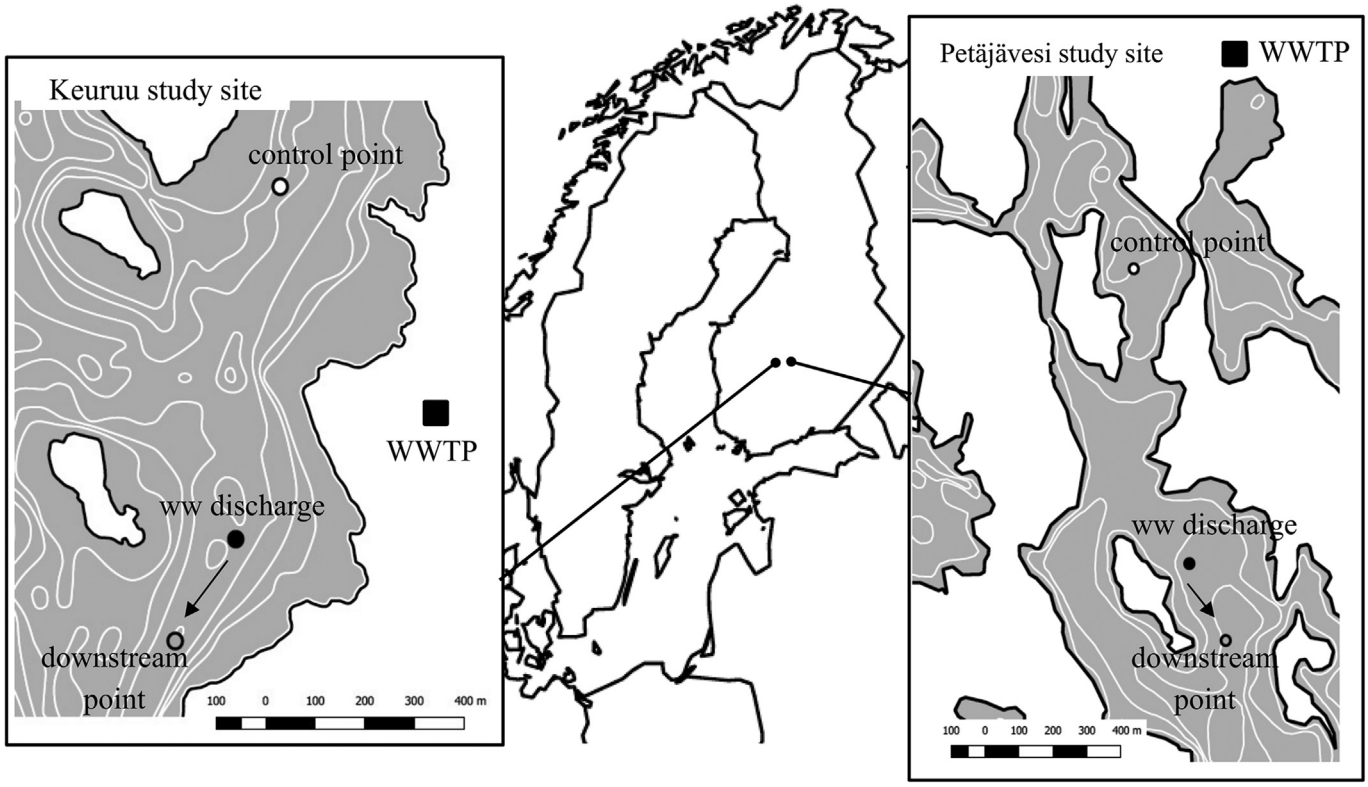

Fig. 1. Map on the study sites, showing the locations of the wastewater treatment plants (WWTP) and three main sampling points (control point, wastewater discharge point and downstream points) at Keuruu and Petäjävesi study sites. Arrow indicates wastewater gradient flowing downstream from the wastewater discharge point, along which the additional sampling points were located.

2.3. Sediment core incubations

After sampling, intact sediment cores were transported to the University of Jyväskylä laboratory and stored at *in situ* temperature in dark until next day. After that, the water above the sediment was replaced with water collected from the sampling sites without disturbing sediment surface, $^{15}NO_3^-$ label ($K^{15}NO_3$, Cambridge Isotope Laboratories) was added to give an initial concentration of 150 μmol/L, and the cores (one core per sampling point) were capped with rubber stoppers and incubated at *in situ* temperature and in dark for 4 h with constant stirring (90 rpm). Pre-incubation time of 5 min was used (Nielsen, 1992), which could have led to appr. 20% underestimation of D14 in lower temperatures due to inhomogeneous mixing of the endogenous $^{14}NO_3^-$ with the exogenous $^{15}NO_3^-$ (Eyre et al., 2002). In addition, the assumptions of IPT (Nielsen, 1992) were verified with concentration series incubations (25, 75, 250 and 400 μmol/L of $^{15}NO_3^-$, one core per concentration per sampling point) at both lake sites in 2014 (11 Feb, 6 May and 14 Oct 2014 in Keuruu, and 8 May, 9 Jun, 11 Aug 2014 in Petäjävesi). In addition, one core was used as an unlabeled control, and one as a time zero control per each sampling point. At the end of the incubations, cores were efficiently mixed and slurry samples were collected for process measurements.

2.4. Isotope analysis

2.4.1. Complete denitrification rates (N_2 production)

Three slurry samples per one core were collected to glass vials (12 mL, Labco), 100 μl of 30% formaldehyde was added to stop the microbial activities, and samples were stored in cold and in dark until isotope analysis. Before IRMS analysis, a helium headspace (~5.5 mL) was added to each sample following Tiirola et al. (2011). The isotope mass areas (*m/z* 28, 29 and 30) and N_2 concentration of the samples were analyzed with Isoprime IRMS connected to Tracegas preconcentrator unit, using a modified N_2O project with no cryotrapping and valves in CO_2 mode. Actual (D14) and "potential" (D15; 150 μmol/L of $^{15}NO_3^-$) denitrification rates, the proportion of coupled nitrification-denitrification (D_n%), and the proportion of denitrification of the NO_3^- in the water above the sediment (D_w%) were calculated as in Rissanen et al. (2013).

