Denitrification kinetics and denitrifier abundances in sediments of lakes receiving atmospheric nitrogen deposition (Colorado, USA)

Michelle L. McCrackin · James J. Elser

Abstract The transport and deposition of anthropogenic nitrogen (N) to downwind ecosystems is significant and can be a dominant source of new N to many watersheds. Bacterially mediated denitrification in lake sediments may ameliorate the effects of N loading by permanently removing such inputs. We measured denitrification in sediments collected from lakes in the Colorado Rocky Mountains (USA) receiving elevated (5–8 kg N ha^{-1} y^{-1}) or low (<2 kg N ha^{-1} y^{-1}) inputs of atmospheric N deposition. The nitrate (NO_3^-) concentration was significantly greater in high-deposition lakes (11.3 µmol l^{-1}) compared to low-deposition lakes (3.3 µmol l^{-1}). Background denitrification was positively related to NO_3^- concentrations and we estimate that the sampled lakes are capable of removing a significant portion of N inputs via sediment denitrification. We also conducted a dose–response experiment to determine whether chronic N loading has altered sediment denitrification capacity. Under Michaelis–Menten kinetics, the maximum denitrification rate and half-saturation NO_3^- concentration did not differ between deposition regions and were 765 µmol N m^{-2} h^{-1} and 293 µmol l^{-1} NO_3^-, respectively, for all lakes. We enumerated the abundances of nitrate- and nitrite-reducing bacteria and found no difference between high- and low-deposition lakes. The abundance of these bacteria was related to available light and bulk sediment resources. Our findings support a growing body of evidence that lakes play an important role in N removal and, furthermore, suggest that current levels of N deposition have not altered the abundance of denitrifying bacteria or saturated the capacity for sediment denitrification.

Keywords Atmospheric nitrogen deposition · Denitrification · Lake · Denitrifier abundance · Sediment

Introduction

The global rate of atmospheric N deposition is expected to reach 125 Tg N y^{-1} by 2050, a 7-fold increase since preindustrial times (Galloway et al. 2004). Ecosystems even in unpopulated areas are subject to anthropogenic influences via the long distance transport of such pollutants (Wolfe et al. 2006). When N is delivered to ecosystems in excess of biological demand, saturation occurs and NO_3^- leaches from the ecosystem (Aber et al. 1998; Tietema 1998). Increases in N deposition will perpetuate saturating conditions for systems already subject to chronic N loading and may push other ecosystems to saturation. As N cascades from soils to

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groundwater, lakes, rivers, and, ultimately, the oceans, denitrification (the microbial conversion of NO_3^- to inert nitrogen gas, N_2) plays an important role in mitigating N loading to coastal areas by permanently removing N (Galloway et al. 2003; Seitzinger et al. 2006). Indeed, lentic ecosystems, such as lakes and reservoirs (hereafter: lakes), have recently been recognized as important biogeochemical sinks for N. Lakes may remove nearly 20 Tg N y^{-1} from watersheds globally, which represents ∼30% of estimated inputs (65 Tg N y^{-1}) to surface waters (Wollheim et al. 2008; Harrison et al. 2009). Such N removal capacity is significant considering that lakes occupy 3% of the land surface. Potential fates for N in lakes include permanent removal by denitrification, long-term storage in sediments, or temporary storage in biomass. Of these mechanisms, denitrification may often account for the majority of N removed globally from these ecosystems (Saunders and Kalff 2001). Given the expected increases in anthropogenic N inputs and the susceptibility of remote ecosystems to atmospherically deposited N, it is important to understand the limits to denitrification in lake sediments.

Just as terrestrial ecosystems may experience N saturation (Aber et al. 1998), aquatic ecosystems may similarly become prone to increased N exports. Models of N saturation were originally developed for terrestrial ecosystems and more recently have been applied to streams (Earl et al. 2006). There is a growing body of evidence that N loading to streams results in decreased efficiency of biotic N removal (Mulholland et al. 2008) and saturation of denitrification has been observed in eutrophic streams (Bernot and Dodds 2005). In lakes, the seasonality of NO_3^- export from lakes has been used to diagnose N saturation in the catchment (Stoddard 1994) but biotic processes have not been explicitly considered in N saturation models for lakes. Recent studies of lakes have found that N deposition has shifted lake phytoplankton and zooplankton nutrient limitation to phosphorus (P, Elser et al. 2009a, 2010), suggesting that anthropogenic N inputs can exceed biological demands in the water column. It is not known if such effects extend to sediment processes such as denitrification.

In soils, chronic N loading has influenced microbial communities and biogeochemical processes by altering environmental conditions, such as pH, the concentration and quality of oC, and the concentration and forms of available N (Lovett and Rueth 1999; Bowden et al. 2004). Nitrogen deposition has reduced soil respiration rates, increased N mineralization rates, and decreased the biomass of heterotrophic bacteria (Wallenstein et al. 2006; Eisenlord and Zak 2010). Similar deposition-induced effects on microbial communities and processes are possible in lake sediments. Indeed, McCrackin and Elser (2010) found sediment denitrification and N_2O production was greater in lakes receiving elevated N deposition. This same study found no difference in potential denitrification (assays amended with non-limiting concentrations of NO_3^- and organic carbon, oC). Generally, potential denitrification is positively correlated with denitrifier biomass (O'Connor et al. 2006); hence, the lack of a difference in potential denitrification rates between high- and low-deposition lakes suggests that N deposition has not altered the abundance of denitrifiers. The abundance of denitrifiers in lake sediments is poorly documented, however, and it is not known how chronic N loading affects their biomass.

Here we report the effects of N deposition on denitrification in sediments of high-elevation lakes in the Colorado Rocky Mountains and on the role of these lakes in NO_3^- removal. We tested the hypothesis that N loading has altered denitrification rates and capacity by estimating background denitrification and performing NO_3^- dose–response experiments with sediments from lakes receiving low (<2 kg ha^{-1} y^{-1}) or elevated (>6 kg ha^{-1} y^{-1}) levels of atmospheric N deposition. We expected N deposition to have a positive effect on denitrification rates by increasing NO_3^- concentrations and a negative effect on denitrification capacity by reducing denitrifier abundance. Reductions in the biomass of denitrifiers could result from deposition-induced changes to the environment, such as acidification (Burns 2004). Alternatively, chronically elevated NO_3^- concentrations in high-deposition lakes could reduce denitrification capacity by weakening the affinity of denitrifying bacteria for NO_3^-. We also measured the abundance of bacterial capable of NO_3^- and nitrite (NO_2^-) reduction in sediments of lakes at both ends of the deposition gradient and related these abundances to denitrification rates.

