

Factors Affecting Denitrification Rate in Barato Lake, Hokkaido, Japan

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Abstract: We examined the sediment denitrification rate and factors affecting the denitrification in Barato lake, an eutrophic oxbow lake in lower Ishikari river basin. Denitrification rate was measured using acetylene inhibition technique on the sediment samples collected during August 2009 to July 2010. The sediment denitrification rate ranged from 0.13 to 2.21 $\mu\text{g N g}^{-1}\text{DM h}^{-1}$ with an average of 0.91 $\mu\text{g N g}^{-1}\text{DM h}^{-1}$ in Barato lake. Denitrification rate showed positive correlation with dissolved nitrate concentration of lake water, indicating that water column nitrate concentration control the sediment denitrification rate in Barato lake. Nutrient amended denitrification assay experiments showed that denitrification rate increased significantly with addition of nitrate regardless of sediment C content, while no significant variation was observed with addition of labile C (glucose). Temperature positively affected the rate of denitrification with five-fold increase in activity on increasing temperature from 5°C to 20°C. Nitrate controlled denitrification followed Michaelis–Menten kinetics with V_{max} as 2.65 $\mu\text{g N g}^{-1}\text{DM h}^{-1}$ and K_m as 0.48 mg/l NO_3^- -N. The value of K_m was comparable with other studies but was well below the median dissolved nitrate concentration (2.95 mg/l NO_3^- -N) indicating that the process of denitrification was saturated in Barato lake.

Key words: Barato lake, denitrification rate, Ishikari river, Michaelis–Menten kinetics.

Introduction

Increasing nitrogen loading of aquatic system due to anthropogenic activities such as over use of nitrogen fertilizer, land use change along with increased recycling of domestic waste is a major environmental concern in recent decades (Pattison et al., 1998; Torre et al., 1992). This increased nitrogen loading leads to coastal hypoxia and eutrophication of fresh water bodies which undermine the use of these water bodies for human need and also affect the aquatic ecosystem structure and function due to noxious algal bloom. For the better management and protection of freshwater bodies it is important to understand processes which lead to removal of nitrogen from aquatic ecosystem. Denitrification, a microbially mediated process in which oxides of nitrogen

(nitrate/nitrite) are reduced to N_2O and N_2 (Payne, 1973) and permanently removed from the system, is one such process. Denitrification rate in an aquatic system is controlled by nitrate loading, availability of organic matter which acts as an electron donor, reduced oxygen concentration and temperature (Thouvenot-Korppoo et al., 2009).

Barato lake, the largest oxbow lake in Ishikari river system formed during the early Showa era (1926–88), is a shallow (mean depth 3.3 m) and eutrophic lake. It receives domestic waste supply from large urban area of Sapporo city (Nakatsugawa and Hamahara, 2004). Barato lake is situated at higher latitude (43°09'56.5"N, 141°21'7.5"E) due to which its surface freezes partly in winter (December–March) and melts during spring season (April–May). This spring melting results in

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increased input of nutrient especially nitrate and phosphate from lake catchments area with melted water, which eventually cause algal blooms and eutrophic condition in Barato lake system. Eutrophication studies in Barato lake often focus on phosphorous, since it is most common nutrient (Hino, 1988; Nakatsugawa and Hamahara, 2004) but there are not enough studies on nitrogen cycling especially denitrification process in Barato lake sediment. Denitrification process plays an important role in eutrophic system by affecting the availability of nitrate to phytoplankton and also restricts the release of phosphorous and heavy metal from sediment to overlaying anoxic water column (Messer and Brezonik, 1984).

The aim of present study was to estimate potential denitrification rate in Barato lake sediment and to identify factors affecting the denitrification rate through laboratory amendment experiment. High nutrient load from urban area and eutrophic condition develop due to natural freezing and melting process makes Barato lake a suitable study area for assessing sediment denitrification process.

Material and Methods

Study Area

The Barato lake is an oxbow lake about 20 km long and 200 m wide, situated in northern Sapporo ($43^{\circ}09'56.5''\text{N}$, $141^{\circ}21'7.5''\text{E}$) (Figure 1). The lake is the former meander channel of the Ishikari River, which was cut off in 1933 during river improvement work. The lake serves multiple purposes such as flood regulation, fishing and recreation for city residents, as well as conservation of riparian environment. The main sources of water exchange in Barato lake are: (1) inflow from rivers (the Sosei, Fushiko, and Hassamu rivers), (2) backflow from Ishikari river through the Shibi channel, (3) discharge from the Barato sewage treatment plant, (4) storm water from areas without a sewerage treatment facility, and (5) groundwater (Nakatsugawa and Hamahara, 2004). Besides supply from waste water treatment plants, runoff from surrounding agriculture area (paddy field) is main source of nutrients to Barato lake.