2.4.2. Truncated denitrification rates (N_2O production)

A slurry sample of 30 ml was collected to a syringe (60 mL) and N_2O was extracted to helium gas, stored in the prevacuumed glass vial (12 mL), and subsequently analyzed with Isoprime IRMS connected to TraceGas preconcentrator unit. N_2O production was calculated as in Dong et al. (2006).

2.4.3. DNRA rates

DNRA rates were measured from the three main sampling points, and from the two additional points (50 and 100 m downstream from wastewater discharge point) in Keuruu. For the analysis, 100 mL of slurried sediment was collected and filtered (GF/C) and NH_4^+ was isolated by alkaline acid trap diffusion (Holmes et al., 1998), where 40 ml of slurry water together with 2 g of NaCl and 0.12 g of MgO and acid traps (triple 1 cm diameter GF/C filters acidified with 20 μl of 2.5 M $KHSO_4$) was added to 250 mL glass bottles and incubated for four days in 35 °C in shaker. After the incubation, trapped samples were dried for two days in a desiccator with sulfuric acid and atm%15 (the proportion of ^{15}N of total N) of samples was analyzed with Thermo Finnigan Flash EA1112 elemental analyzer connected to a Thermo Finnigan DELTAplus

Advantage IRMS. DNRA rate was calculated as in Christensen et al. (2000).

2.5. Laboratory analysis

Samples for DIN species (NH_4^+, NO_x^-) were filtered through glass fibre filters (Whatman GF/F) and measured as in Rissanen et al. (2011). Furthermore, the porosity and the proportion of organic matter of the sediment (LOI%) were determined for each sampling point as in Rissanen et al. (2011).

2.6. Lake Keurusselkä 3-D flow model

To estimate the contact time between wastewater effluent and lake bottom in the dynamic real world conditions, we used a hydrodynamic simulation. A 3-D model and a simulation model of Lake Keurusselkä, with the wastewater effluent as a conservative tracer, was built using the open source European Union Public License (EUPL) COHERENS code (Luyten, 2013). COHERENS solves the three-dimensional hydrodynamic equations using the finite difference method, assumes hydrostatic balance, and uses the Boussinesq approximation when solving buoyancy. The modelled area is depicted in Supplemental Fig. 2. The model input data included lake bathymetry, wastewater discharge volume and concentrations, lake inflows and outflows, and weather (temperature, precipitation, wind speed and direction, humidity, air pressure). The bathymetry, inflow and outflow data was provided by Finnish Environment Institute, wastewater data by Keuruu WWTP and weather data by Finnish Meteorological Institute (FMI). Additional verification data of water currents was obtained using an Acoustic Doppler Current Profiler (ADCP) device at 15 min intervals over a period of 1 Apr – 22 Jun 2015.

We used version 2.9 of the COHERENS code to develop a nested Lake Keurusselkä high resolution model. First, the entire lake was modelled using a coarse (250 m) horizontal resolution. The results of this simulation were used as boundary conditions for a high resolution (10 m) model around and including the wastewater outlet. Both resolution models used eleven terrain-following vertical layers. Vertical mixing was based on the k-ε turbulence scheme (k: turbulent energy; ε: the rate of dissipation of turbulent energy), TVD (total variation diminishing) advection scheme was used for momentum, and tracers and explicit horizontal diffusion was disabled. In the coarse simulation model, we used time-dependant flows as open boundary data. Using the high resolution model, we simulated a total of eight 48 h long situations: Four different seasons (winter, spring, summer, autumn) and two wastewater discharge pipe configurations (original pipe and diffuser pipe system) for each season. The original wastewater pipe releases the effluent into a single calculation cell (10m × 10m) at ~1 m from the bottom, while the sediment diffuser pipe system spreads the effluent into three cells at the bottom of the water column. The simulation periods were chosen to coincide with field sampling occasions. Relevant input data was used for each season, and the winter scenario also included the effect ice and snow cover. The background concentration of wastewater effluent in the lake was set to zero at the beginning of each modelling scenario, which enabled us to determine how the effluent behaves during the first few hours after the release (see Supplemental video).

Supplementary video related to this article can be found at https://doi.org/10.1016/j.watres.2018.03.068.

The simulation data was saved at 10 min intervals for post-process calculation of the contact time of wastewater and the sediment surface. We defined the contact time to be an arbitrary time interval multiplied by the fraction of wastewater effluent in the bottom layer of the water column vs. the total effluent in the water column during the same time interval. For example, if 20% of all effluent in the model is found in the layer closest to bottom during some 10 min interval, the contact time would be $0.2 \times 600\ s = 120\ s$. This means that in a stable system, when time approaches infinity, the contact time will approach *time interval/number of layers* because the effluent will be nearly fully mixed in the water column. In this method, the only varying parameter is the wastewater discharge pipe configuration. Thus, we could calculate how much the sediment diffuser pipe system altered the contact time, area and concentration of wastewater as compared to the original wastewater pipe during the 48h simulation period.

2.7. Data analysis

Data from Keuruu and Petäjävesi was analyzed separately. The concentration series sediment core data from the three main sampling points (upstream, wastewater discharge point and downstream) was reported as the average of two, or as the average ± standard error (SE) of three to five cores per sampling occasion. Otherwise, no replicate cores were taken (Supplemental Table 1). The relative DNRA rate (%DNRA) was calculated as the contribution of DNRA on total nitrate reduction ($D14 + N_2O + DNRA$) and the relative N_2O production (%N_2O) as the ratio between the N_2O production and total denitrification (i.e. $N_2 + N_2O$) using averaged values per each sampling date and site.

The interactions between transformation rates (D14, D15, N_2O, %N_2O, DNRA, %DNRA) and environmental factors (temperature, oxygen concentration, NO_x^-, NH_4^+, LOI%) were studied using Spearmann rank correlation separately before and after installing the sediment diffusor. For this, samples from 13 Aug 2014, 16 Oct 2014, 31 Aug 2016 and 25 Oct 2016 were used in Petäjävesi data (Supplemental Table 1). In Keuruu data, all sampling dates were included. The differences in N transformation rates and environmental factors before and after the sediment diffusion method experiment were studied with Wilcoxon Signed-Rank Test (Supplemental Table 1). All statistical analyses were conducted using R version 3.3.3 (R Core Team, 2017).