Methods

Study site

We sampled 20 lakes located in the Rocky Mountains of Colorado (USA) between June and August 2008. The eastern slopes of the Rocky Mountains near the Niwot Ridge Long Term Ecological Research site (NWT) and the Loch Vale Watershed Research site (LVW) in Rocky Mountain National Park receive atmospheric N deposition from fossil fuel combustion and agricultural sources in Denver and eastern Colorado (Nanus et al. 2003; Burns 2004). The rate of inorganic N (NO_3^- + NH_4^+) deposition has increased over the past 20 years to 6–8 kg ha^{-1} y^{-1} from background levels of <2 kg ha^{-1} y^{-1} (nadp.sws.uiuc.edu). Lakes in central and western Colorado near the Rocky Mountain Biological Laboratory (RMBL, Gothic, CO) and the Mountain Studies Institute (MSI, Silverton, CO) receive <2 kg ha^{-1} y^{-1} atmospheric inputs of N. Lakes near NWT and LVW are considered to be in the high-deposition region and lakes near RMBL and MSI are considered to be in the low-deposition region. Sampled lakes were within 6 km of a trailhead. Mean annual temperatures at high elevations are <2°C and the lakes are generally covered with ice between November and June (Baron et al. 2000). Catchment vegetation below the tree line

(~3,300 m) is characterized by Englemann spruce and subalpine fir forests. Alpine tundra is found above the tree line. With the exception of Estes Lake, the sampled lakes were small, generally ~0.1 km^2, and the watersheds were unpopulated (Fig. 1).

The sampled lakes occupy geologically diverse bedrock (Kent and Porter 1980). Precambrian-age granite, gneiss, and schist dominate the underlying geology in the vicinity of Rocky Mountain National Park. Near RMBL geologic parent materials include Mesozoic sedimentary rocks and Paleozoic metamorphics and intrusives. The San Juan Mountains surrounding MSI are characterized by extensive volcanic deposits and felsic gneisses and granites of the Uncompahgre formation.

Field sampling and laboratory procedures

We sampled 10 lakes in both the high- and low-deposition regions during summer 2008 (Table 1). Fieldwork was staggered so that sampling of lakes in each region was not strongly skewed by date. High-deposition lakes were sampled in late June and late July 2008 and low-deposition lakes were sampled in early July and early August 2008. An inflatable boat was used for sampling each lake. The depth of the lake was measured with a hand-held echo sounder. Water temperature and dissolved
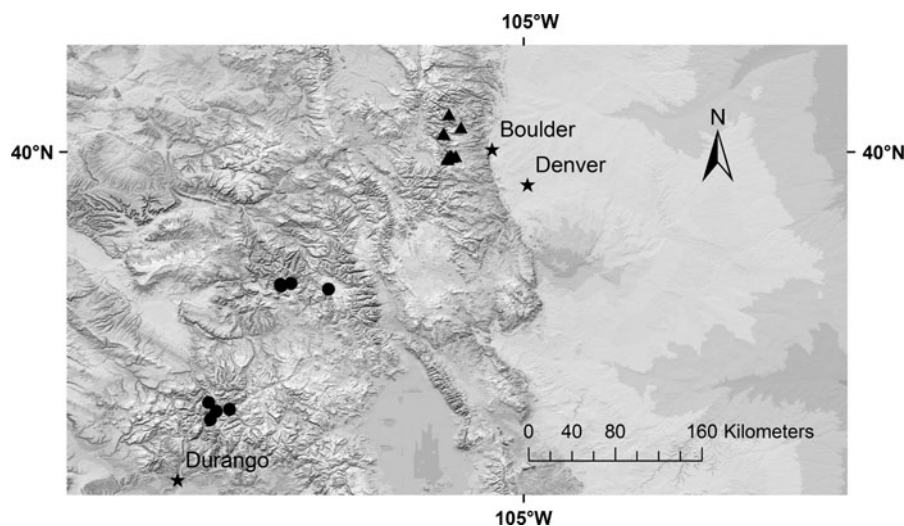
Fig. 1 Locations of the main study areas in Colorado, USA. Lakes in the Eastern area receive high levels of atmospheric deposition (5–8 kg N ha^{-1} y^{-1}), while lakes in Central and Western areas receive low levels of deposition (<2 kg N ha^{-1} y^{-1}). *Solid triangles* denote lakes sampled in the high-deposition region, while *solid circles* indicate lakes sampled in the low-deposition region

Table 1 Sampling dates and various parameters for the study lakes

	Sample date	Elevation (m)	Lake depth (m)	Water		Sediment			
				NO_3^- (μmol l^{-1})	DOC (mmol l^{-1})	Total C (mmol g^{-1})	Total N (mmol g^{-1})	Total P (mmol g^{-1})	OM
High-deposition lakes									
Brainard	6/26/08	3,154	3	12.6	0.27	10.4	0.94	0.20	0.18
Dream	7/4/08	3,032	4	16.6	0.28	11.4	0.91	0.09	0.12
Estes	6/29/08	2,277	>20	6.4	0.45	4.7	0.33	0.09	0.28
Green Lake 1	7/25/08	3,421	9	9.5	0.33	10.1	0.84	0.10	0.13
Green Lake 3	7/25/08	3,416	5	15.6	0.43	5.5	0.40	0.13	0.09
Isabelle	6/30/08	3,314	40	19.3	0.39	3.5	0.27	0.08	0.07
Long	7/5/08	3,219	3	12.4	0.72	7.0	0.66	0.06	0.11
Mitchell	7/23/08	3,280	1.5	10.8	0.63	7.2	0.59	0.07	0.17
Nymph	7/4/08	2,965	1	5.2	0.55	19.3	1.15	0.06	0.23
Red Rock	6/26/08	3,112	1	4.3	0.93	18.8	1.45	0.10	0.16
Mean				11.3	0.50	9.8	0.75	0.10	0.15
SE				1.3	0.05	1.8	0.13	0.01	0.02
Low-deposition lakes									
Andrews	7/11/08	3,284	6	3.4	1.44	2.4	0.19	0.13	0.19
Clear	7/10/08	3,633	>25	6.3	0.53	4.6	0.36	0.07	0.04
Dollar	8/6/08	3,059	5	0.4	0.60	15.1	1.04	0.08	0.18
Highland Mary	7/15/08	3,708	30	2.9	0.77	5.0	0.44	0.18	0.10
Irwin	8/7/08	3,148	5	0.5	0.35	5.3	0.49	0.07	0.11
Little Molas	7/8/08	3,329	6	4.1	0.84	4.6	0.36	0.07	0.18
Lost	8/6/08	3,010	10	3.3	0.18	6.9	0.61	0.08	0.09
Lost Slough	8/13/08	2,939	4	0.3	0.29	4.1	0.30	0.06	0.12
Potato	7/18/08	2,983	17	11.4	1.47	9.3	0.79	0.05	0.13
Spring Creek	8/12/08	3,040	8	0.3	1.45	7.7	0.62	0.11	0.12
Mean				3.3	0.79	6.5	0.52	0.09	0.13
SE				1.0	0.14	1.2	0.08	0.01	0.02
High vs. low deposition				High > low	High = low	High = low	High = low	High = low	High = low
P				0.001	n.s.	n.s.	n.s.	n.s.	n.s.