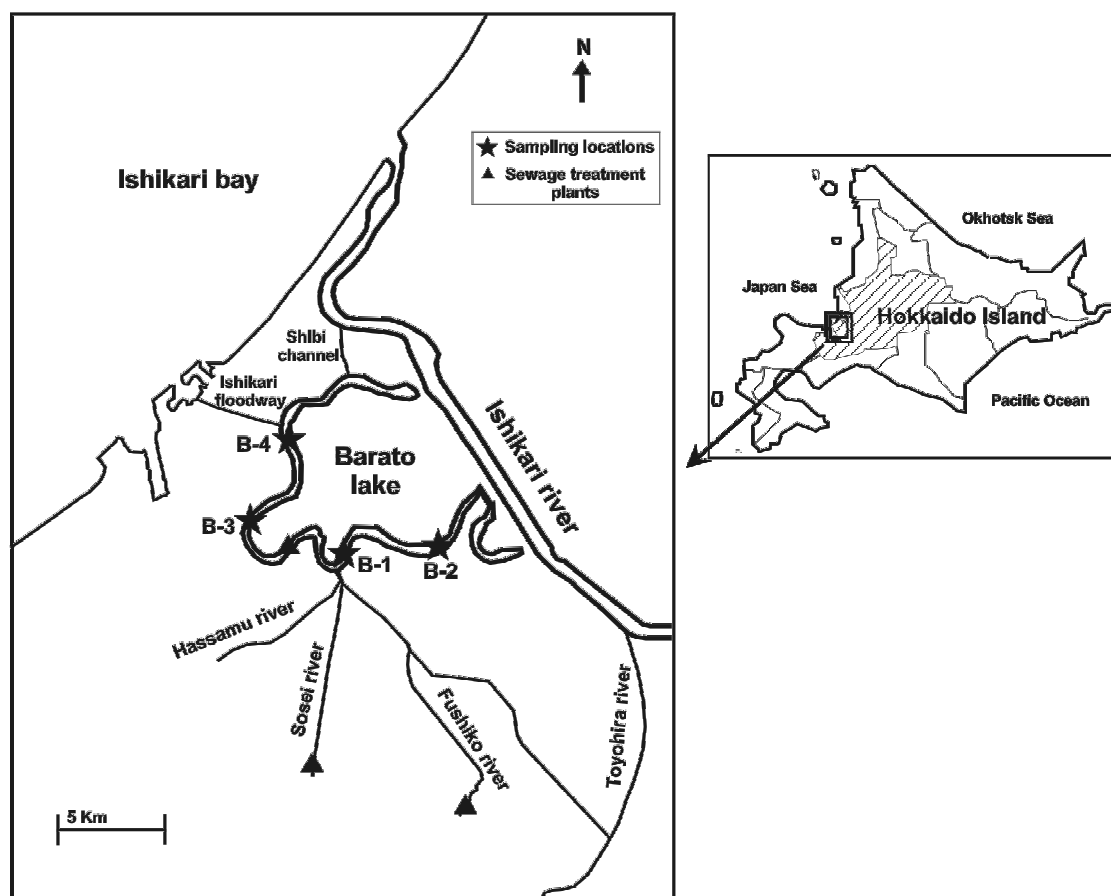


Figure 1: Sampling locations map of Barato lake.

Field Sampling

Four sampling locations were selected in Barato lake depending upon accessibility and water samples from all these locations and sediment samples from three locations were collected during summer (June–August), autumn (September–November), winter (December–March) and spring (April–May) of 2009–10 (Figure 1). However, samples were not collected during September 2009 and January and February 2010. Water samples were collected using stainless steel bucket attached to string from top of bridge and stored in an acid-cleaned polyethylene bottle. Water temperature, pH and ORP were measured onsite by using mercury thermometer, pH meter (Shindengen pH BOY-P2 model) and ORP meter (DKK•TOA, RM-20P), respectively. In the laboratory, water samples were filtered through pre-combusted (3 hours at 400 °C) and pre-weighted Whatman GF/F fibreglass filters (47 mm) and stored at 4°C until analysis. Sediment samples were collected using Eckman Barge Bottom Grab Samplers and top 0–10 cm sediment samples were transferred into a sterilized plastic bag. Samples were then transported in an ice-box to the laboratory and were stored at 4°C until processed.

Sample Analysis

Water samples were analysed for dissolved nitrate (NO_3^-), nitrite (NO_2^-), ammonium ion (NH_4^+), phosphate (PO_4^{3-}) and silica (SiO_2) by photo-metrically using auto analyser (Quattro; Bran + Luebbe, Germany). Dissolved Organic Carbon (DOC) was analysed using TOC analyser (Shimadzu TOC-5000A, Japan). Before DOC analysis, water samples were acidified with 1N HCl solution and purged with N_2 gas for removal of inorganic carbon content. Sediment sample were oven dried at 60 °C and dried sample were homogenized and analysed for total organic carbon (TC_{org}) and total nitrogen (TN) in a CN elemental analyser (Fison NA 1500). Samples were treated with 1.2 N HCl for removing inorganic carbonate present in the sediment before analysis of total organic carbon. Loss on ignition (LOI %) was measured by calculating the weight loss after heating dry sediment sample to 550 °C for 6 hours. The grain sizes were measured using a Horiba laser scattering particle size distribution analyser (LA-920, Japan). The percentages of grain size group: clay (0.02–4 μm), silt (4–63 μm) and sand fraction (63–500 μm) were determined (Zhong et al., 2010).