The areal potential denitrification capacity was calculated for Keuruu data by multiplying mean D15 for each sampling date with the estimated wastewater-influenced lake area (33.3 ha). For this, only D15 measured using the same $^{15}NO_3^-$ concentration (150 μmol/L) was used. By comparing the calculated areal denitrification potential (mg NO_x^- d^{-1}) to the long-term data on nitrate input (mg NO_x^- d^{-1}) coming from the WWTP, we could estimate how much sediment denitrification could potentially remove incoming wastewater nitrate per day (proportion of NO_x^- potentially removed through denitrification). We calculated the estimate for each sampling date (season) before and after sediment diffusion experiment and compared those to finally estimate how much sediment diffusion method can promote areal N removal through denitrification. Similar calculations were conducted using D14 to demonstrate the true "areal denitrification capacity", "proportion of NO_x^- removed through denitrification" and the improvement in the latter one.

3. Results

Both study sites had strong seasonal variation in the physico-chemical characteristics (e.g. temperature, oxygen) at wastewater-influenced and control sampling points (Table 1, Supplemental Table 2). In Petäjävesi, LOI% was generally higher and oxygen concentration lower than in Keuruu. At the wastewater-influenced sampling points in both Keuruu and Petäjävesi, NO_x^- concentrations were always higher than NH_4, except in winter 2015 in Keuruu, when the nitrification process collapsed in WWTP (Table 1).

Table 1
Physico-chemical characteristics of wastewater-influenced sampling points before and after sediment diffusion in Keuruu and in Petäjävesi. Values are presented as mean ± SE. The results of Wilcoxon signed rank test are presented.

Keuruu		temperature (°C)	LOI%	O_2 (mg/L)	NO_x^- (μmol/L)	NH_4^+ (μmol/L)	n
Before sediment diffusion (2014)	winter	2.59 ± 0.70	18.81 ± 3.33	9.34 ± 0.51	103.57 ± 82.14	15.71 ± 1.43	
	spring	7.00 ± 0.07	26.09 ± 0.48	10.75 ± 0.09	40.14 ± 19.14	1.21 ± 0.14	
	summer	15.5 ± 0.42	26.38 ± 2.66	3.89 ± 0.77	49.57 ± 17.93	13.17 ± 11.10	
	autumn	8.82 ± 0.02	13.54 ± 0.26	9.63 ± 0.14	16.67 ± 4.11	1.75 ± 0.18	
After sediment diffusion (2015)	winter	0.91 ± 0.21	9.07 ± 3.34	12.85 ± 0.24	61.57 ± 7.21	121.05 ± 8.60	
	spring	8.70 ± 0.04	8.76 ± 0.64	11.15 ± 0.39	16.60 ± 1.25	4.57 ± 0.29	
	summer	18.70 ± 0.03	7.52 ± 0.53	7.39 ± 0.13	43.37 ± 17.10	5.32 ± 1.32	
	autumn	7.49 ± 0.01	10.90 ± 1.15	10.42 ± 0.09	23.20 ± 10.71	6.78 ± 2.57	
total 2014		8.48 ± 1.76	21.21 ± 2.19	8.41 ± 1.02	51.92 ± 20.36	7.96 ± 3.26	8
total 2015		8.95 ± 2.40	9.32 ± 0.77	10.34 ± 0.76	36.19 ± 7.81	34.43 ± 18.98	8
Wilcoxon test		NS	P = 0.012	P = 0.025	NS	NS	

Petäjävesi		temperature (°C)	LOI%	O_2 (mg/L)	NO_x^- (μmol/L)	NH_4^+ (μmol/L)	n
Before sediment diffusion(2014)	summer	13.80 ± 0.50	14.07 ± 1.65	0.52 ± 0.08	27.78 ± 6.95	11.68 ± 8.07	
	autumn	6.72 ± 0.05	17.32 ± 1.56	9.09 ± 0.28	19.76 ± 1.91	3.17 ± 0.15	
After sediment diffusion (2016)	summer	12.89 ± 0.70	34.81 ± 5.15	5.73 ± 0.83	19.79 ± 9.36	3.28 ± 0.82	
	autumn	3.54 ± 0.43	28.98 ± 1.17	9.86 ± 0.44	241.92 ± 230.06	121.51 ± 116.21	
total 2014		9.94 ± 1.14	15.84 ± 1.19	5.20 ± 1.36	23.41 ± 3.37	6.82 ± 3.50	7–11
total 2016		7.79 ± 1.52	31.63 ± 2.46	7.98 ± 0.77	140.95 ± 125.19	70.85 ± 66.55	7–11
Wilcoxon test		P = 0.008	P = 0.003	P = 0.013	NS	NS	

3.1. Nitrate reduction processes along wastewater gradient

In general, the underlaying IPT assumptions on independence of D14 of and D15 increasing with labeled nitrate were met at the three main sampling points in Keuruu and Petäjävesi (Supplemental Fig. 3). However, on 6 May 2014, there was no positive trend between D15 and labeled nitrate at the downstream sampling site in Keuruu, which was most likely due to the missing data from the cores incubated with the two lowest concentrations of $^{15}NO_3^-$. Furthermore, on 11 Feb 2014, D14 was increasing with labeled nitrate at the wastewater-influenced sampling points in Keuruu. Nitrate reduction rates were highest at the wastewater-influenced sampling points (Figs. 2–3). At both study sites, the complete (N_2 production; D14) and potential (D15) denitrification rates tended to decrease when moving downstream from the discharge point (Figs. 2–3). DNRA rates measured in Keuruu had a similar decreasing pattern (Fig. 2). Truncated denitrification (N_2O production) or relative N_2O production rates (%N_2O) did not show any clear pattern between the wastewater-influenced sampling points (Figs. 2–3). In Keuruu, where all three nitrate reduction processes were measured, denitrification was the main nitrate reduction process (86 ± 12% of all nitrate reduction). There was no difference in the contribution of DNRA in nitrate reduction (% DNRA) between the wastewater-influenced sampling points (9 ± 2%) and the control point (11 ± 5%).