Results of statistical tests comparing high- and low-deposition lakes are shown for each parameter. Nonsignificant results are indicated by n.s

oxygen at the sampling depth were measured with a YSI model 85 temperature-oxygen probe (YSI, Yellow Springs, Ohio, USA). Water was collected just above the sediments using a battery-powered submersible pump and then filtered with Pall A/E glass fiber filters. Water samples were frozen until analysis for dissolved organic carbon (DOC) on a Shimadzu TOC 5000 (Shimadzu Corporation, Kyoto, Japan) and for nitrate plus nitrite (hereafter: NO_3^-) concentration on a Lachat Quick Chem 8000

autoanalyzer (Hach Corporation, Loveland, Colorado, USA).

Surface sediments were collected using a LaMotte dredge from a water depth of approximately 10 m or at the maximum lake depth if the lake was <10 m. The dredge collected sediments from an area of 221 cm^2 to a depth of $\sim$7 cm. Sediments were returned to the laboratory and processed within 24 h of collection. Denitrification was measured using the acetylene inhibition method (Yoshinari and Knowles

1976). To estimate the background rate of denitrification, three replicate 100-g subsamples of homogenized sediments from each lake were slurried with 80 ml of water collected from above the sediments. Bottles were purged of oxygen with N_2 and acetylene was added to block the reduction of N_2O to N_2. After vigorous shaking, we collected 10-ml samples from the headspace volume (about 550 ml) at the onset of anoxic conditions (0 h) and at 4 h. Incubations were conducted at 4°C (approximate annual temperature at sediment depth) in dark conditions. Gas samples were analyzed for N_2O on a Varian CP-3800 gas chromatograph (Agilent Technologies, Santa Clara, CA, US) with an electron-capture detector. The denitrification rate was determined as the production of N_2O during the incubations on the basis of dry sediment mass and was converted to an areal basis using the sediment bulk density for each lake (Richardson et al. 2004).

We conducted a dose–response experiment to evaluate the capacity of sediments to denitrify additional NO_3^- inputs. For each lake, three replicate 50-g subsamples of homogenized sediments were slurried with 40 ml of one of five different incubation media. The incubation media were: distilled water as a control, 5 µmol NO_3^--N l^{-1}, 50 µmol NO_3^--N l^{-1}, 500 µmol NO_3^--N l^{-1}, and 5,000 µmol NO_3^--N l^{-1}. We also measured potential denitrification by amending sediments with a medium consisting of 8.3 mmol oC (dextrose and acetate) l^{-1}, 7.1 mmol NO_3^--N l^{-1}, 450 µmol phosphate (PO_4^{3-})-P l^{-1}, for N:P ratio of 16. For the denitrification capacity and potential denitrification assays, we induced anoxia and amended the slurries with acetylene as described above. We collected 3.5-ml initial samples and final samples at the end of the 4-h incubation at 17–20°C in dark conditions, both after vigorous shaking. These slurries were incubated at room temperature because we were interested in maximal denitrification rates, not rates that are representative of in situ conditions, and for comparison with a previous study (McCrackin and Elser 2010). Gas samples were analyzed for N_2O on a Shimadzu 14A gas chromatograph (Shimadzu, Kyoto, Japan) with an electron-capture detector. Denitrification in response to experimental resource enrichments is reported on the basis of dry sediment mass that was converted to an areal basis using the sediment bulk density.

We investigated the influence of atmospheric N deposition and individual lake factors on NO_3^-- and NO_2^--reducers (together as denitrifiers) in a subset of 16 lakes by estimating the culturable populations of these organisms using a most probable number (MPN) technique (Staley and Griffin 1981). To determine the MPN of denitrifiers, we suspended 4-g subsamples of homogenized sediment in 25 ml of 25% Ringer's solution, forming a slurry from which 100 µl was inoculated into each well of a 96-well micro-titer plate by 10-fold serial dilutions (Johnson et al. 2007). Prior to inoculation with sediment slurry, 100 µl of NO_3^- broth (9.9 mmol NO_3^- l^{-1}, Difco Laboratories, Detroit, MI, USA) was added to each well. Plates were incubated in a dark, anoxic environment in a vacuum desiccator for 1 week. Positive growth was determined by the addition of diphenylamine to test for the presence of NO_3^- and N(1-napthyl)-ethylene-diamine-dihydrochloride to test for the presence of NO_2^-. Wells that indicated the presence of NO_2^- were scored positive for NO_3^--reducing bacteria and wells that indicated the absence of both NO_3^- and NO_2^- were scored positive for NO_2^--reducing bacteria. Scores were translated to the MPN of each microorganism using a probability table developed by Rowe et al. (1977).

Interactions with benthic periphyton may influence denitrifier communities because the availability of oxygen, oC, and NO_3^- in sediments will fluctuate with the availability of light as the balance shifts between production and respiration. To understand the effects such interactions, we measured photosynthetically active radiation (PAR) at the lake surface and at the depth from which sediments were collected using a LI-COR data-logger and LI-192 Underwater Quantum Sensor (LI-COR Corporation, Lincoln, NE, USA). The light extinction coefficient (k) was calculated as $k = -z * LN(I_z/I_0)$, where z is the depth from which sediments were collected, I_Z is the irradiance at depth z, and I_0 is the irradiance at the lake surface.

