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Dissimilatory nitrate reduction pathways in an oligotrophic freshwater ecosystem: spatial and temporal trends

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ABSTRACT: Elevated nitrate (NO_3^-) concentrations can cause eutrophication, which may lead to harmful algal blooms, loss of habitat and reduction in biodiversity. Denitrification, a dissimilatory process that removes NO_3^- mainly as dinitrogen gas (N_2), is believed to be the dominant NO_3^- removal pathway in aquatic ecosystems. Evidence suggests that a less well-studied process, dissimilatory nitrate reduction to ammonium (DNRA), which retains nitrogen (N) in the system, may also be important under favorable conditions. Using stable isotope tracers in sealed microcosms, we measured the potential for NO_3^- losses due to DNRA and denitrification in an oligotrophic freshwater ecosystem. We took sediment and water samples at runoff and baseflow, across several ecotypes. Our objective was to quantify the relative importance of DNRA compared to denitrification with changes in ecotype and season. Potential denitrification rates ranged from 0 to 0.14 ± 0.03 µgN gAFDM^{-1} d^{-1}. Potential DNRA rates ranged from 0 to 0.0051 ± 0.0008 µgN gAFDM^{-1} d^{-1}. Denitrification losses peaked at the inflow stream ecotype at 96.2% of total dissimilatory NO_3^- removal, whereas losses due to DNRA peaked in the lake ecotype at 34.4%. When averaged over the entire system, denitrification peaked at baseflow (31.2%), while DNRA peaked at runoff (2.9%). Although NO_3^- transformations due to denitrification were higher than DNRA in all ecotype and temporal comparisons, our results suggest that DNRA is also important under favorable conditions.

KEY WORDS: DNRA · Denitrification · Nitrogen transformations · Ecotype · Season

INTRODUCTION

Anthropogenic activities have affected the global nitrogen (N) cycle substantially. Current estimates suggest that creation of reactive N has increased by 120% since 1970 due to agriculture and industry and the rate is still increasing dramatically (Galloway et al. 2008).

Riverine export of total N increased globally by up to 30% between 1970 and 2000 (Seitzinger et al. 2010). Increased N loading in riverine systems can cause local eutrophication and can increase N fluxes to coastal systems. This loading adds to the problem of coastal eutrophication and, in extreme cases, can lead to hypoxic zones such as that in the Gulf of Mexico (Rabalais et al. 2001). The main biological process for removal of N as nitrate (NO_3^-) from freshwater systems is the microbial process of denitrification (Seitzinger 1988). However, a competing process, dissimilatory nitrate reduction to ammonium (DNRA), retains N in the system in a bioavailable form (Tiedje et al. 1982). It is important to understand the processes that remove or transform NO_3^- in order to manage aquatic ecosystems properly and prevent potential problems such as harmful algal blooms (Davis & Koop 2006).

Respiratory denitrification (hereafter denitrification) is a dissimilatory process of facultatively anaerobic microbes in the absence of oxygen (O_2 < 10 µM, Tiedje 1988). NO_3^- is reduced to NO_2^-, NO, N_2O and

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finally N_2 (Ye et al. 1995). The final reduction products, nitrous oxide (N_2O), a potent greenhouse gas (Ramaswamy et al. 2001), and dinitrogen gas (N_2), are lost from the system into the atmosphere (Delwiche & Bryan 1976). In the presence of O_2, most denitrifying bacteria will switch to the physiologically preferred process of aerobic respiration at the expense of NO_3^- reduction. (Megonigal et al. 2004). Denitrification may be diminished by the presence of free sulfides, which can inhibit the enzymes responsible for the final 2 stages of the process (Burgin & Hamilton 2007).

DNRA is a microbial process that transforms NO_3^- to ammonium (NH_4^+) via formation of NO_2^- in anaerobic or low O_2 environments. The final N form, NH_4^+, is bioavailable and readily immobilized by microbes and plants, or transformed by nitrification (Bengtsson et al. 2003). There are 2 DNRA pathways; fermentative and chemolithoautotrophic. Fermentative DNRA microbes reduce NO_3^- to NO_2^- to produce ATP. The subsequent reduction of NO_2^- to NH_4^+ is an electron sink that allows re-oxidation of NADH (Tiedje 1988). Chemolithoautotrophic DNRA is the transformation of NO_3^- to NH_4^+, linked to oxidation of reduced sulfur (S) compounds. This sulfur-driven NO_3^- reduction leads to production of N_2 and N_2O via respiratory denitrification. However, because higher concentrations of free sulfides may inhibit the final steps of denitrification, (Brunet & Garcia-Gil 1996, Burgin & Hamilton 2007) reduction to NH_4^+ via DNRA should dominate. The fermentative microbes are favored by non-sulfidic sediments with high C:N ratios, whereas the chemolithoautotrophic microbes prefer sediments where S oxidizers dominate and H_2S is present in appreciable concentrations (Burgin & Hamilton 2007). While most DNRA active microbes are anaerobes (Tiedje 1988), recent evidence suggests they can also tolerate low levels of O_2, while continuing to reduce NO_3^-, especially at high C:N ratios (Fazzolari et al. 1998, Silver et al. 2001).