3.2. The influence of the sediment diffusion method on nitrate reduction processes

Based on 3D modelling, the diffuser pipe system increased the contact time of wastewater with the sediment surface during the first few hours after release when compared to the original configuration (Supplemental Fig. 4). This was observed especially in spring and in autumn. In winter (ice-covered time), the effect was lowest but still positive. In lake-scale, the wastewater concentration near the sediment increased with the sediment diffusion method in all seasons. However, no changes in total contact area were seen (Fig. 4).

Nitrate concentrations did not increase after the sediment diffusion at the study sites (Wilcoxon, $P > 0.05$; Table 1). At both study sites, oxygen concentration at the bottom was higher after the sediment diffusion, and LOI% was lower in Keuruu, but higher in Petäjävesi (Wilcoxon, $P < 0.05$, Table 1). Sediment diffusion method did not increase D14 (Keuruu: before 881 ± 230 after 767 ± 163, Petäjävesi: before 451 ± 80 after 2689 ± 2088, Wilcoxon, $P > 0.05$), but the overall potential denitrification rates (D15) increased at both sites (Keuruu: before 1056 ± 184 after 2048 ± 408, $Z = -2.20$, $P = 0.028$; Petäjävesi: before 820 ± 91 after 2087 ± 469, Wilcoxon, $Z = -2.22$, $P = 0.026$). There was no difference in N_2O, %N_2O or % DNRA before and during the diffuser pipe system ($P > 0.05$), but DNRA rates decreased significantly in Keuruu (before 218 ± 82 after 70 ± 28, $Z = -2.10$, $P = 0.036$).

The correlation patterns between the nitrate reduction rates and environmental factors before and after the sediment diffusion varied between the two study sites (Table 2). In Petäjävesi, D14 did not correlate with inorganic N concentrations, but increased with oxygen and LOI% before the sediment diffusion. After the optimization, it correlated positively with nitrate and ammonium. Before the optimization, D15 correlated positively with D14, oxygen and LOI% and negatively with temperature, but after that, the correlation to LOI% remained but D15 was higher when temperature was high and oxygen concentration low. Before the optimization, N_2O production and %N_2O increased with temperature and decreased with oxygen, and %N_2O decreased with LOI%. After the wastewater discharge was spatially optimized, both actual N_2O production and %N_2O decreased with LOI%.

In Keuruu, D14 was always related to nitrate concentration, and after the diffuser pipe system, also to ammonium (Table 2). Before the sediment diffusion method, D15 was higher in higher temperatures and in lower oxygen concentrations. After the optimization, it correlated with D14, but not with any environmental factors. Before the optimization, %N_2O increased with oxygen but after that only with decreasing LOI%. The DNRA rates always followed nitrate and oxygen concentrations and were higher when temperature was low, but they also followed ammonium after the sediment diffusion. A similar relationship was found between %DNRA and temperature and oxygen in 2014, but not after the sediment diffusion. In both years, %DNRA was higher when LOI% was low.

3.3. The influence of sediment diffusion method on areal nitrate removal capacity

We estimated that denitrification could potentially remove 3–10% of WWTP NO_x^- load (mg d^{-1}) coming to the area in Keuruu