Sediment dry mass was determined after drying subsamples at 105°C for 48 h and organic matter (OM) content was determined as mass loss on ignition at 550°C for 4 h. Total C and N contents of dried, ground sediments were measured with a PerkinElmer CHN elemental analyzer (Perkin-Elmer, Waltham, MA). Total P content of combusted

sediment was measured colorimetrically following extraction with 0.5 M hydrochloric acid using the acid-molybdate technique (Lukkari et al. 2007).

Statistical analysis

Under the assumption that the denitrification process is a single step that converted NO_3^- to $N_2O + N_2$, the Michaelis–Menten model was used to determine dose–response relationships of denitrification to experimental additions of NO_3^- (Betlach and Tiedje 1981; Silvennoinen et al. 2008).

$$\text{Denitrification rate} = (V_{max} * [NO_3^-]) / (K_m + [NO_3^-]) \quad (1)$$

The maximum denitrification rate is V_{max}, and the NO_3^- concentration that results in 50% of the maximum denitrification rate is K_m, also referred to as the affinity constant (Laverman et al. 2006). The parameters V_{max} and K_m were estimated separately for each lake, for lakes in the high- and low-deposition region, and together for all sampled lakes by fitting the "ligand-binding, one-site saturation model" in SigmaPlot Version 10 (SSI, San Jose, CA, USA) to measured denitrification rates.

We performed t tests or, where the assumption of normality was not met, Mann–Whitney U tests to compare water concentrations of NO_3^- and DOC, sediment organic matter, sediment nutrient pools, MPN of denitrifiers, and denitrification rates between lakes in high- and low-deposition regions. The Wilcoxon test was used to compare potential denitrification and maximum denitrification rates (V_{max}). Multiple-linear regression was used to identify relationships among predictor variables and the NO_3^- affinity constant (K_m), maximum denitrification (V_{max}), estimated background denitrification, potential denitrification, and the MPN of NO_3^-- and NO_2^--reducers. Predictor variables included water concentrations of NO_3^- and DOC, sediment organic matter, sediment C, N, and P content, ratios of sediment C:N, C:P, and N:P, the PAR light extinction coefficient, and denitrifier abundances. Models were selected by considering all subsets on the basis of adjusted R^2 and AIC. We evaluated multi-colinearity and selected final models for which tolerance values were >0.5 for all predictor variables. When necessary, response and predictor variables were transformed to improve normality. Statistical tests were performed using JMP (SAS Institute, Inc.) Version 8.0.1, with $\alpha = 0.05$.

Results

Water and bulk sediment characteristics

Water and bulk sediment characteristics for all lakes are reported in Table 1. The mean concentration of NO_3^- was greater in high-deposition lakes compared to low-deposition lakes at 11.3 and 3.3 μmol l^{-1}, respectively. High- and low-deposition lakes did not differ in DOC concentrations, which averaged 0.64 mmol l^{-1} across all lakes. There were no significant differences in sediment OM, total C, N, and P content, or ratios of C:N, C:P, and N:P between lakes in high- and low-deposition regions ($P > 0.05$). Lakes in the low-deposition region were generally thermally stratified whereas lakes in the high-deposition region were not. All lakes were oxic at the depth where sediments were collected and concentrations of dissolved oxygen just above the sediments did not differ between stratified and non-stratified lakes.

Denitrification assays

Under Michaelis–Menten kinetics, the maximum denitrification rate for the study lakes, V_{max}, was between 8 and 1,688 μmol N m^{-2} h^{-1} and was negatively related to sediment C:P (Tables 2 and 3). The half-saturation constants, K_m, for individual lakes ranged between 3 and 1,100 μmol l^{-1} NO_3^- and were negatively related to sediment OM content. When the Michaelis–Menten model was fit to the data for high- and low-deposition regions separately, V_{max} was comparable between high- and low-deposition lakes, at 797 and 700 μmol N m^{-2} h^{-1}, respectively. The half-saturation constant for high-deposition lakes was nearly half that of low-deposition lakes (207 and 448 μmol l^{-1} NO_3^-, respectively), however, these values were not statistically different ($P > 0.05$). For all lakes, the best-fit parameters V_{max} and K_m were 765 μmol N m^{-2} h^{-1} and 293 μmol l^{-1} NO_3^-, respectively ($R^2 = 0.98$, Fig. 2).

Table 2 Denitrification rates for the study lakes

	Sample depth (m)	Denitrification potential (μmol N m^{-2} h^{-1})	V_{max} (μmol N m^{-2} h^{-1})	"Background" denitrification (μmol N m^{-2} h^{-1})
High-deposition lakes				
Brainard	3	2,115	1,692	3.8
Dream	4	430	201	61.9
Estes	10	2,352	1,827	0.9
Green Lake 1	10	1,499	1,106	0.0
Green Lake 3	9	876	659	1.7
Isabelle	10	1,833	1,652	0.0
Long	3	733	561	7.0
Mitchell	1.5	505	507	0.0
Nymph	1	5	8	3.7
Red Rock	1	487	291	0.0
Mean		1,083	850	7.9
SE		269	212	6.4
Low-deposition lakes				
Andrews	6	734	790	0.0
Clear	10	498	1,188	0.9
Dollar	5	1,005	615	0.0
Highland Mary	10	260	174	0.7
Irwin	5	1,826	1,190	–
Little Molas	6	460	453	0.7
Lost	10	1,439	1,528	0.0
Lost Slough	4	437	268	0.0
Potato	10	23	17	2.2
Spring Creek	8	961	986	0.0
Mean		764	722	0.5
SE		196	158	0.3
High vs. low deposition		High = low	High = low	High = low
P		n.s.	n.s.	n.s.

V_{max} the maximum denitrification rate is the best-fit value for the dose–response experiment for individual lakes, *"Background" denitrification* is in response to addition of lake water, *n.s.* nonsignificant results. Results of statistical test comparing high- and low-deposition lakes are shown for each parameter

Estimated background denitrification (slurries amended with lake water) was positively related to and best predicted by NO_3^- (Table 3; Fig. 3). These denitrification rates averaged 4.4 μmol N m^{-2} h^{-1} ($\pm$3.3 SE) for all lakes and did not differ between high- and low-deposition regions ($P > 0.05$, Table 2). Potential denitrification (in response to non-limiting concentrations of oC, NO_3^-, and P) ranged from 5 to >2,000 μmol N m^2 h^{-1} and was negatively related to the sediment C:P ratio (Tables 2 and 3). There was no difference in potential denitrification between high- and low- deposition lakes,

which across all lakes averaged 924 μmol N m^{-2} h^{-1} ($\pm$159 SE) and was nearly 200 times greater than the mean estimated background denitrification rate. Rates of potential denitrification and maximum denitrification (V_{max}) did not differ statistically between lakes ($P > 0.05$, Table 2).