The main factors believed to govern the balance between denitrification and DNRA in freshwater sediments are the ambient O_2 concentration (Fazzolari et al. 1998, Silver et al. 2001), the C:N ratio (Tiedje 1988), and the presence of free sulfides (H_2S, S^{2-}) or elemental S (Brunet & Garcia-Gil 1996, Burgin & Hamilton 2007). Other possible contributing factors include the presence of macrophytes (Nijburg & Laanbroek 1997a,b) and ambient temperature (Ogilvie et al. 1997, Scott et al. 2008, Nizzoli et al. 2010).

Spatial and temporal variations in the balance between denitrification and DNRA in freshwater ecosystems have been studied by relatively few researchers, and studies seldom quantify variation in both space (e.g. between different ecotypes) and time. Accordingly, we aimed to elucidate NO_3^- losses due to potential DNRA and potential denitrification, across a stream-lake interaction zone of a sub-alpine watershed. Our objective was to quantify the relative importance of DNRA compared to denitrification with changes in ecotype and season.

MATERIALS AND METHODS

Sample sites

The sampling area, Warm Springs Creek and Bull Trout Lake, is an oligotrophic stream-lake system in a sub-alpine watershed in the Sawtooth Mountains in Idaho, USA. Sediment cores and water samples were obtained from 7 sites (Fig. 1) We sampled in June

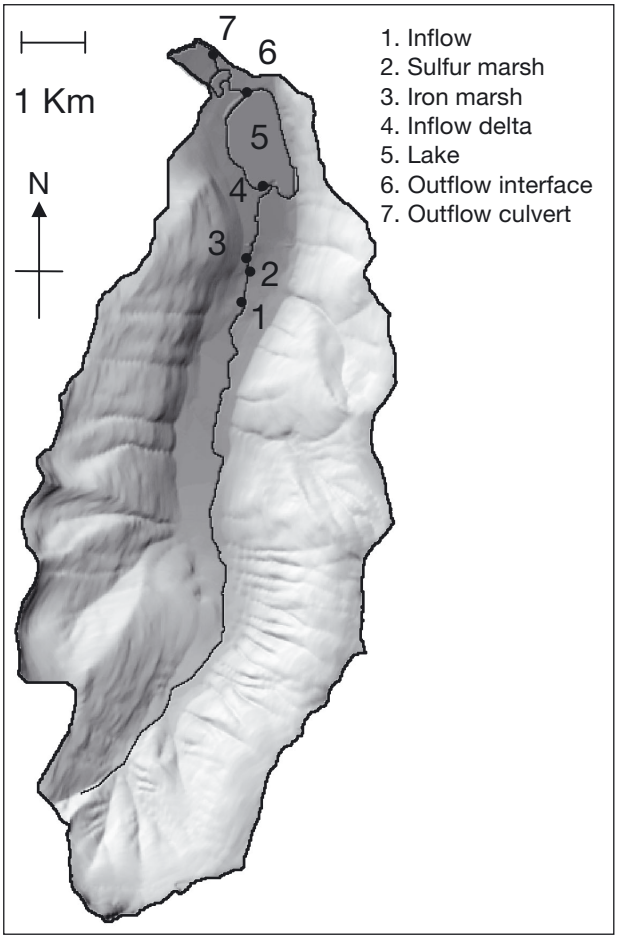

Fig. 1. Field sites at Bull Trout Lake and Warm Springs Creek in the Sawtooth Mountains in southern Idaho, USA

2008, during snowmelt (runoff), close to peak discharge, measured at ~ 858 l s^{-1} at Site 1 (K. J. Goodman pers. comm.). Samples were again taken at baseflow in August 2008, with a discharge of about 154 l s^{-1} at Site 1 (K. J. Goodman pers. comm.). Peak discharge occurred on about the same date for all sites, as did baseflow.

Site 1 was located in-stream, approximately 1.5 km upstream from the lake (Fig. 1). Site 2 was in a lateral pool just downstream of Site 1 in the delta marsh, and contained abundant emergent plants on the outskirts of the pool. Site 3 was about 1 km upstream from the lake in an algae filled, stagnant side channel in the delta marsh. Site 4 was at the stream-lake interface at the head of the lake. Site 5 was located at about 3 m depth in the littoral zone of the lake where submerged macrophytes were plentiful. Site 6 was at the outflow stream-lake interface at the bottom of the lake. Site 7 was in-stream, approximately 100 m downstream of the lake. Sites 1 and 4 were categorized as the inflow stream ecotype. Sites 2 and 3 were categorized as the marsh ecotype. Sites 5 and 6 were taken as the lake ecotype, (Site 6 was right at the edge of the lake where the water temperature and sediment consistency indicated lake conditions). Site 7 was the outflow stream ecotype.