Denitrification Assay

Potential denitrification rate was measured using the Denitrifying Enzyme Activity (DEA) Assay (Groffman et al., 1999) an acetylene inhibition based technique with some modification. Prior to sediment slurry addition, chloramphenicol was added to 160 ml borosilicate glass media bottle to a final concentration of 5 mM. Chloramphenicol is an antibiotic that inhibits *de novo* protein synthesis and reduces bottle effects associated with laboratory slurry incubations, allowing for more accurate estimates of in situ rates (Arango et al., 2007). Approximately 25 ml of sediment slurry prepared with addition of distilled water was added into glass media bottle and lake water was added to reach final volume of 75 ml.

Oxygen was purged from each bottle by continuously pumping helium at 35 ml min^{-1} for 7 min and shaking bottle every 30 second. About 16 ml of acetylene was added to reach a final concentration of 10%, and sample bottles were placed on a shaker table and shaken (125 r/min) in dark at fixed temperature. 1 ml headspace sub sample were taken out at 0, 30, 60 and 120 minutes for measurement of N_2O gas concentration in headspace of media bottle. The original positive pressure in media bottle was maintained by addition of 1 ml replacement gas (10 part acetylene/90 part helium). We analysed head space sub samples by manually injecting 1000 μl into a gas chromatograph (Shimadzu GC-8A, Japan) equipped with a porapak Q column (80/100 mesh; 2m length) and electron-capture detector (injector temperature 300 °C; column temperature 80 °C; detector temperature 300 °C; ultra pure mixed gas (95% Ar + 5% CH_4) as carrier gas with flow rate 10 ml min^{-1}). Total concentration of N_2O at each sampling period was calculated using the appropriate Bunsen coefficient to determine the amount of gas dissolved in water at a given temperature (Tiedje, 1982). Denitrification rate was calculated as the slope of best fit curve in the N_2O concentration against time plot. N_2O production rate was expressed as denitrification rate by converting N_2O to N and normalizing by substratum dry mass (DM) ($\mu\text{g N g}^{-1} \text{DM h}^{-1}$).

Treatment Experiments

In treatment experiment nitrate, carbon and temperature were manipulated to determine their effect on denitrification rate in Barato lake sediment. To analyse the effect of nitrate concentration on denitrification rate, rate measurements were done at four different concentrations of nitrate (KNO_3) (0, 2, 10 and 20 $\text{mg/l NO}_3^- \text{N}$). All four treatments received non-limiting concentrations of

carbon (20 mg/l glucose-C) and were incubated at 25 °C to eliminate the effect of co-substrate limitation (Herrman et al., 2008). Denitrification rate measurements were carried out by following method mentioned in denitrification assay section but in case of treatment experiment deionized water was added to each bottle with a corresponding amendment to reach final volume of 75 ml. Using deionized water as compared to lake water allowed us to calculate the exact amount of substrate each media bottle received during the treatment experiment. Effect of carbon (glucose) concentration on denitrification activity was also tested for four different concentrations 0, 2, 10 and 20 mg/l glucose-C. All treatments received non-limiting concentration of nitrate (20 mg/l NO_3^- -N) and were incubated at 25°C. The effect of temperature on denitrification was tested at high (20°C) and low (5 °C) temperature on slurries receiving non-limiting concentrations of nitrate (20 mg/l NO_3^- -N) and carbon (20 mg/l glucose-C).

Data Analysis and Enzyme Kinetics

One way ANOVA was used to determine significant differences among different treatment experiments using SPSS 10.0 (SPSS, Inc., Chicago, IL). The data met the assumption of the analysis (normality and homoscedasticity). Further post hoc analysis was done using

Tukey test. Denitrification rates in the nitrate enrichment experiment were fit to Michaelis–Menten type kinetics using single rectangular hyperbola function on SigmaPlot 2001 (SPSS, Inc., Chicago, IL) with the following equation:

$$\text{Denitrification rate} = \frac{V_{\max}(\text{NO}_3^-)}{K_m(\text{NO}_3^-)}$$

The two parameters, V_{\max} and K_m , in the Michaelis–Menten equation describe how denitrification responds logarithmically to increasing nitrate concentration until reaching saturation. V_{\max} is the maximum denitrification rate achieved and K_m is the nitrate concentration at which the denitrification rate reaches half its maximum value, i.e., $V_{\max}/2$ (Herrman et al., 2008).