Most probable number of denitrifiers

The abundances of sediment denitrifiers varied between lakes, but did not differ between deposition regions ($P > 0.05$, Table 4). The mean abundances of

Table 3 Comparison of multiple regression models for measured response variables

Response variable	Predictor variables	R^2	P	Equation
K_m	Sediment OM	0.26	0.02	$\text{Log } K_m = -3.15 \text{ arcsine OM} + 3.61$
V_{max}	Sediment C:P	0.34	0.008	$\text{Log } V_{max} = -1.2 * \text{low sediment C:P} + 4.96$
Denitrification rate (μmol N m^{-2} h^{-1})	[NO$_3^-$]	0.22	0.04	$\text{Log denitrification rate} = 0.42 * \log [\text{NO}_3^-] + 0.07$
Potential denitrification rate (μmol N kg h^{-1})	Sediment C:P	0.28	0.03	$\text{Log potential denitrification rate} = -1.12 * \log \text{sediment C:P} + 4.84$
MPN NO$_2^-$-reducers	Sediment C:N	0.24	0.04	$\text{Log MPN NO}_2^-\text{-reducers} = -7.46 \log \text{sediment C:N} + 10.87$
MPN NO$_3^-$-reducers	PAR, sediment OM	0.28	0.047	$\text{Log MPN NO}_3^-\text{-reducers} = 3.80 * \log \text{PAR} + 2.56 \text{ arcsine OM} + 3.78$

R^2 denotes goodness-of-fit values adjusted for the number of parameters in the model

PAR is the light extinction coefficient, k

NO$_3^-$- and NO$_2^-$-reducing bacteria were 38 ($\pm$7 SE) $\times 10^4$ and 15 ($\pm$5 SE) $\times 10^2$ bacteria g^{-1} dry sediment, respectively, across all lakes. Regression analysis revealed that the MPN of NO$_3^-$-reducing microbes was negatively related to available light and positively related to sediment OM (Table 3). The abundance of NO$_2^-$-reducing bacteria was negatively related with the sediment C:N ratio.

Discussion

The Front Range of the Colorado Rocky Mountains receives <8 kg N ha^{-1} y^{-1} atmospherically, which represents 25–40% of estimated N mineralization rates in high-elevation soils (Fisk and Schmidt 1995). In contrast, N deposition rates in the eastern US and Europe are as high as 11–20 kg N ha^{-1} y^{-1} (Tørseth and Semb 1998; Bergstrom and Jansson 2006) and represent 5–17% of estimated soil N mineralization rates (Groffman et al. 2006; Zak et al. 2006). Thus, while current rates of N deposition are lower in Colorado compared to other regions, there is potential for atmospheric N loading to have a disproportionate ecological effect because it represents a relatively large fraction of N that is cycled. Indeed, atmospheric N loading has produced measurable changes in high-elevation ecosystems in Colorado. The ratio of C:N in foliage of old-growth forests has decreased and soil N content and soil N mineralization rates have increased compared to reference sites (Baron et al. 2000). Nitrogen deposition has also changed the composition and biomass of the diatom community, altered stoichiometric ratios of N to P in the water

column, and shifted phytoplankton nutrient limitation (Elser et al. 2009b). In the present study, we found no evidence that N deposition has altered denitrification capacity or abundances of denitrifying bacteria in sediments. Our data suggest that catchment- and lake-specific factors have a stronger influence on the denitrifier community than atmospheric N loading. Additionally, there is potential for N deposition to influence the instantaneous rate of denitrification by increasing NO$_3^-$ concentrations. Denitrification was positively related to NO$_3^-$, which was greater in high deposition regions although denitrification rates did not differ between deposition regions.
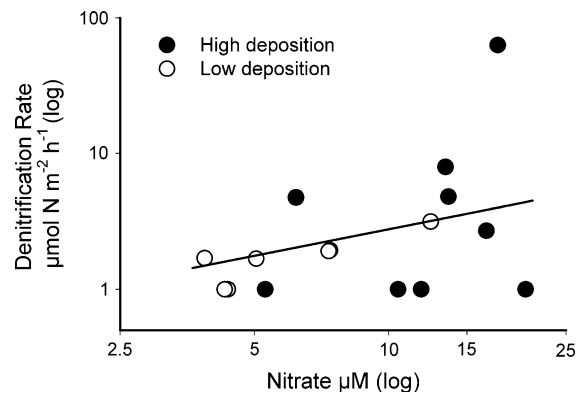
Factors influencing denitrification rates