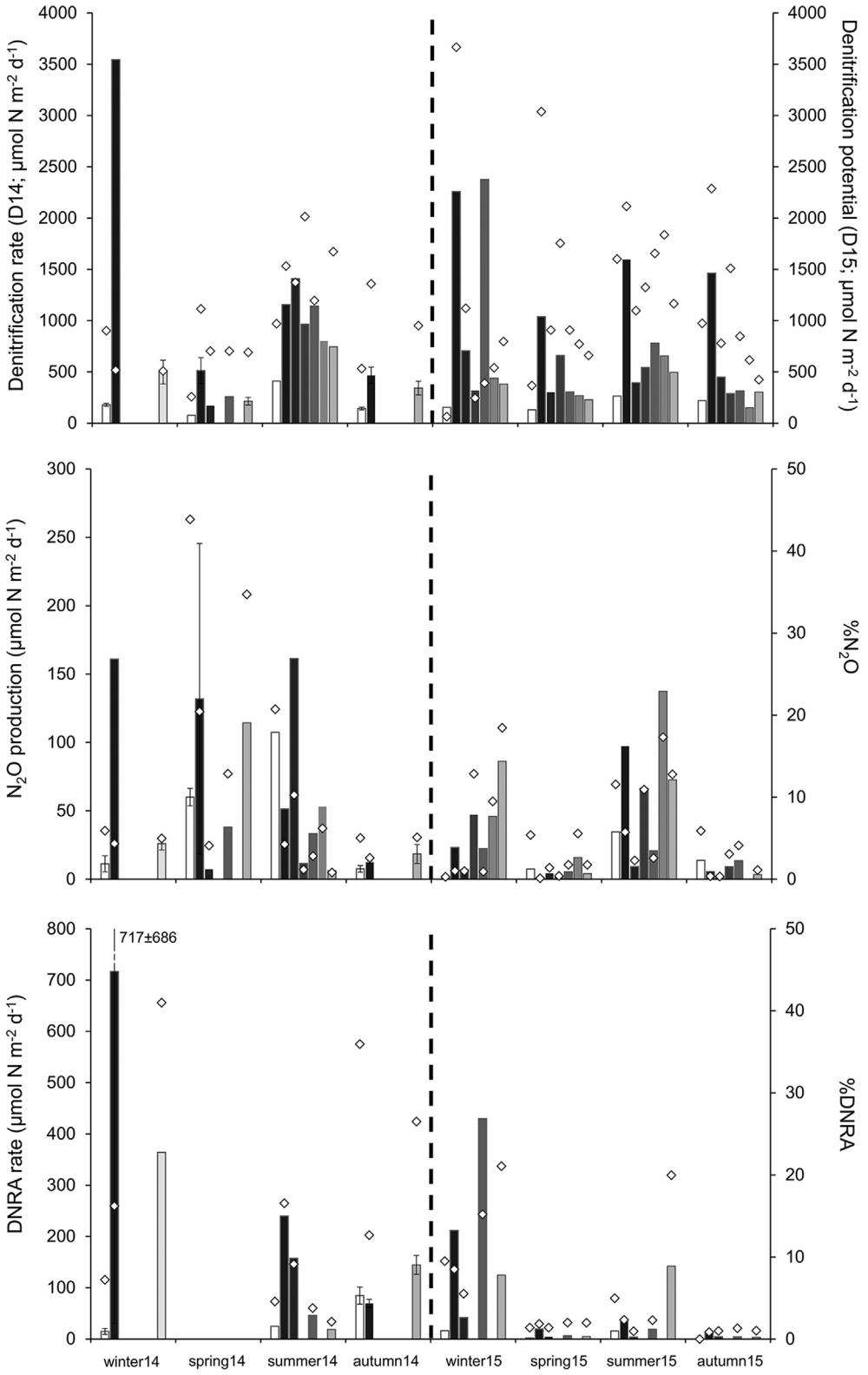

Fig. 2. A) Complete denitrification (D14) rates (bars) and denitrification potential (D15; dots), B) N_2O production (bars) and relative N_2O production (%N_2O; dots), and C) DNRA rates (bars) and relative DNRA (%DNRA; dots) in Keuruu. Dashed line indicates the beginning of the sediment diffusion of the wastewater discharge. White bar indicates denitrification rate at the control sampling point, black bar represents wastewater discharge point and color gradient follows the wastewater gradient, lowest sampling point being light grey.

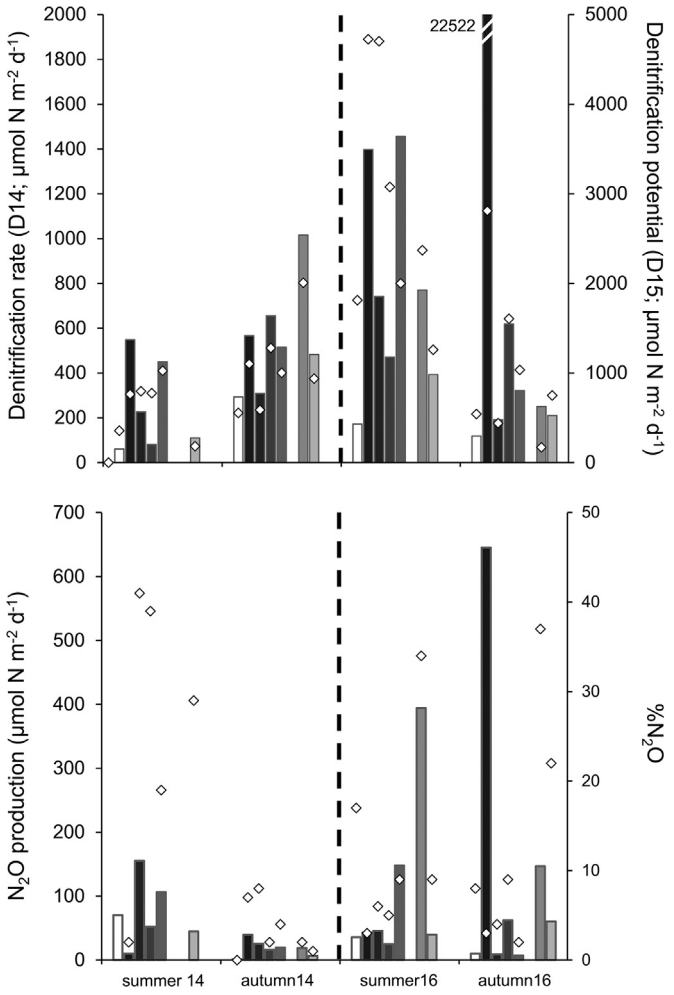

Fig. 3. A) Complete denitrification (D14) rates (bars) and denitrification potential (D15; dots), and B) N_2O production (bars) and relative N_2O production (%N_2O; dots) in Petäjävesi. Dashed line indicates the beginning of the sediment diffusion of the wastewater discharge. White bar indicates denitrification rate at the control sampling point, black bar represents wastewater discharge point and color gradient follows the wastewater gradient, lowest sampling point being light grey.

(Table 3). After the implementation of the sediment diffusion method, the removal was improved in winter, spring and autumn, whereas it declined slightly in summer. However, when the removal estimate was based on true denitrification values measured (D14), denitrification could remove 1–15% of WWTP NO_x^- load, and sediment diffusion method improved removal only in spring and autumn (Table 3).

4. Discussion

As expected, highest nitrate reduction rates were observed close to the wastewater discharge points. The measured N_2 production rates (D14; 150–3500 µmol N m^{-2} d^{-1} in Keuruu and 80–23500 µmol N m^{-2} d^{-1} in Petäjävesi) at these points were comparable to, or even exceeded the previous reports from eutrophic/hypertrophic lakes (900–1200 µmol N m^{-2} d^{-1}: Mengis et al., 1997; 290–1700 µmol N m^{-2} d^{-1}: Risgaard-Petersen et al., 1999) and wastewater-influenced estuarine sediments (40–370 µmol N m^{-2} d^{-1}: Bonaglia et al., 2014), and denitrification was the main nitrate reduction pathway at all wastewater-influenced sampling points. Both denitrification and DNRA rates followed the wastewater gradient, decreasing downstream as mixing and dilution decreased the wastewater and nitrate concentration. There was also some seasonal variation, as in Keuruu, complete denitrification and DNRA were highest in winter, contradicting with the previous studies from boreal lakes, in which highest rates have been found in summer when nitrate and organic matter concentrations as well as temperature are high (Ahlgren et al., 1994; Rissanen et al., 2011). This is probably due to the contact rate between wastewater and sediment being naturally high during the winter, as also the highest nitrate concentrations were observed then in Keuruu. Then, we also saw D14 increasing with labeled nitrate, which suggest that anammox could contribute to N_2 production (Risgaard-Petersen et al., 2003). However, high nitrate and organic matter concentrations at wastewater-influenced sampling points should not favor anammox, as has been demonstrated earlier in wastewater-influenced estuary sediments (Bonaglia et al., 2014). It is possible that the amount of labeled nitrate was not high enough as compared to the natural nitrate concentrations or too short pre-incubation period (Eyre et al., 2002), leading to inhomogeneous mixing of label with natural nitrate. This was observed only in winter 2014 in Keuruu, when the nitrate concentration was the highest at the wastewater-influenced sites. Agreeing with previous results on genetic nitrous oxide reduction potential in Keuruu (Saarenheimo et al., 2017), N_2O production was not following wastewater nitrate gradient, but was possibly related to seasonal factors and organic matter availability. Although the absolute DNRA rates were occasionally high at the wastewater-influenced sampling sites, the average contribution of DNRA in nitrate reduction was similar between wastewater and control points. In general, observed %DNRA was comparable to the ones recorded in eutrophic lake during high C availability (15%; Nizzoli et al., 2010), but not as high as in aquaculture-influenced sediments (300%; Christensen et al., 2000) or in wastewater-contaminated estuary sediments (50–1700%; Bonaglia et al., 2014). Although wastewater-influenced sampling points seem to have higher amount of organic matter (LOI %), the organic matter quality could be less favorable for the fermentative DNRA bacteria (Akunna et al., 1993). In addition, the amount of nitrate was always significantly higher at the wastewater sampling points, lowering C:N, which should favor denitrifying bacteria (Kraft et al., 2014). The habitat characteristics may also be more dynamic and turbid, following the wastewater discharge volume, and this could suppress the growth of DNRA bacteria (Nogaro and Burgin, 2014).