Microcosms

Four sample cores were obtained from each site on both dates. However only 3 cores were collected at Site 7 during runoff and none were collected for Site 3 at baseflow as it had dried out. Sediment from at least 15 cm below the water-sediment interface was extracted using a coring device. The cores were measured and the top 10 cm (6 cm for the lake samples) of sediment discarded. The rest of each sediment sample was then pushed out into a plastic bag and sealed and the core depth was recorded. Lake samples were taken using a Wildco® standard KB core sampler (Rickly Hydrological) at runoff and with hand deployed cores using SCUBA diving at baseflow. Water samples were also taken at each site.

On return to the lab the sediments were weighed out into Mason jars, topped off with sample water, sealed and shaken. After settling, the overlying water was sampled for $^{15}N_2$, $^{15}N_2O$, $^{15}NH_4^+$, $^{14+15}NH_4^+$ and $^{14+15}NO_3^-$ and then the jars were topped off with the appropriate sample water again, sealed, shaken and stored in the dark for 24 h to assure anoxia. Duplicate samples were taken so that the O_2 levels could be checked for anoxia. We did not extract sorbed NH_4^+ using KCl and therefore it is possible that our potential DNRA rates are underestimated.

Stable isotope tracer (0.4 ml and 0.8 ml to runoff and baseflow samples, respectively, of 50.32 mg l^{-1} $Na^{15}NO_3$-N solution, 99 atom %) and nutrient solutions (1.0 ml of 25 mg l^{-1} KNO_3-N + 4 mg l^{-1} KH_2PO_4-P + 1.5 g l^{-1} Dextrose-C solution) were added with a syringe through a gas impermeable septa to each microcosm (sediment jar) at T_0. We calculate that addition of ^{15}N tracer enriched the nitrate pool to 90 atom percent and 70 atom percent, at runoff and baseflow respectively. The addition of $^{15}NO_3$-N increased the $^{14+15}NO_3$-N mass in each microcosm on average from 2.1 µg to 47.2 µg at runoff and from 16.9 µg to 82.1 µg at baseflow. Because of both this increase in N and the addition of the nutrient solution we consider the rates we present here as potential rates. Microcosm septa were re-sealed with Aquaseal Urethane Repair Adhesive (McNett), incubated in the dark at 20°C for approximately 11 h and sampled once more for $^{15}N_2$, $^{15}N_2O$, $^{15}NH_4^+$, $^{14+15}NH_4^+$ and $^{14+15}NO_3^-$.

Chemistry

All $^{14+15}NO_3^-$ and $^{14+15}NH_4^+$ samples were run on an Astoria Pacific flow injection analyzer using methods adapted from the phenolhypochlorite method (Solorzano 1969) for NH_4^+ and the cadmium reduction method (Grasshoff 1976) for NO_3^-. Dissolved organic carbon (DOC) samples were run on an OI Corporation model 700 TOC analyzer using the protocol outlined by Bernard (1984). ^{15}N (N_2, N_2O and NH_4^+) samples were run on a continuous flow Isotope Ratio Mass Spectrometer (IRMS) or on a Europa ANCA-SL elemental analyzer at the UC Davis and Marine Biological Laboratory stable isotope facilities, respectively.

Potential denitrification and DNRA rates were calculated as the change in $^{15}N_2$ and $^{15}NH_4^+$ nitrogen mass, respectively, over time per gram of ash free dry mass (AFDM) of sediment (given as µgN $gAFDM^{-1}$ d^{-1} and corrected for initial ambient $^{15}NO_3$-N mass). Both microbial processes were also calculated as percent transformation of $^{15}NO_3$-N mass per day (to $^{15}NH_4$-N mass for DNRA and $^{15}N_2$-N mass for denitrification) corrected for initial ambient $^{15}NO_3$-N mass. $^{15}N_2O$ production was measured but not attributed to either of these 2 processes. DNRA was also measured as a percentage of total dissimilatory NO_3^- removal, with the total being made up of denitrification plus DNRA plus N_2O production. Note that we measured

denitrification as production of $^{15}N_2$-N and our method did not distinguish between denitrification and anammox.

Percent organic matter was measured as the percentage of pre-dried sample burned off by the ashing process (sample heated to 450°C in muffle furnace for 2 h). AFDM was taken as the mass of the pre-dried sample remaining after ashing.