We found that sediments possess considerable capacity for denitrification regardless of the atmospheric N loading rate (Table 2). The potential denitrification rate was not statistically different from the maximum denitrification rate (V_{max}, Table 2), suggesting that denitrifiers are primarily limited by NO$_3^-$ without secondary limitation by oC or P. Potential denitrification did not differ between regions, consistent with assays from lakes at the extreme high and low ends of a gradient of N deposition in Norway (McCrackin and Elser 2010). Interestingly, the potential denitrification rates for Colorado lakes were three times that of Norwegian lakes, perhaps because of differences in the underlying denitrifying communities between regions. In this study, both potential denitrification and V_{max} were negatively related to sediment C:P ratios. Phosphorus has been found to limit heterotrophic microbial growth and respiration (Cleveland
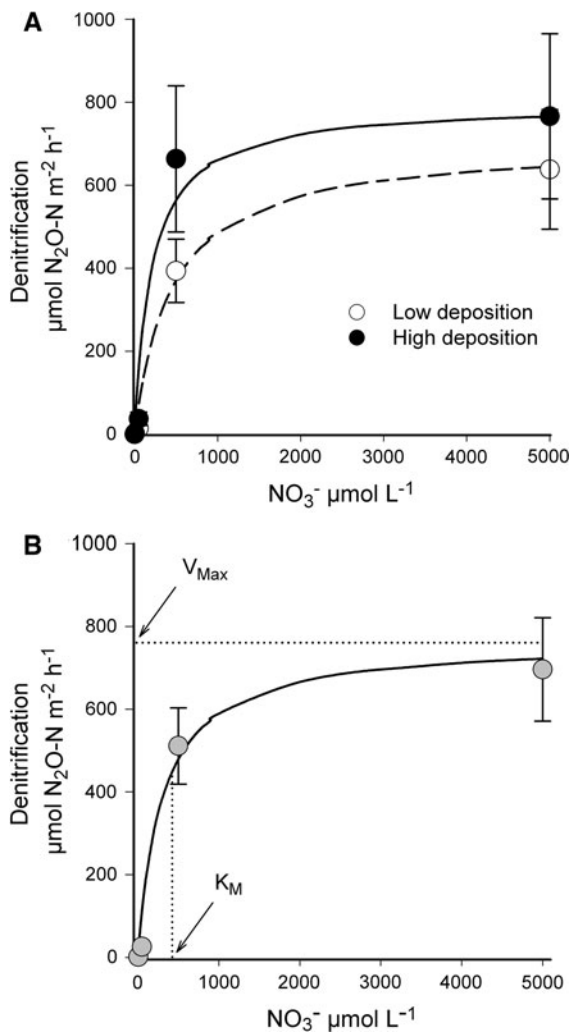
Fig. 2 Results of dose–response experiments. **a** Points are mean denitrification rates for high- and low-deposition lakes (±SE, $n = 10$ for each region) at five NO_3^- concentrations. *Lines* are the predicted denitrification rate using Eq. 1. **b** Points are mean denitrification rates for all sampled lakes (±SE, $n = 20$) The *solid line* shows the predicted denitrification rate using Eq. 1, where V_{max} is 765 μmol N m^{-2} h^{-1} and K_m is 293 μmol l^{-1} NO_3^-

Fig. 3 Relationship between denitrification and NO_3^- concentrations for lakes in the high- (*solid circles*, $n = 10$ lakes) and low-deposition (*open circles*, $n = 10$ lakes) regions ($R^2 = 0.22$, $P = 0.04$)

It is possible that sediment C:P influences the composition of the denitrifying community or correlates with some other factor that is important for maximal denitrification rates. Further research is needed to understand the factors that are important for potential denitrification in lake sediments, but we found no evidence that N loading plays a role.

Estimated background denitrification rates (in response to additions of lake water) did not differ between deposition regions. In these assays, denitrification rates were best predicted by NO_3^- concentrations, which were significantly greater in high deposition lakes. Similar assays conducted with sediments of Norwegian lakes receiving N deposition found denitrification rates were greater in lakes receiving elevated N deposition (McCrackin and Elser 2010). It is unclear why the results differ between studies, but incubation temperatures (4°C for Colorado and 15°C for Norway) could have played a role. Regardless, the estimated background denitrification rate was up to three orders of magnitude less than both the maximum and potential denitrification rates. These results are not strictly comparable because of temperature differences between the incubations, but suggest that sediment denitrifiers are currently performing below the maximal capacities.

The best-fit NO_3^- affinity constant for all lakes in the dose–response experiment, 298 μmol l^{-1} NO_3^-, was ~35 times greater than the mean lake water NO_3^- concentration. Diffusional barriers between NO_3^- and active sites of enzymes and the affinity of

et al. 2002; Cotner and Biddanda 2002), but it is not clear how relative sediment P content influences denitrification rates in the potential denitrification assays where oC and P were supplied in non-limiting concentrations. Rather, we would have expected potential denitrification—and possibly V_{max}—to be positively related to the abundance of denitrifiers, as has been reported in other studies (O'Connor et al. 2006); however, no such relationships were observed.

Table 4 Most probable number of bacteria capable of NO_3^- and NO_2^--reduction by lake

	Sample depth (m)	PAR	Most probable number	
			Nitrate reducers g^{-1} ($\times 10^4$)	Nitrite reducers g^{-1} ($\times 10^2$)
High-deposition lakes				
Brainard	3	0.71	90.9	19.7
Dream	4	0.59	3.0	6.8
Green Lake 1	9	0.82	18.9	0.3
Green Lake 3	10	0.81	75.7	24.7
Isabelle	10	1.25	65.7	5.8
Long	3	0.89	38.1	30.4
Mitchell	1.5	0.22	30.4	71.1
Nymph	1	0.18	5.1	1.1
Red Rock	1	0.21	39.5	0.3
Mean			40.8	17.8
SE			10.9	8.1
Low-deposition lakes				
Andrews	6	1.33	65.2	5.7
Clear	10	0.72	2.9	4.1
Dollar	5	1.17	40.4	0.5
Highland Mary	10	1.15	4.0	6.0
Irwin	5	0.65	15.2	5.5
Little Molas	6	0.81	65.9	3.1
Lost	10	0.84	57.0	24.2
Potato	10	0.90	32.0	46.2
Mean			35.3	11.9
SE			9.9	5.9
High vs. low deposition			High = low	High = low
P			n.s.	n.s.

PAR is the light extinction coefficient, k

Table 5 Comparison of half-saturation constants for denitrification for aquatic ecosystems

Location	Incubation temperature °C	Method	K_m (µmol N)	Reference
20 Colorado lakes, CO, USA	17–20	Sediment slurry, static	298	This study
Temmesjoki River, Finland	15	Intact sediment core	20	Silvennoinen et al. (2008)
Barataria Basin Estuary, LA, USA	26–29	In situ chamber	433	Yu et al. (2006)
Scheldt Estuary, Netherlands and Belgium	20	Sediment slurry, shaken	200–400	Laverman et al. (2006)
San Francisco Bay, CA, USA	20	Sediment slurry, stirred	50	Oremland et al. (1984)
N/A	30	Pure culture, stirred	<15	Betlach and Tiedje (1981)
Kysing Fjord, Denmark	12	Sediment slurry	344	Oren and Blackburn (1979)
Tokyo Bay, Japan	16	Sediment slurry, stirred	27	Koike et. al (1978)

denitrifiers for NO_3^- influence K_m. Generally low values for K_m in pure cultures reflect the absence of diffusion barriers and a bias of bacterial taxa that are selected for fast growth and high affinity for NO_3^- (Laverman et al. 2006). The K_m that we observed is within the range of that that reported in the literature,