Results and Discussion

Water Nutrient and Sediment Characteristics

Barato lake water and sediment characteristics in terms of physical and chemical parameters are summarized in Table 1. The average and range of values obtained during the surveyed periods are presented. Lake water pH ranged from neutral to slightly alkaline. The high ORP values for all the sampling sites indicated oxic condition of water column during whole sampling event. Nitrate was

Table 1: Water and sediment characteristics of Barato lake (Data represent mean of monthly measurements \pm SE; Water sample ($n = 10$); Sediment sample ($n = 4$); Particle size were analysed on the sample collected during November 2009)

Parameters	Sampling locations				
	B1	B2	B3	B4	Barato lake (average)
pH	7.2 \pm 0.1	7.7 \pm 0.1	7.4 \pm 0.1	7.4 \pm 0.1	7.4 \pm 0.1
ORP (mV)	203 \pm 93	196 \pm 58	214 \pm 50	220 \pm 32	208 \pm 58
NH_4^+ -N (mg/l)	0.38 \pm 0.36	0.28 \pm 0.23	0.61 \pm 0.39	0.45 \pm 0.35	0.43 \pm 0.34
NO_3^- -N (mg/l)	4.67 \pm 2.18	0.81 \pm 0.44	3.3 \pm 1.25	2.82 \pm 1.19	2.95 \pm 1.97
NO_2^- -N (mg/l)	0.08 \pm 0.05	0.02 \pm 0.01	0.09 \pm 0.06	0.08 \pm 0.05	0.07 \pm 0.05
DOC-C (mg/l)	5.88 \pm 0.52	6.70 \pm 0.46	5.84 \pm 0.39	5.04 \pm 0.33	5.86 \pm 0.42
PO_4^{3-} -P (mg/l)	0.04 \pm 0.01	0.02 \pm 0.01	0.02 \pm 0.01	0.02 \pm 0.01	0.02 \pm 0.01
SiO_2 -Si (mg/l)	9.95 \pm 2.54	5.05 \pm 2.39	8.09 \pm 2.23	8.11 \pm 2.32	7.86 \pm 2.90
Sediment OM (% dry weight)	12.9 \pm 1.6	9.8 \pm 1.6	19.2 \pm 7.2	NA	14.3 \pm 5.9
Sedimentary Total Organic Carbon (% m/m)	4.03 \pm 0.73	3.60 \pm 2.28	4.16 \pm 2.28	NA	3.93 \pm 1.68
Sedimentary Total Nitrogen (% m/m)	0.34 \pm 0.11	0.42 \pm 0.27	0.44 \pm 0.24	NA	0.4 \pm 0.19
Clay (% V/V)	34.5	37.6	17.6	NA	—
Silt (% V/V)	65.5	62.4	33.4	NA	—
Sand (% V/V)	0.0	0.0	48.992	NA	—

NA: Not analysed

dominant component within dissolved inorganic N-species. Its values ranged from 0.24 to 8.41 mg/l NO_3^- -N with an average value of 2.95 mg/l NO_3^- -N. Nitrate concentration showed significant spatial variation ($F_{2,89} = 11.66$, $P < 0.0001$) within the Barato lake, with maximum mean concentration observed at station B1 (4.67 mg/l) and lowest mean was observed at station B2 (0.81 mg/l). Station B2 fall into more stagnant zone of Barao lake, receiving less inflow compared to other section of lake (Nakatsugawa and Hamahara, 2004). Seasonal variation of nitrate concentration in Barato lake is given in Figure 2. Nitrate concentration showed significant seasonal variation ($F_{2,89} = 5.54$, $P = 0.003$) with maximum mean concentration observed during winter (4.52 mg/l) and lowest mean was observed during summer (1.54 mg/l). The relatively higher concentration during winter could be due to exclusion of solutes during lake ice formation as part of lake system partially freezes during winter period with low in-stream biological activity due to low temperature condition in winter (Cai et al., 2008). NO_2^- -N concentration varied from 0.001 to 0.18 mg/l with a mean value of 0.07 mg/l in Barato lake. NH_4^+ -N concentration varied from 0.03 to 1.20 mg/l with a mean value of 0.43 mg/l in Barato lake. NH_4^+ -N concentration did not show significant spatial variation ($F_{2,89} = 1.49$, $P = 0.235$) within the Barato lake, however,

sample collected from station B3 has higher mean value (0.61 mg/l). NH_4^+ -N concentration did not show significant seasonal variation within the Barato lake, however higher mean concentration (0.60 mg/l) was observed in samples collected during winter. NH_4^+ -N concentration in lake water can be mainly attributed to discharge from sewage treatment plant and runoff from agriculture field in the catchment area along with precipitation. Dissolved inorganic phosphate concentration usually remain low in lake system and varied from 0.01 to 0.07 mg/l PO_4^{3-} -P with an average value of 0.02 mg/l PO_4^{3-} -P. PO_4^{3-} -P concentration showed significant spatial variation ($F_{2,89} = 10.48$, $P < 0.0001$) in Barato lake, with maximum mean observed at station B1 (0.04 mg/l) and lowest mean at B2 (0.01 mg/l). Season variation in PO_4^{3-} -P concentration did not show significant variation ($F_{2,89} = 1.94$, $P = 0.142$), however relatively higher mean concentration (0.29 mg/l) was observed during winter. Besides weathering of phosphate bearing mineral in catchment area effluent from sewage treatment plant and runoff from surrounding agriculture area were main source of phosphate in lake water. Dissolved silica concentration varied from 2.02 to 12.74 mg/l SiO_2 -Si with an average value of 7.86 mg/l SiO_2 -Si. Dissolved silica concentration showed significant spatial variation ($F_{2,89} = 6.83$, $P = 0.001$) in Barato lake