Statistical analysis

For pairwise comparisons of data groups we used the multiple response permutation procedure (MRPP) in the USGS statistical package Blossom (Cade & Richards 2005). This non-parametric analysis accommodates data with heterogeneous variances, non-normal distributions and small sample sizes. One-sample, single tailed *t*-tests (R statistical software, www.r-project.org) were used to evaluate whether the N transformations measured were significantly greater than zero.

RESULTS

Biogeochemistry

The lake and wetland ecotype sediments contained the most organic matter, 9.9 and 7.0% by mass, respectively. The inflow and outflow ecotypes only contained 0.4 and 1.3% organic matter, respectively. DOC was measured at Sites 1, 6 and 7 and then averaged to give total available C values (ambient + added DOC) of 2340 µg and 2040 µg per microcosm equivalent volume at runoff and baseflow, respectively. NH_4^+ and NO_3^- were measured in microcosms from all sites, averaged, and combined to give total available N values (ambient + added DIN) of 51.0 µg and 87.9 µg per microcosm at runoff and baseflow, respectively.

Spatial trends

Potential rates of denitrification and DNRA varied spatially and temporally. The potential denitrification rate ranged from 0 to 0.14 ± 0.03 µgN gAFDM^{-1} d^{-1} over the entire study, while potential DNRA rates ranged from 0 to 0.0051 ± 0.0008 µgN gAFDM^{-1} d^{-1}. DNRA rate was significantly higher at Site 6, the interface between the lake and the outflow, on both dates (although only marginally significant at baseflow, p = 0.098). Mean rates of DNRA and denitrification were significantly greater than zero in approximately one half of the samples (Fig. 2). Denitrification rates were not significantly greater than zero at any site during runoff but were greater than zero at more than half of the sites during baseflow (Fig. 2). Rates of N_2O production were also measured but due to low values and high variation all but one result were non-significant, and this one rate was negligible compared to denitrification and DNRA (Site 4: $1.2 \times 10^{-6} \pm 4.7 \times 10^{-7}$ µgN gAFDM^{-1} d^{-1}, p = 0.010, results not shown).

The highest denitrification rate of the samples taken at runoff was measured at Site 4 (0.06 ± 0.03 µgN gAFDM^{-1} d^{-1}, p = 0.033, Fig. 2). The maximum DNRA rate was 0.0051 ± 0.0008 µgN gAFDM^{-1} d^{-1} (p < 0.050), measured at Site 6. Denitrification rate exceeded DNRA rate at Site 6 in June by an order of magnitude (p = 0.050). All other pairwise comparisons between denitrification and DNRA were not statistically significant (p > 0.050).

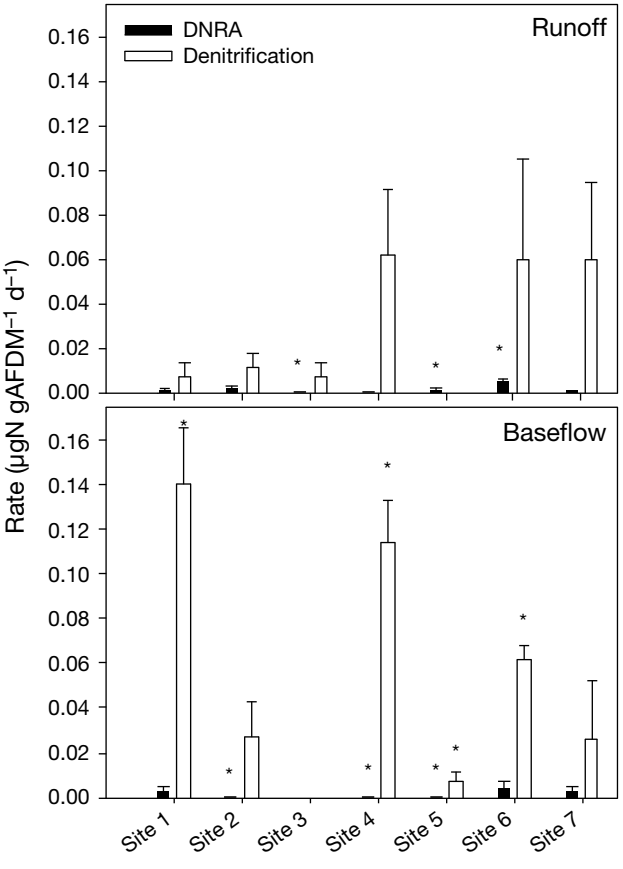

Fig. 2. Potential mean (±SE) rates of denitrification and dissimilatory nitrate reduction to ammonium (DNRA) (µgN gAFDM^{-1} d^{-1}) for each site, sampled at runoff and baseflow. Asterisks denote p < 0.050, with the exception of Baseflow, Site1, p = 0.058, Site 2, p = 0.057, Site 5, p = 0.060

The baseflow data set results show averages of the 2 microbial processes to be statistically different ($p < 0.0001$, Fig. 2), with the maximum rate of denitrification exceeding that of DNRA by nearly 3 orders of magnitude ($p = 0.016$, Fig. 2). Denitrification rate was highest at Site 1 (0.14 ± 0.03 µgN gAFDM^{-1} d^{-1}), but means across sites were not significantly different ($p > 0.050$). DNRA rates ranged from 0.0002 ± 0.0001 µgN gAFDM^{-1} d^{-1} at Site 2 to 0.0006 ± 0.0002 µgN gAFDM^{-1} d^{-1} at Site 5, although means were not statistically different ($p > 0.050$).