suggesting that denitrifier function is comparable across a variety of aquatic ecosystems (Table 5). That said, half-saturation constants less than 50 µmol l^{-1} NO_3^- have also been reported, although these values might result from differences in methods, such as shaking or stirring incubations, or from unique denitrifier assemblages with high NO_3^- affinities. Across the sampled lakes, we found a positive relationship between NO_3^- affinity and sediment OM content, suggesting that that the presence of humic compounds has not reduced the diffusion of NO_3^- or the activity of denitrifying enzymes (Håkanson and Jansson 2002). Perhaps resources available in sediment OM, such as labile oC or trace metal cofactors of denitrification enzymes, alter the composition or performance of the denitrifier community. Overall, however, there was no evidence that N deposition has saturated denitrification in sediments of the lakes we sampled. While atmospheric N loading has increased NO_3^- concentrations in high-deposition areas (11.3 vs. 3.3 µmol N l^{-1}), these lakes are unproductive compared to those in agricultural landscapes. In eutrophic lakes, NO_3^- concentrations can exceed 100 µmol N l^{-1} and background denitrification rates as high as 300 µmol N m^{-2} h^{-1} have been measured (Wetzel 2001; Pina-Ochoa and Alvarez-Cobelas 2006). Both of these values are less than half of the K_m and V_{max}, respectively, that we observed in the dose–response experiment. It is not known, however, how K_m and V_{max} vary between lakes of different trophic status and it would be interesting to study denitrification kinetics in highly N-loaded lakes to determine whether sediments of eutrophic lakes are more likely to saturate than those that we sampled.

Factors influencing denitrifier abundance

Very little is known about how denitrifiers are distributed within or between lakes. Across the lakes we sampled, the light extinction coefficient was a co-predictor of NO_3^--reducer abundance. The biomass of NO_3^--reducers was greater where less light was available to the sediments, perhaps because light influences the abundance of autotrophs and, as a result, interactions with benthic bacteria. It is possible that competition with algae for N or other resources reduces the abundance of denitrifiers. Studies of coastal sediments found that periphyton in littoral

areas suppresses nitrification—and hence denitrification—by out-competing nitrifying bacteria for ammonium (Sundbäck et al. 2004). In addition to less competition for resources with autotrophs, bacteria in profundal sediments also experience a relatively stable thermal regime and are not subject to seasonal changes, such as freeze–thaw cycles, as are microbes in littoral sediments (Nielsen et al. 2001; Sharma et al. 2006). Sediment resources were also important predictors of denitrifier abundances. The MPN of NO_3^--reducers was positively related to sediment OM and the MPN of NO_2^--reducers was negatively related to sediment C:N ratio (increasing sediment N relative to C). Taken together, the relationships between the MPN of denitrifiers and sediment OM, C:N, and available light suggest that denitrifier biomass is more strongly influenced by catchment- and lake-specific factors than by anthropogenic N inputs. This is consistent with a recent meta-analysis of N fertilization studies that found bacterial biomass did not change significantly in response to N additions, although denitrifying bacteria were not specifically considered (Treseder 2008).

Most probable number techniques measure the growth of culturable bacteria and, thus, underestimate abundances of in situ populations. Also, certain members of the bacterial community may respond disproportionately to the incubation conditions. Thus, the application of molecular techniques would be useful to further characterize the abundance and composition of denitrifiers and investigate possible deposition-related effects. We compared our results to those determined with similar methods and found that the abundances of lake sediment denitrifiers were within the ranges reported for desert, riparian, and prairie ecosystems, but less than that for grazed and agricultural soils (Sotomayor and Rice 1996; Cannavo et al. 2004; Johnson et al. 2007; McCarty et al. 2007). The MPN's may not be directly comparable because of differences in incubation conditions. It is notable, however, that despite significant differences in environmental conditions across ecosystems (such as the quality of oC and the temperature and moisture regimes) the abundances of denitrifiers are fairly comparable. Unlike nitrification, which is limited to few taxa, denitrification can be performed by biochemically diverse microorganisms (Knowles 1982). Indeed, denitrifiers have flexible metabolic pathways and use electron acceptors other than N oxides,

perhaps allowing them to develop similarly sized communities in a wide range of ecosystems.

Estimated N removal in high-elevation lakes

In catchments subject to N loading, seasonal patterns of NO_3^- export are an indicator of N saturation (Stoddard [1994](#); Aber et al. [1998](#)). Nitrogen saturation begins with increased seasonal NO_3^- concentrations in water that exits the watershed. Later stages occur when NO_3^- concentrations are elevated in all seasons, with no decline during the growing season and when watershed NO_3^- exports exceed inputs. Symptoms of advanced stages of N saturation have been observed in catchments of the Colorado Rocky Mountains and atmospheric N deposition has been linked with elevated NO_3^- concentrations in high-elevation lakes and streams (Williams et al. [1996](#); Burns [2004](#)). Here, catchment storage and removal of inorganic N is between 21 and 97%, varying largely due to differences in forest and soil cover (Sickman et al. [2002](#)), although lakes were not specifically considered. Musselman and Slauson ([2004](#)) surveyed high-elevation lakes in Colorado and found that NO_3^- concentrations decreased between lake inlets and outlets. While the sediments of the lakes we sampled show considerable denitrification capacity, the role that sediments play in N removal also depends on hydrologic and physical factors (Kelly et al. [1987](#)). The fraction of N removed by lakes generally correlates negatively with lake depth and positively with water residence time (Dillon and Molot [1990](#); Molot and Dillon [1993](#)). Across the sampled lakes, estimated background denitrification rates were best predicted by NO_3^- concentrations and these data permit us to make a preliminary estimate of the magnitude of N removal via denitrification.

We estimated the fraction of N that may be removed in the sampled lakes based on the background denitrification rate as in Harrison et al. ([2009](#)) as $R_N = 1 - \exp(-V_f/H)$, where V_f is the piston velocity for N (m y^{-1}) in lake sediments and H is the areal water discharge from the lake, hydraulic load (m y^{-1}). Using the method of Kelly et al. ([1987](#)), the piston velocity was determined as $V_f = $ denitrification rate/$[NO_3^-]$, with the estimated background denitrification rate and NO_3^- concentration for each lake. The resulting mean piston velocity was 2.1 m y^{-1}, with a range of 0–23.8 m y^{-1},

comparable to values reported by Harrison et al. ([2009](#)). The hydraulic load was determined as in Wollheim and Vorosmarty ([2006](#)) as $H = \bar{z}/\tau$, where the mean lake depth, $\bar{z}$ (m), was assumed to be one-third of the maximum depth of each lake and the water residence time, τ (y), was assumed to be 0.083 (1 month) or 1 year, which is in the range of residence times reported for lakes in areas where we sampled.