The 3D modelling results confirmed that the sediment diffusion method enhanced the contact time between wastewater effluent and sediment in Keuruu. This was seen especially in spring and in autumn, which are the mixing periods in the boreal lakes. We did not expect any enhancement in winter, when wastewater is naturally at the lake bottom and the water residence time is long. Also in summer, heavy wastewater, having high conductivity, is supposed to stay at the bottom. Although sediment diffusion method increased near-bottom wastewater concentration, it did not increase the total wastewater-influenced area, indicating that the diffuser pipe system was probably not long enough. In our sampling data, we did not see a significant increase in the nitrate concentration in the bottom water after the diffuser pipe system. It seems that process rates and modelling results are more reliable estimates of the effect of sediment diffusion method on N dynamics than only nitrate concentration. Interestingly, we saw higher oxygen concentration at both study sites after the sediment diffusion method, while organic matter concentration increased in Petäjävesi and decreased in Keuruu, both factors being important in controlling nitrate reduction. Increased oxygen was most likely reflecting the higher concentration of fully oxygen saturated wastewater effluent near the sediment surface. The lower LOI% in Keuruu is in agreement with previous study from wastewater-influenced river

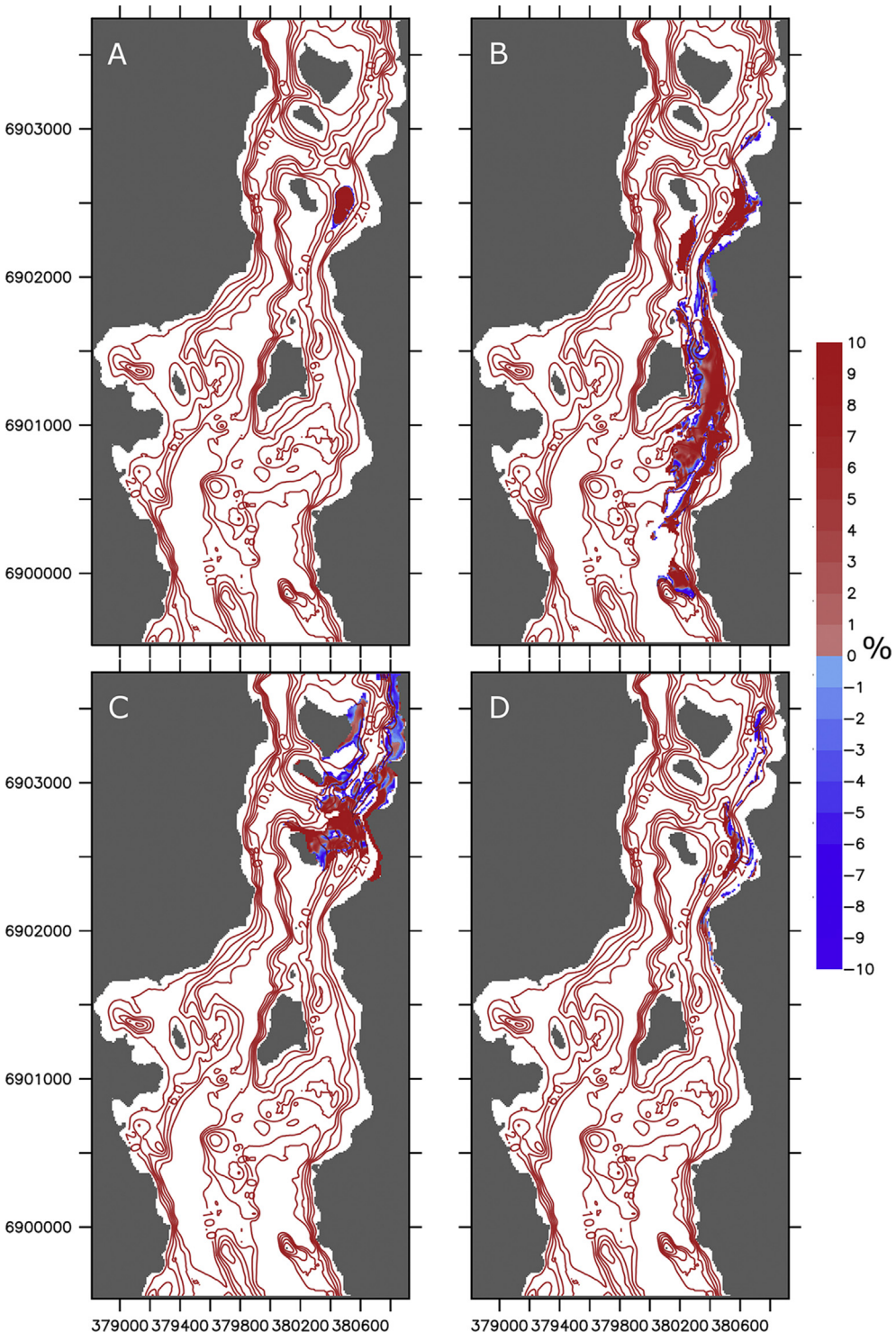

Fig. 4. The relative difference (%) in near-bottom wastewater concentration between the sediment diffuser pipe system and original discharge pipe configuration at Keuruu study site after 40h simulation in A) winter, B) spring, C) summer and D) autumn scenarios. Red indicates relatively higher and blue relatively lower concentration after the sediment diffusion method implementation. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

sediments (Lofton et al., 2007), and could be due to more turbid conditions after the sediment diffusion. In Petäjävesi, the wastewater discharge point has naturally lower water velocity and lower wastewater effluent volume, suggesting that after the sediment diffusion, WWTP-derived organic matter could be sedimentated more efficiently to the bottom, resulting in higher LOI%. However, the composition and quality of organic matter probably changed at both study sites after the implementation of the sediment diffusion method.