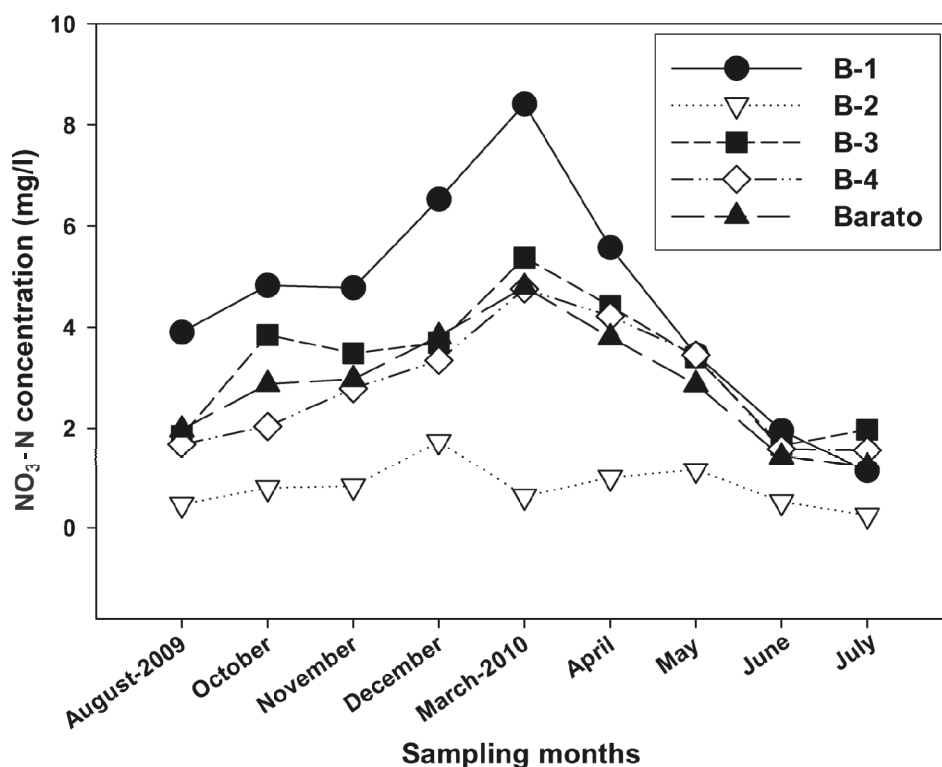


Figure 2: Seasonal variation of dissolved nitrate concentration in Barato lake.

with maximum mean concentration observed at B1 (9.95 mg/l) and lower mean observed at station B2 (5.05 mg/l). Dissolved silica concentration did not show significant seasonal variation ($F_{2,89} = 1.60$, $P = 0.208$) in Barato lake. However, samples collected during winter showed higher mean concentration of (9.52 mg/l). Silica is an important nutrient and it is necessary for the growth of diatom and mainly attributed to silicates weathering occurring in the lake catchment area. Dissolved organic carbon is the main source of carbon available to denitrifiers for the oxidation of nitrate during denitrification process. DOC-C concentration varied from 3.38 to 8.55 mg/l with a mean value of 5.86 mg/l in Barato lake.

Barato lake sediment organic carbon (TC_{org}) concentration varied from 0.96 to 6.22% with an average value of 3.93% and total nitrogen (TN) varied from 0.11 to 0.64% with an average value of 0.40%. The organic matter content (LOI % dry weight) in sediment samples varied from 5.7 to 26.4% with an average value of 14.3% in Barato lake sediment samples (Table 1). TC_{org} , TN and organic matter content did not show significant spatial variation (95% confidence limit) in Barato lake. Grain size distribution showed spatial variation with silt fraction (4–63 μm) dominating the distribution (more than 60% particle) and the clay fraction (0.02–4.0 μm) accounting for less than 40% of the particle at sampling location B-1 and B-2, but in case of sampling location B-3 sand fraction (63–500 μm) dominated the distribution pattern (49% of particle) and silt and clay fraction accounted for 33.4% and 17.6%, respectively.

Sediment Denitrification

The potential denitrification rate in Barato lake sediments are shown in Table 2. The potential denitrification rate varied from 0.13 to 2.21 $\mu g N g^{-1} DM h^{-1}$ with an average of 0.91 $\mu g N g^{-1} DM h^{-1}$ in Barato lake. Denitrification rates measured in our study (0.13–2.21 $\mu g N g^{-1} DM h^{-1}$) fall in the range of values reported for other aquatic ecosystems: like lake sediments, 0.04–2.27 $\mu g N g^{-1} DM h^{-1}$ (Zhong et al., 2010); river sediments, up to 3.64 $\mu g N g^{-1} DM h^{-1}$ (Gracia-Ruiz et al., 1998) and stream sediments, 0.01–4.77 $\mu g N g^{-1} DM h^{-1}$ (Arango et al.,