The mean percent transformation of NO_3-N due to denitrification was lowest in the wetland ecotype (12.7 ± 4.4%, Fig. 3) and highest in the stream ecotypes (36.1 ± 8.0%, inflow stream, Fig 3). However, the only statistically significant difference between denitrification values was between the wetland and inflow ecotypes ($p = 0.028$), so there was no statistically significant spatial trend.

Percent NO_3-N transformation per day due to DNRA, averaged over both seasons, increased downstream from the wetland ecotype (0.5 ± 0.2%) to peak at the lake ecotype (3.6 ± 0.7%, Fig. 3). MRPP analysis showed the lake maximum to be significantly different to all other ecotypes ($p < 0.050$, with the exception of comparison to outflow, which was marginally significant, $p = 0.086$)

The rate of N_2O production was considerably lower than that of DNRA per ecotype ($p < 0.001$, Fig. 3), with the exception of the wetland ecotype, which had approximately equal transformations of N due to DNRA and N_2O production (wetland DNRA = 0.5 ± 0.2%, wetland $N_2O = 0.5 \pm 0.2$%, $p = 0.641$).

We calculated DNRA as a percentage of total dissimilatory NO_3^- removal (with the total being defined as the sum of denitrification, DNRA and N_2O production) to evaluate the relative importance of this process as a NO_3-N removal pathway. Nitrogen transformations due to DNRA were greatest at the lake site (34.4 ± 21.9%, Fig. 4) and lowest at the inflow stream site (3.7 ± 2.8%, Fig. 4). Ecotypes were not significantly different to each other except for comparisons between the inflow and lake ($p = 0.043$) and between the inflow and outflow ($p = 0.075$, only marginal significance). DNRA seems to be a potentially more important pathway for NO_3-N removal in the lake than in any of the other ecotypes in our study.

Temporal trends

Transformation of N due to denitrification was potentially more important during baseflow (31.2 ± 4.9%) compared to runoff (19.9 ± 6.0%) when averaged across sites ($p = 0.011$, Fig. 5). In contrast, NO_3-N transformation due to DNRA was higher at runoff (2.9 ± 0.7%) than at baseflow (1.3 ± 0.4%, $p = 0.027$; Fig. 5). Similarly N_2O production was higher at runoff (0.2 ± 0.1%) than at baseflow (0.03 ± 0.02%, $p = 0.037$; Fig. 5).

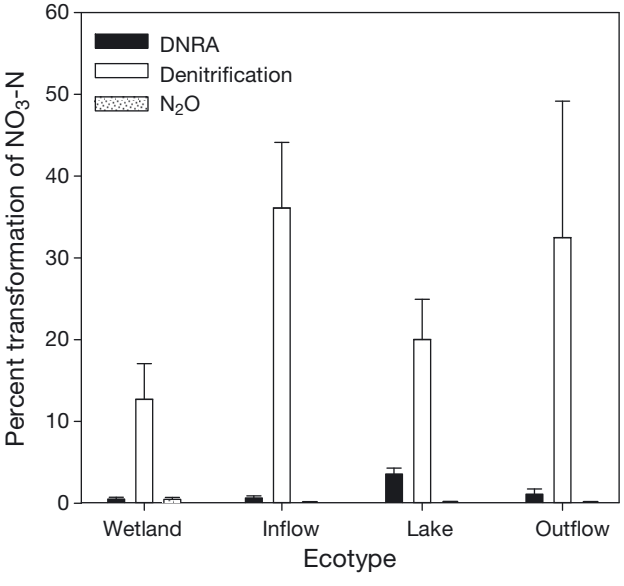

Fig. 3. Means (±SE) dissimilatory nitrate reduction to ammonium (DNRA), denitrification and N_2O production, measured as percent NO_3-N transformation per day (calculated by mass), per ecotype down the watershed (left to right)