Potential denitrification rates (D15) were higher after the diffuser pipe system at both study sites, and this was observed especially at the wastewater discharge sampling point, where D15 were on average three times higher. Since D15 was not related to the environmental nitrate concentrations, wastewater promoted it

Table 2
Significant correlations between environmental factors in bottom water and sediment and complete denitrification (D14), potential denitrification (D15), truncated denitrification (N_2O), and relative N_2O production (%N_2O) at two study sites, and DNRA rates and relative DNRA rate (%DNRA) in Keuruu before and after the sediment diffusion.

Keuruu					
Before sediment diffusion			After sediment diffusion		
	Correlation coefficient	P value		Correlation coefficient	P value
D14 vs. NO_x^-	0.81	<0.001	D14 vs. NO_x^-	0.72	<0.01
D14 vs. NH_4^+	0.74	<0.01	D14 vs. NH_4^+	0.75	<0.01
N_2O vs. NO_x^-	0.71	<0.01	N_2O vs. O_2	−0.58	<0.05
N_2O vs. NH_4^+	0.59	<0.05	N_2O vs. LOI%	−0.56	<0.05
%N_2O vs. O_2	0.61	<0.05	%N_2O vs. LOI%	−0.68	<0.01
D15 vs. O_2	−0.65	<0.05	D15 vs. D14	0.63	<0.05
D15 vs.T	0.86	<0.001	DNRA vs. NO_x^-	0.73	<0.001
DNRA vs. NO_x^-	0.64	<0.01	DNRA vs. NH_4^+	0.78	<0.001
DNRA vs. O_2	0.50	<0.05	DNRA vs. O_2	0.56	<0.05
DNRA vs. T	−0.68	<0.01	DNRA vs. T	−0.67	<0.01
%DNRA vs. O_2	0.75	<0.001	%DNRA vs. LOI%	−0.87	<0.001
%DNRA vs. T	−0.91	<0.001			
%DNRA vs. LOI%	−0.77	<0.001			
Petäjävesi					
Before sediment diffusion			After sediment diffusion		
	Correlation coefficient	P value		Correlation coefficient	P value
D14 vs. O_2	0.85	<0.001	D14 vs. NO_x^-	0.93	<0.001
D14 vs.T	−0.85	<0.001	D14 vs. NH_4^+	0.92	<0.001
D14 vs. LOI%	0.74	<0.01	N_2O vs. NO_x^-	0.84	<0.001
N_2O vs. O_2	−0.80	<0.01	N_2O vs. NH_4^+	0.81	<0.001
N_2O vs. T	+0.80	<0.01	N_2O vs. LOI%	−0.68	<0.01
%N_2O vs. O_2	−0.95	<0.001	%N_2O vs. LOI%	−0.70	<0.01
%N_2O vs. T	0.95	<0.001	D15 vs. O_2	−0.72	<0.01
%N_2O vs. LOI%	−0.59	<0.05	D15 vs.T	0.92	<0.001
D15 vs. D14	0.92	<0.001	D15 vs. LOI%	0.59	<0.05
D15 vs. O_2	0.81	<0.01			
D15 vs.T	−0.81	<0.01			
D15 vs. LOI%	0.66	<0.05			

Table 3
Mean wastewater NO_x^- input, areal denitrification potential, proportion of wastewater NO_x^- potentially removed through denitrification, and improvement of that, as well as real areal denitrification capacity, proportion of wastewater NO_x^- removed through denitrification, and improvement of that after the sediment diffusion method in Keuruu.

		mean wastewater NO_x^- input	areal denitrification potential	proportion of NO_x^- potentially removed through denitrification	improvement	areal denitrification capacity	proportion of NO_x^- removed through denitrification	improvement
		(kg d^{-1})	(kg d^{-1})	(% d^{-1})	(%)	(kg d^{-1})	(% d^{-1})	(%)
winter	before	63.9	2.4	3.7%	120%	9.4	14.7%	−17%
	after		5.2	8.2%		7.6	12.1%	
spring	before	131.9	4.2	3.2%	48%	1.3	1.0%	61%
	after		6.2	4.7%		2.2	1.6%	
summer	before	69.1	7.2	10.4%	−4%	4.8	6.9%	−21%
	after		6.9	10.0%		3.8	5.5%	
autumn	before	68.0	5.4	7.9%	17%	1.9	2.8%	22%
	after		6.3	9.2%		2.3	3.4%	

through some other mechanism. We have previously shown that wastewater shapes the sediment microbial community composition significantly by bringing in WWTP microbes and modifying habitat characteristics (Saarenheimo et al., 2017), so it is likely that by altering oxygen and organic matter concentrations and quality, wastewater favored certain microbes, which directly or indirectly contributed to denitrification. The connection between wastewater and D15 is further corroborated with the found correlations between D15 and D14 in Keuruu, and D15 and LOI% in Petäjävesi. The connection between D15 and D14 was found in Petäjävesi even before the sediment diffusion, and our recent study showed that wastewater has indeed stronger impact on microbial community there than in Keuruu (Saarenheimo et al., 2017). Complete denitrification (D14) and D_w% (data not shown) followed inorganic N concentrations at both study sites, and did not significantly increase after the sediment diffusion, but the method successfully decreased seasonal effects (e.g. oxygen or temperature) on N_2 production, suggesting that the conditions were more favorable to denitrification microbes throughout the year. Furthermore, although higher oxygen concentration could have supported nitrification and coupled nitrification–denitrification (Bonaglia et al., 2013), we did not find a significant increase in the proportion of coupled nitrification–denitrification (D_n%) after the sediment diffusion (data not shown). One reason for D14 not increasing after the implementation of the sediment diffusion method could be inhomogenous mixing of labeled and natural nitrate during IPT incubations, due to inadequate pre-incubation period and higher OPD at cold temperatures (Eyre et al., 2002). However, within-season temperatures remained similar in Keuruu, so possible underestimation of D14 happened at both study years. In Petäjävesi,

there was a slight decrease in temperatures after the sediment filtration system, but since only summer and autumn samples were included in the data, the temperature-driven D14 underestimation was probably rather small. We think that best explanation for D14 not increasing is that inorganic nitrogen concentration remained similar and there was no increase in the proportion of coupled nitrification-denitrification after the implementation of the sediment diffusion method.