2007). Denitrification process shows significant spatial and temporal variation in aquatic ecosystem due to changes in sediment properties and nutrient concentration with space and time (Arango et al., 2007; Ricardson et al., 2004; Zhong et al., 2010). However, no significant spatial and temporal variations (95% confidence limit) in denitrification rate were observed in Barato lake. The effect of seasonal temperature variation on denitrification rate may be suppressed due to incubation of sediment samples at room temperature before starting of experiment. Denitrification rate showed strong correlation ($r^2=0.79$) with dissolved NO_3^- -N concentration in lake water sample indicating strong control of nitrate concentration on denitrification rate in Barato lake sediment (Figure 3). This agrees well with other denitrification studies that found similar correlation between denitrification rates and dissolved nitrate concentration (Ogilvie et al., 1997; Zhong et al., 2010). Seitzinger (1988) reported that nitrate produced in the sediment via nitrification of ammonia appears to be the major substrate source for denitrification in most aquatic sediments. However, in the aquatic systems with significant nitrate concentration in the overlying water, diffusion of overlying nitrate to sediment denitrifying sites control the rate of sediment denitrification (Christensen et al., 1990). Dissolved organic carbon (DOC) concentration had no correlation with denitrification rate in Barato lake. Similar results have been reported for other aquatic systems (Arango et al., 2007; Inwood et al., 2007). Denitrification rate usually increases with increasing temperature (Maag et al., 1997) but in case of Barato lake denitrification rate was relatively low during summer season coinciding with low dissolved nitrate concentration, which indicates that availability of dissolved nitrate exert stronger control over denitrification rate compared to temperature effect (Hasegawa and Okino, 2004).

Treatment Experiments

Nitrate Amendments

Nitrate amendment had significant positive effect on denitrification rate ($F_{4,06} = 8.98$; $P = 0.006$). Comparing

Table 2: Spatial and temporal variation in denitrification rate in Barato lake

Sampling locations	Denitrification rate ($\mu g N g^{-1} DM h^{-1}$)			
	Summer	Autumn	Winter	Spring
B1	0.58 \pm 0.22	1.36 \pm 0.01	2.22 \pm 0.02	1.14 \pm 0.11
B2	0.13 \pm 0.06	0.45 \pm 0.02	—	0.50 \pm 0.02
B3	0.67 \pm 0.08	0.21 \pm 0.02	1.58 \pm 0.08	1.16 \pm 0.03
Barato lake (average)	0.46 \pm 0.12	0.67 \pm 0.02	1.90 \pm 0.05	0.93 \pm 0.05

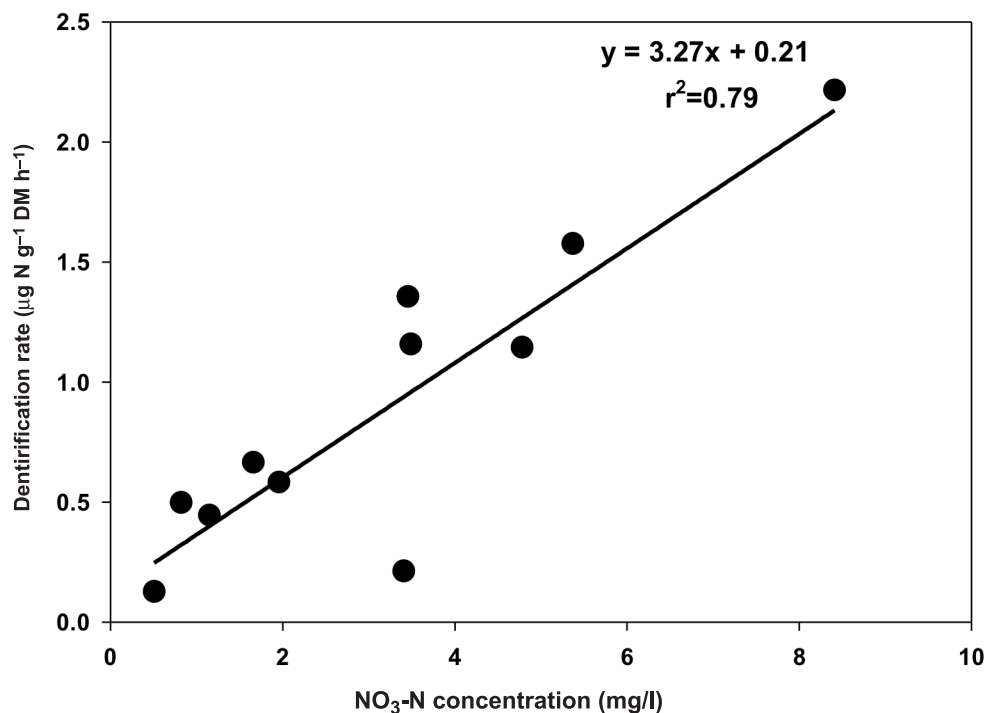


Figure 3: Co-relation plot between potential denitrification rate and dissolved nitrate concentration in Barato lake.