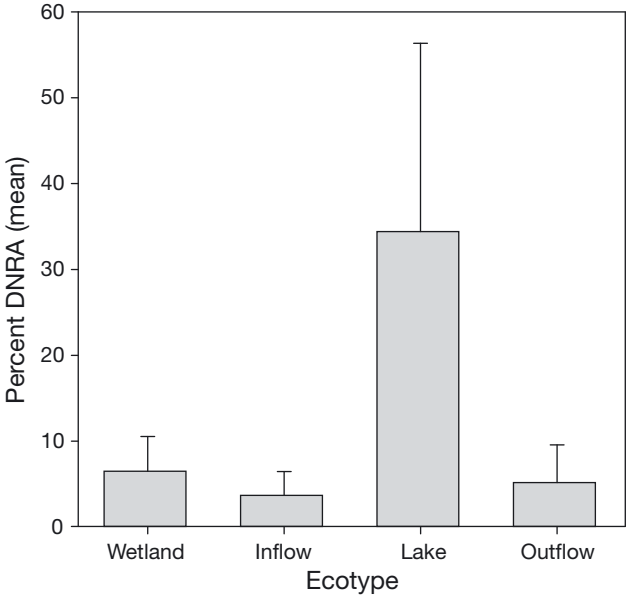

Fig. 4. Average (±SE) dissimilatory nitrate reduction to ammonium (DNRA) as a percentage of total dissimilatory nitrate removal per ecotype

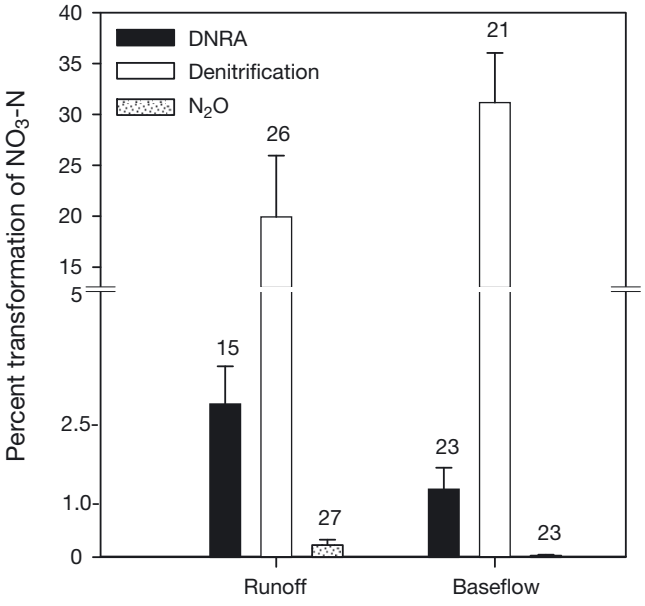

Fig. 5. Average (±SE) dissimilatory nitrate reduction to ammonium (DNRA), denitrification and N_2O values, measured as percent transformation of NO_3-N per day (calculated by mass), at runoff (June samples) and at baseflow (August samples). Multiple response permutation procedure (MRPP) analysis gave p = 0.0270 for DNRA, p = 0.0114 for denitrification and p = 0.0369 for N_2O. The number of observations (n) is indicated above each bar

DISCUSSION

Spatial patterns in NO_3^- losses by dissimilatory pathways

The lake sediments were relatively productive in the littoral zone compared to the other ecotypes as confirmed by the calculated percent organic matter. The wetland and lake ecotype sediments contained considerably more organic matter than the inflow and outflow ecotype sediments. Additionally, the top 5 to 6 cm of each lake core collected at Site 5 was visibly green, and Site 6 samples were noted as smelling strongly of sulfides. Owing to high organic matter content, sediments from lake and wetland ecotypes were relatively highly reducing, as they all went anoxic within 30 min of being sealed in the dark, whereas microcosms from the other ecotypes took close to 11 h. Highly reducing sediments that contain free sulfides (S^{2-} or H_2S) can facilitate the chemolithoautotrophic DNRA process (Buresh & Patrick 1981, Burgin & Hamilton 2007), while at the same time free sulfides also inhibit the enzymes that sustain the final steps of the denitrification process (Brunet & Garcia-Gil 1996, Burgin & Hamilton 2007). Therefore the presence of highly reducing sediments and hence free sulfides may have suppressed denitrification in our samples while potentially enhancing the DNRA process.

High importance of DNRA to total dissimilatory NO_3-N transformation in lake sediments also may be attributed to the presence of macrophytes. The presence of certain macrophytes in low nitrate sediments may greatly increase the proportion of DNRA to denitrification, possibly due to increased C availability from root exudates and elevated O_2 levels, (Nijburg & Laanbroek 1997b). Aerenchymatous plants release O_2 into the root zone when healthy (Nijburg et al. 1997), and this process in turn selects for DNRA over denitrification as DNRA is less inhibited by O_2 presence than denitrification, especially at high C:N ratios (Fazzolari et al. 1998). *Potamogeton praelongus* and *Elodea canadensis* (identified as aerenchymatous macrophytes) were abundant in Bull Trout Lake and were present at Site 5. Macrophytes were not substantially present in the inflow and outflow stream ecotypes.