The increased oxygen concentration can explain the decrease in DNRA observed in Keuruu, as re-oxygenation of sediments favors denitrification over DNRA (De Brabandere et al., 2015). As the oxygen concentration increased after the sediment diffusion, obligate anaerobic DNRA bacteria were possibly suppressed (Nogaro and Burgin, 2014). Decrease in DNRA rate coincided also with decreased LOI%, implying that lower carbon availability suppressed DNRA (Kraft et al., 2014). We did not analyze DNRA in Petäjävesi, where LOI% increased. However, it was rather unlikely that DNRA would have been promoted there, as we saw no significant decrease in D14, and D15 even increased, and conditions were probably too oxygen-rich and turbid for DNRA bacteria. Furthermore, the quality of sediment organic matter can be even more important in governing the end-product of nitrate reduction than the carbon content. WWTP-derived organic matter is considered to be more biodegradable and protein-rich than natural organic matter (Nam and Amy, 2008), which should favor especially denitrifying microbes (Barnes et al., 2012).

Sediment diffusion method did not affect N_2O production or $\%N_2O$. It is possible that in addition to denitrification, some proportion of N_2O was derived from nitrification. For example, the negative correlation (see Goreau et al., 1980) found between N_2O and oxygen in Keuruu after the sediment diffusion suggests that nitrification could be the main source of N_2O. This correlation was also observed in Petäjävesi before the sediment diffusion. Organic matter concentration seems to be the main factor controlling N_2O production and $\%N_2O$, especially after the sediment diffusion, when nitrate levels were probably not limiting (Zhao et al., 2014). In general, higher carbon availability facilitates complete denitrification (Weymann et al., 2010). The interaction between N_2O and LOI% more likely reflects spatial distribution of N_2O production rather than the effect of sediment diffusion (as LOI% decreased but $\%N_2O$ did not increase in Keuruu after the sediment diffusion), since after the sediment diffusion, $\%N_2O$ was constantly higher at downstream sampling points where LOI% was lower. Interestingly, both N_2O and $\%N_2O$ were higher in Petäjävesi than in Keuruu, although LOI% was higher there even before the sediment diffusion. In Petäjävesi, the loading of allochthonous carbon from the surrounding catchment area is substantial and lake color is darker than in Keuruu. This suggests that a significant proportion of sediment organic matter might be recalcitrant and less favorable for denitrifiers, although seems to support the denitrification process until N_2O, agreeing with previous studies on carbon-amended denitrification rates at the Baltic Sea oxic–anoxic interface (Bonaglia et al., 2016) and in boreal lakes sediments (Myrstener et al., 2016). However, further studies are needed to understand the importance of wastewater organic matter quantity and quality in governing these different nitrate reduction processes.

Areal denitrification calculations based on D15 revealed that natural sediment denitrification could potentially remove 3–10% of wastewater nitrate input at Keuruu site, and sediment diffusion can increase the rate by 17–120%. When using true denitrification values (D14), the proportion of wastewater nitrate removed was 1–15%, and sediment diffusion increased the rate by 22–61%. Although these calculations are based on process rates measurements from sampling points and calculated only on the 33 ha study area, we can expect that they are rather realistic, as our field observations have shown that wastewater can be detected during the first 400 m downstream from the discharge site and is after that efficiently diluted. We did not take the improvement in the wastewater contact time or concentration into account in the calculations, so in reality the improvement in the total areal nitrate removal capacity was probably higher. Previous estimates on denitrification nitrate removal potentials have been ~2% of incoming nitrate from river sites (Lofton et al., 2007) and 60–70% from constructed wetlands (Lee et al., 2009). Sediment diffusion method enhanced areal nitrate removal in spring and in autumn, which followed the pattern in contact time. However, potential nitrate removal was increased also in winter, although wastewater is then naturally near the sediment surface, and thus no improvement in contact time was expected. Furthermore, no change in contact time was observed in winter based on 3D modelling. What makes it especially interesting is that in winter after installing the sediment diffusor pipe, nitrification process in WWTP collapsed for several months, and wastewater effluent consisted mainly of ammonium nitrogen. Possibly nitrification benefitted from the better oxygenation, compensating the problems observed in the function of the WWTP and feeding efficiently denitrification community, increasing the areal nitrate removal potential. When using D14-based estimate in the calculations, no improvement in the wastewater nitrate removal was seen in winter, which can be explained with low nitrate concentration after the sediment diffusion, as nitrification collapsed in WWTP. Here, D15-based estimate is probably more realistic, since it is always based on the same amount of label, which corresponds the wintertime nitrate concentrations in Keuruu, when WWTP nitrification is functional.

5. Conclusions

The full-scale experiments showed that sediment diffusion method can create more favorable conditions for the sediment microbes, and thus increase the denitrification potential. Furthermore, they showed that in general, wastewater promotes nitrate reduction, supporting especially N_2 production through complete denitrification. As the nitrate-rich wastewater had a longer contact time with the sediment, sediment diffusion method enhanced the total areal wastewater nitrate removal, especially in spring and autumn, when wastewater would have otherwise been mixed with the lake water. However, in order to utilize sediment microbes more efficiently in wastewater N removal, diffuser pipe system should be further modified to increase the effluent contact time and area with the sediment. Through this methodology, nitrate removal can be enhanced with low costs in treatment plants where nitrification is part of the process. As the implementation of the sediment diffusion method is easy and inexpensive, it would be especially recommended for supplementing nitrate removal in small and medium-sized nitrifying WWTPs, where the construction of post-nitrification processes is not economically feasible.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.watres.2018.03.068.

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