untreated samples (0 mg/l NO₃⁻-N) with nitrate amendments of 2, 10 and 20 mg/l NO₃⁻-N the increase in denitrification rate was significant. However, comparing 2, 10 and 20 mg/l NO₃⁻-N treatments among themselves showed no significant change (Tukey's $P = 0.05$) (Figure 4). This implies the rate of denitrification reached near saturation with 2 mg/l NO₃⁻-N treatment. The nitrate treatment had denitrification rates of 0.02 ± 0.02 , 2.12 ± 1.17 , 2.61 ± 1.75 and 2.51 ± 1.55 $\mu\text{g N g}^{-1} \text{DM h}^{-1}$ for 0, 2, 10 and 20 mg/l NO₃⁻-N concentration respectively. There was 106 fold increases in denitrification rate over control on addition of 2 mg/l NO₃⁻-N. Ricardson et al. (2004) reported that NO₃ addition resulted into 30 fold increase in denitrification rate over controls in Mississippi river sediment samples. Upper sugar creek watershed sediment samples showed 74 fold increase in denitrification rate after addition of 2 mg/l NO₃⁻-N over control (Herrman et al., 2008). Previous studies also indicated the nitrate limitation on sediment denitrification rate in aquatic ecosystem (Table 3). The response of denitrification rate for nitrate amendment showed positive correlation ($P < 0.001$, $r^2 = 0.99$) for Michaelis-Menten kinetics. It is non-linear single rectangular hyperbola regression. On the basis of regression line, estimated V_{max} was $2.65 \mu\text{g N g}^{-1} \text{DM h}^{-1}$ and estimated K_m was 0.48 mg/l NO₃⁻-N (Figure 4). Denitrification rates studies in literature usually express rates in different units (rate per dry weight unit (DM), rate per ash-free dry mass (AFDM)

and rate per area unit), which make it difficult to compare V_{max} with other studies but value of K_m is relatively easy to compare. Reported values of K_m also known as half-saturation constant for different aquatic ecosystem are given in Table 4. The K_m values for Barato lake were comparable with other lake studies. Value of K_m reflects two important facts: how quickly denitrifying microbial population is saturated with nitrate and how close denitrification is to saturation (Herrman et al., 2008). The low K_m value of Barato lake showed that the denitrifying microbial population gets saturated at low nitrate values. Low K_m value also indicates that the lake will reach saturation in terms of denitrification at lower nitrate values. The annual average nitrate ion concentration in Barato lake was 2.95 mg/l NO₃⁻-N, which is far greater than the K_m value. If dissolved nitrate concentration is much higher than the K_m value, then the denitrification process would have to be nitrate saturated and denitrification rate will not change with changes in nitrate concentration (Garcia-Ruiz et al., 1998). Thus the denitrification activity in Barato lake was saturated throughout the year.

Temperature Variation

We choose 5 °C and 20 °C as incubation temperature because they represented the range of temperature observed in lake water during whole sampling event. The denitrification rate increased significantly with increase in temperature from 5 °C ($0.18 \pm 0.03 \mu\text{g N g}^{-1} \text{DM h}^{-1}$)

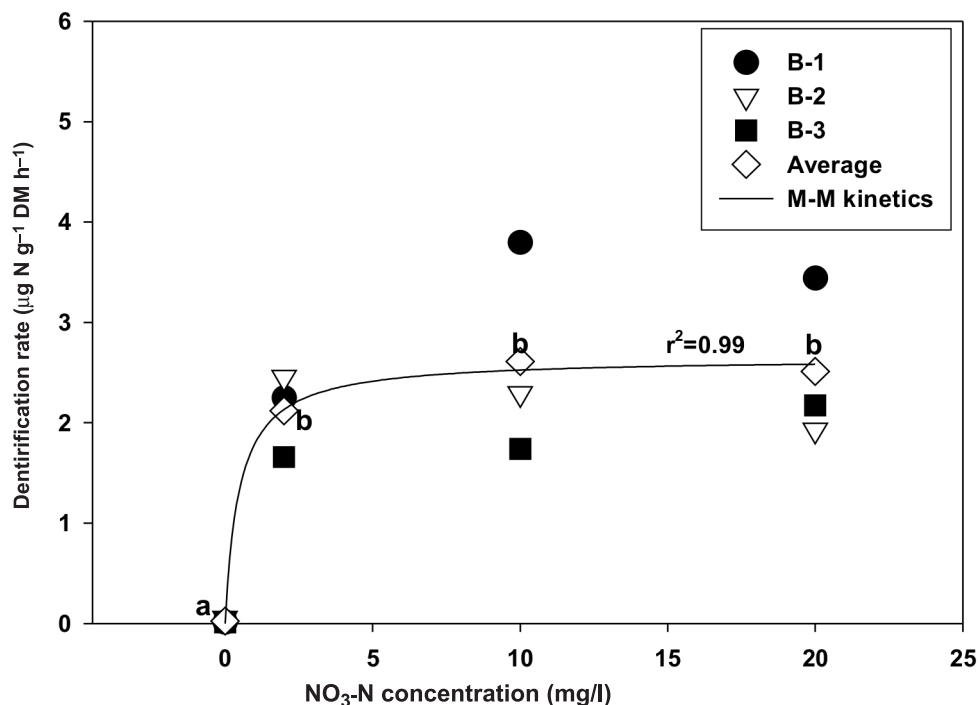


Figure 4: Response curve of potential denitrification rate with increasing nitrate concentration. Treatments with the same letter are not significantly different (Tukey's $P = 0.05$). Regression analysis was conducted using Michaelis-Menten kinetics in Barato lake.