Temporal variation in NO_3^- losses via dissimilatory pathways

The data in this study show that denitrification is potentially more important during baseflow than runoff, while the opposite is true for DNRA. A similar temporal trend was observed in a fringing marsh-aquifer ecotone where, seasonally, the denitrification:DNRA ratio was 25-fold lower at runoff (0.6) than at baseflow, suggesting that NO_3^- removal was significantly higher during baseflow conditions. However water temperatures varied by about 2°C between seasons and were therefore unlikely to account for this trend (Tobias et al. 2001).

Denitrification and DNRA may be carried out by different competing species of microbes, with ambient conditions selecting for or against denitrifiers (Tiedje 1988, Megonigal et al. 2004). The relative changes in denitrification and DNRA from runoff to baseflow could be explained by this competition, which could be governed by a shift in the balance of available nutrients. Denitrification is generally favored by more C-limited conditions, and DNRA by sediments more enriched with available C, specifically with high C:N ratios, (Tiedje 1988, Omnes et al. 1996, Kelso et al. 1997). Fazzolari et al. (1998) measured DNRA at changing C:N ratios and found that in all but one case an

increase in C:N ratio correlated to an increase in NH_4^+ production via DNRA. Our nutrient data showed average dissolved C:N ratios (DOC:DIN) in our microcosms of 46 at runoff and 23 at baseflow. The higher ratio at runoff is expected in this system, due to increased DOC inputs with snowmelt from the watershed. B. McGlynn (pers. comm.) found C:N ratios of 35 at runoff and 22 at baseflow in the Warm Springs Creek/Bull Trout Lake system (average of 4 sites in the lake, inflow and outflow). Inputs to the inflow stream peaked at runoff in late May, when inflow DOC was measured at 2.81 mg l^{-1}, and stayed high through the first week of June. Baseflow average was measured as only 0.65 mg l^{-1} (K. J. Goodman pers. comm.).

Temperature can influence the balance of denitrification and DNRA, and mounting evidence points towards a summer DNRA maximum. Ogilvie et al. (1997) reported that denitrifying bacteria were better than fermentative nitrate-ammonifiers at scavenging NO_3^- at low temperatures (5°C) and vice versa at high temperatures (20°C). DNRA was only measured at Lake Waco wetlands in Texas during the summer months when temperatures averaged 28.6°C, as compared to a winter average of 8.4°C (Scott et al. 2008). Nizzoli et al. (2010) found that DNRA was appreciably higher in Lake Verde in summer samples (13°C) compared with the winter (5°C). However, modeled DNRA was favored in more extreme temperatures (<14°C and >17°C) whereas denitrifying microbes preferred a narrow range of 14 to 17°C (Kelly-Gerreyn et al. 2001). Although our microcosms were all incubated at 20°C, different ambient temperatures between seasons may have selected for different microbial populations at the time of sample collection.

Potentials

All rates and percent transformations mentioned in this study refer to potential values, although the nutrient concentrations we employed were not outside the realms of natural variation at this study site (Hall et al. 2009, Marcarelli & Wurtsbaugh 2009). The addition of N, C and P to the microcosms to remove low-level nutrient limitation, and the use of ^{15}N as a tracer, altered the available nutrient pool and influenced the rates of localized microbial processes (Burgin & Hamilton 2008). Therefore, it was not possible to measure actual *in situ* rates of denitrification and DNRA for our sites in this experiment.

Sediment depths

To obtain results from microbial communities controlled for O_2 concentration, we removed the top portion of sediment (6 to 10 cm) from each core. Highly oxygenated sediments (due to significant hyporheic flow in our lotic systems and a low density floc of episammic algae in the top 6 cm of loosely packed lake sediment) may have boosted the importance of DNRA relative to denitrification as the latter process is known to be inhibited by the presence of even low concentrations of O_2, whereas DNRA is more tolerant of oxygen (Fazzolari et al. 1998). Even though all of our microcosms were forced to be anoxic, sampling the oxygenated sediment could bias the microbial community composition in favor of O_2 tolerant microbes.

N_2O

N_2O production represented a small transformation of NO_3 compared to the processes of DNRA and denitrification. N_2O could be attributed to either DNRA or denitrification as it is believed to be an intermediate in both pathways (Tiedje 1988, Welsh et al. 2001, Burgin & Hamilton 2008).

Therefore, both DNRA and denitrification may be underestimated. However, because N_2O production was either not significantly different from zero, or negligible, this underestimation would be small relative to the measured rates of DNRA and denitrification. Thus, in this study N_2O production rates were only used to complete the calculation of total dissimilatory nitrate reduction.