Nitrate concentrations in lakes in the Colorado Rocky Mountains vary seasonally due to the timing of snowmelt and biological assimilation (Campbell et al. [2002](#)). In the Loch Vale watershed, stream water NO_3^- concentrations have been found to vary between 10 and 35 μmol l^{-1} seasonally (Baron and Campbell [1997](#)). Concentrations tend to peak in lake May, decrease during June and July, and increase in the fall. Assuming lakes follow a similar pattern, the timing of our sampling likely missed peak NO_3^- concentrations. We expect the denitrification rate would increase with additional NO_3^-; thus, variability in concentrations contributes to uncertainty in our estimates. Indeed, temporal variation in sediment denitrification has been attributed to seasonal changes in NO_3^- concentrations (Hasegawa and Okino [2004](#)). Consequently, we calculated a 95% confidence interval (CI) of N removal based on the standard error of mean R_N to reflect uncertainty and variability in these parameters.

In the case of extremely short (1 month) residence times, we estimate that the sampled lakes could remove an average 11% (4–25% CI) of N inputs across all lakes, 18% (10–38% CI) for high-deposition lakes and <1% (0–2% CI) for low-deposition lakes. For a 1 year residence time, denitrification potentially removes an average 26% (9–43% CI) across all lakes, 41% (10–72% CI) for high-deposition lakes, and 9% (0–18% CI) for low-deposition lakes. There are many uncertainties in scaling from laboratory measurements of denitrification to whole-lake estimates of N removal. To refine these estimates, existing models of N removal (e.g. Harrison et al. [2009](#)) could be modified and downscaled. Further work is needed to understand the in situ denitrification rate and how it varies within and between lakes, and the seasonality of NO_3^- concentrations. Obtaining such data from remote, high-elevation lakes is challenging. While our estimates are approximate, they suggest that denitrification in

lake sediments can be an important biogeochemical sink for N.

Atmospheric N deposition is the dominant source of new N to high-elevation watersheds in the Front Range of the Colorado Rocky Mountains (Sickman et al. 2002). The source of NO_3^- to sediment denitrifiers, however, is not clear. In Colorado, NO_3^- and NH_4^+ are 61 and 39%, respectively, of N deposition (Baron et al. 2000). Thus, deposition can deliver NO_3^- to the lake directly or through watershed runoff, and indirectly through nitrification resulting from elevated NH_4^+ inputs. Indeed, isotopic studies have found that nitrification in talus slopes is a significant source of NO_3^- to streams in the Colorado Front Range (Campbell et al. 2002; Nanus et al. 2008). In lake sediments, the extent to which nitrification and denitrification are coupled depends on concentrations of NO_3^- in bottom waters (Dong et al. 2000; Seitzinger et al. 2006). Where NO_3^- concentrations are <10 μM, over 90% of denitrification may tightly coupled with nitrification. Where NO_3^- concentrations are >10 μM, such as in high-deposition lakes, sedimentary denitrification may predominantly consume NO_3^- that diffuses from the water column. Hence, the source of NO_3^- to sediment denitrifiers likely varies between deposition regions.

One factor that may have influenced our results is water pH. Due to their underlying geology, lakes in the high-deposition region are sensitive to atmospheric pollutants (Clow et al. 2003; Nanus et al. 2009). Increased pH and decreased acid neutralizing capacity have been observed in the surface water of lakes in the Colorado Front Range. Indeed, Elser et al. (2009b) sampled lakes in the same regions and reported that pH averaged 6.5 in high-deposition lakes and 7.1 in low-deposition lakes. The optimum pH range for denitrification is between 7 and 8 and there is generally a positive relationship between pH and denitrification rates (Knowles 1982). Interestingly, whole-lake studies found that experimental acidification enhanced, not repressed, denitrification rates (Rudd et al. 1988). Accordingly, it is not clear whether reduced pH in high-deposition lakes had a positive or negative effect on our denitrification measurements.

Another factor that could have influenced our results is thermal stratification. Lakes in the low-deposition region tended to be thermally stratified, while lakes in the high-deposition region were not, likely because lakes were deeper in the former region. Stratification may have reduced sedimentation rates by trapping particles above the thermocline (Håkanson and Jansson 2002). Here, and in previous work, we found that sediment denitrification was not limited by oC so it is unlikely that stratification influenced the MPN results or denitrification measured in response to the addition of lake water (McCrackin and Elser 2010). Additionally, all of the sampled lakes are unproductive and we found no evidence of anoxia in the hypolimnion. We did not measure the oxygen status of the sediments, however, our measurements of denitrification are likely typical of deeper, anoxic layers (Wetzel 2001). Lastly, we only collected water from above the sediments, so we do not know how NO_3^- concentrations vary within the lake.

In sum, N deposition has reduced pH, changed the composition and biomass of the diatom community, altered the stoichiometric ratios of N to P in the water column, and shifted plankton nutrient limitation in Colorado lakes and elsewhere (Baron et al. 2000; Elser et al. 2009a, b). Future population growth in metropolitan Denver and agricultural intensification in eastern Colorado will result in increased N inputs to the Front Range of the Colorado Rocky Mountains and will further stress ecosystems that currently show signs of N saturation (Burns 2004). While lakes in the Colorado Front Range receive less atmospheric N deposition than those in other areas, they may be more sensitive to deposition because of exposed bedrock and sparse catchment vegetation (Clow et al. 2003) and because atmospherically delivered N represents a significant portion of the N that is cycled. Our data show that atmospheric N deposition is associated with elevated lake NO_3^- concentrations, but that chronic N loading has not altered the capacity of denitrifying communities or the abundance of NO_3^-- and NO_2^--reducing bacteria in lake sediments. Sediment denitrifiers are limited primarily by NO_3^-, regardless of background N inputs. We estimate that denitrification in lakes is an important sink for NO_3^- that can mitigate N loading to downstream ecosystems. Additionally, if atmospheric N deposition rates increase substantially, our findings suggest that lake sediments possess significant capacity to remove much of this anthropogenic N.

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