Table 3: Effect of nitrate, carbon and temperature treatments on potential denitrification rates

Water body	Method	Effect on potential denitrification*			References
		Nitrate	Carbon	Temperature	
Barato lake, Japan	Acetylene inhibition, sediment slurry	+	—	+	Present study
Upper sugar creek watershed, USA	Acetylene inhibition, sediment slurry	+	—	+	Herrman et al., 2008
South Platte river, USA	Acetylene inhibition, sediment slurry	—	+	+	Pfenning and McMahon, 1996
Mississippi river, USA	Acetylene inhibition, sediment core	+	—	ND	Richardson et al., 2004
Taihu lake, China	Acetylene inhibition, sediment core	+	—	+	Zhong et al., 2010
Suwa lake, Japan	Acetylene inhibition, sediment core	ND	ND	+	Hasegawa and Okino, 2004
Douro estuary, Portugal	Acetylene inhibition, sediment slurry	+	—	ND	Teixeira et al., 2010

* +: significant effect; —: no significant effect; ND: no data available.

to 20 °C ($0.92 \pm 0.12 \mu\text{g N g}^{-1} \text{DM h}^{-1}$) ($F_{7,70} = 107.19$; $P = 0.001$). There was 5 fold increase in the denitrification activity on increasing temperature from 5 °C to 20 °C. Herrman et al. (2008) reported 2 fold increase in denitrification rate when incubation temperature changed from 8 °C to 25 °C. Study of South Platte River Basin showed 77% decrease in denitrification rate when incubation temperature decreased from 22 °C to 4 °C

(Pfenning and McMahon, 1996). Increase in water temperatures results in higher microbial activity, causing denitrifying bacteria to metabolize NO_3^- -N faster (Zhong et al., 2010).

Carbon Amendments

Carbon amendments had no significant effect on denitrification rate ($F_{4,07} = 1.18$; $P = 0.001$). The glucose

Table 4: Comparison of K_m values (mg/l NO_3^- -N) obtained with Michaelis–Menten kinetics from various aquatic systems

<i>Water body</i>	<i>Sediment/soil type</i>	K_m (mg/l NO_3^- -N)	<i>References</i>
Lake	Sediment slurry	0.48	This study
Lake	Sediment slurry	0.20–1.40	Hordijk et al., 1987
River	Sediment slurry	1.0	Herrman et al., 2008
Sandy soil	soil core	1.0–100	Strong and Fillery, 2002
River	Sediment core	0.20–9.0	Garcia-Ruiz et al., 1998
Estuary	Sediment slurry	0.38–0.74	Seitzinger, 1988

(carbon) treatment had denitrification rates of 2.05 ± 0.27 , 2.21 ± 0.38 , 2.65 ± 0.75 and $2.79 \pm 0.67 \mu\text{g N g}^{-1} \text{DM h}^{-1}$ for 0, 2, 10 and 20 mg/l glucose-C. Other studies also indicated that carbon concentration has no significant effect on denitrification rate; however, some study had documented a C limitation (Table 3). Sediments from the South Platte river were reported to be carbon limited. However, authors also reported that fine-grained surficial sediment did not indicate a significant increase in N_2O production rate with increasing carbon (acetate) concentration due to the relatively high organic carbon of sediment (Pfenning and McMahon, 1996). The high organic carbon content of Barato lake sediment most likely provided sufficient substrate to support denitrification activity.

The results of our study indicate that though Barato lake sediments showed significant denitrification activity, it is not effective in removing nitrogen load from the system due to saturation of denitrification process. So in future, any increase in nitrogen load due to anthropogenic activities may further deteriorate the lake water quality and undermine its uses. However, other retention process such as sedimentation and uptake by aquatic plants also has to be taken into account for proper understanding of nitrogen retention process in Barato lake system.

Conclusion

The potential denitrification rate varied from 0.13 to $2.21 \mu\text{g N g}^{-1} \text{DM h}^{-1}$ with an average of $0.91 \mu\text{g N g}^{-1} \text{DM h}^{-1}$ in Barato lake sediment. Sediment denitrification rate in Barato lake was positively correlated with the dissolved nitrate concentration in lake water, however no correlation was observed with DOC concentration. Laboratory amendment experiment showed carbon concentration had no significant effect on denitrification rate but rate increases significantly with increase in nitrate concentration and temperature. Denitrification rate followed Michaelis–Menten kinetics with increasing nitrate concentration (V_{max} $2.65 \mu\text{g N g}^{-1} \text{DM h}^{-1}$ and

K_m 0.48 mg/l NO_3^- -N). K_m value is lower than annual average dissolved nitrate concentration, indicating saturation of denitrifying activity in Barato lake throughout the year.

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