Anammox

Anammox, the combination of NO_2^- (from reduction of NO_3^-) and NH_4^+ to form N_2 gas under anaerobic conditions (Dalsgaard et al. 2005) has not been addressed in this study. This process is mainly of interest in marine systems, where it contributes up to 67% of total N_2 production in continental shelf sediments (Thamdrup & Dalsgaard 2002). In one freshwater system anammox accounted for 7 to 13% of the total production of N_2 but this was only measured in the water column (Schubert et al. 2006). Because we did not measure anammox in this study it is therefore possible that our denitrification figures are overestimated. However, anammox rates are believed to

be higher in eutrophic rather than oligotrophic sediment conditions (Megonigal et al. 2004) with relatively high NO_3^- concentrations (Rysgaard et al. 2004) and low labile carbon concentrations (Jetten et al. 1998). It would, therefore, seem probable that this process would be minimal in our system.

Cation exchange

In our study, we used a simple mixing method versus the KCl extraction method (Morin & Morse 1999). Cation exchange in our sediments may have reached equilibrium in less than 2 h (Rosenfeld 1979), and so the exchange of NH_4 between the sediment surface and dissolved fraction should have been at equilibrium before the microcosm experiments started. It is possible that the $^{14}NH_4$ and $^{15}NH_4$ fractions could have interchanged during our microcosm experiments (Seitzinger et al. 1991, Gardner et al. 2006), and therefore DNRA may be underestimated due to this mechanism.

Global comparisons

Measured as percent of the total dissimilatory NO_3^- removal at each ecotype, our DNRA results can be compared to global data as reviewed by Burgin & Hamilton (2007). Our results range from 0 to12 % at the inflow stream ecotype to 6 to 99 % at the lake ecotype and overlap with global freshwater data (Freshwater lakes: Nijburg & Laanbroek 1997b, Nizzoli et al. 2010; wetlands: Ambus et al. 1992, Scott et al. 2008; streams: Kelso et al. 1999, Omnes et al. 1996; Fig. 6). According to this small sample of global data, and data presented by Burgin & Hamilton (2007), wetland and lake ecotypes in general have higher percent DNRA than stream ecotypes. The results of this study agree with this finding. However, in this study, the lake ecotype had by far the highest proportion of DNRA as a percentage of total dissimilatory nitrate removal, but was also most variable (34.42 ± 21.92 %, Figs. 4 & 6).

We infer that denitrification accounts for the main proportion of dissimilatory nitrate removal in each ecotype (Fig. 4). Optimal conditions for DNRA in freshwater sediments are still poorly defined. The results in this study show that DNRA varies spatially and temporally and has potential to rival denitrification in the sediments of some freshwater ecotypes, particularly those with high organic matter content.

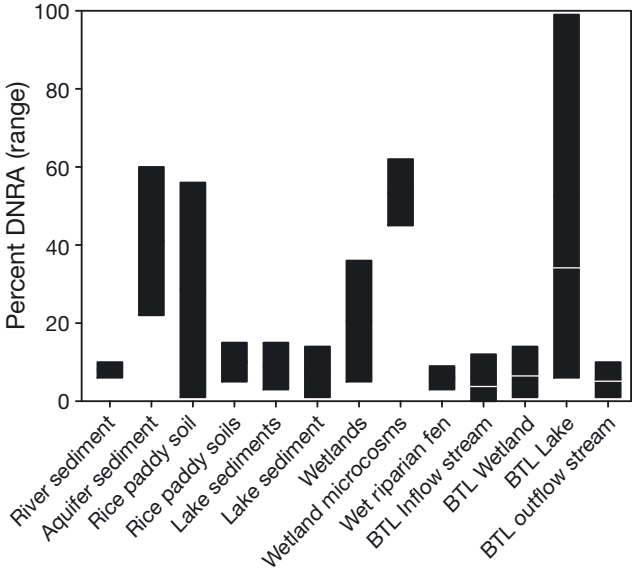

Fig. 6. Ranges of dissimilatory nitrate reduction to ammonium (DNRA) as percent of total dissimilatory nitrate removal. Data were obtained from the following sources, left to right: Kelso et al. (1997), Bengtsson & Annadotter (1989), Buresh & Patrick (1978), Yin et al. (2002), Nizzoli et al. (2010), Nijburg & Laanbroek (1997b), Scott et al. (2008), Matheson et al. (2005), Ambus et al. (1992). BTL prefix signifies ranges from Bull Trout Lake measured in the present study, with averages for these data displayed as white bands

CONCLUSIONS

DNRA was measured in each ecotype and season and, whilst not as prevalent as denitrification, was significant in this study. The lake ecotype was found to be the most favorable environment for DNRA, with a third of all dissimilatory nitrate reduction being attributed to it in our microcosms. DNRA was significantly higher during runoff compared to baseflow conditions, although temperature was kept constant between the 2 seasonal experiments and so did not contribute directly to the observed difference. We conclude that DNRA may be more important during runoff conditions compared to baseflow, with the opposite being true for denitrification.

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