Running head: Denitrification versus DNRA

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

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ABSTRACT

Elevated 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 nitrate (NO_3^-) mainly as dinitrogen gas (N_2), is widely believed to be the dominant NO_3^- removal pathway in aquatic ecosystems. Evidence suggests a lesser studied process, dissimilatory nitrate reduction to ammonium, (DNRA), that transforms NO_3^- to ammonium (NH_4^+) and hence retains nitrogen (N) in the system, may be at least as important as denitrification 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 aquatic ecosystem. We took sediment and water samples at runoff and baseflow, across several ecotypes. We hypothesized that the relative importance of DNRA compared to denitrification would vary spatially and temporally, because of variations in ambient conditions related to ecotype and season.

Potential denitrification rates ranged from 0 to 0.14 $\pm$ 0.03 μgN $gAFDM^{-1}$ d^{-1}. Potential DNRA rates ranged from 0 to 0.0051 $\pm$ 0.0008 μgN $gAFDM^{-1}$ d^{-1}. Denitrification losses peaked at the inflow stream ecotype at 96.16 % of total dissimilatory NO_3^- removal, whereas losses due to DNRA peaked in the lake ecotype at 34.42 %. When averaged over the entire system, denitrification peaked at baseflow (31.17 %), while DNRA peaked at runoff (2.93 %)

Although NO_3^- transformations due to denitrification were higher than DNRA in all ecotype and temporal comparisons, our results suggest that DNRA may be more important than denitrification under favorable conditions.

KEY WORDS DNRA, denitrification, nitrogen transformations, lake-stream interactions.

INTRODUCTION

Anthropogenic activities have had a profound effect on the global N cycle. Current estimates suggest that creation of reactive N has increased by 120 % since 1970 due to agriculture and industry and the rate is still dramatically increasing (Galloway et al. 2008). A significant fraction of this anthropogenically-mobilized reactive N ends up in inland aquatic ecosystems. Riverine export of TN was calculated to increase globally by up to 30% between 1970 and 2000 (Seitzinger et al. 2010). Increased N loading in riverine systems can cause local problems with eutrophication and can increase N fluxes to coastal systems. This 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 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). In order to properly manage aquatic ecosystems and prevent potential problems such as harmful algal blooms (Davis and Koop 2005) it is important to understand the processes that remove or transform NO_3^-.

Respiratory denitrification (hereafter denitrification) is a dissimilatory process usually carried out by 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 finally N_2 (Ye et al. 1995). The final reduction products, nitrous oxide (N_2O, a potent greenhouse gas, Ramaswamy et al. 2001) and N_2, are then lost from the system into the atmosphere (Delwiche and 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 also be

63 diminished by the presence of free sulfides, which can inhibit the enzymes responsible for the
64 final two stages of the process (Burgin and Hamilton 2007).
65 DNRA is a microbial process that transforms NO_3^- to NH_4^+ via formation of NO_2^- in
66 anaerobic or low O_2 environments. The final N form, NH_4^+, is highly bioavailable and can be
67 readily immobilized by microbes and plants, or can be transformed by nitrification (Bengtsson et
68 al. 2003). There are two DNRA pathways; fermentative and chemolithoautotrophic.
69 Fermentative DNRA microbes reduce NO_3^- to NO_2^- as a way of producing ATP / energy. The
70 subsequent reduction of NO_2^- to NH_4^+ is believed to be used as an electron sink to allow re-
71 oxidation of NADH, (Tiedje 1988). Chemolithoautotrophic DNRA is the transformation of NO_3^-
72 to NH_4^+, linked to free sulfide / elemental sulfur oxidation. This sulfur-driven NO_3^- reduction can
73 also lead to production of N_2 and N_2O via respiratory denitrification, however since higher
74 concentrations of free sulfides are believed to inhibit the final steps in the denitrification
75 sequence, (Brunet and Garcia-Gil 1996, Burgin and Hamilton 2007) reduction to NH_4^+ via
76 DNRA should dominate. Burgin and Hamilton (2007) summarized that the fermentative
77 microbes are favored by non-sulfidic sediments with high C:N ratios, whereas the
78 chemolithoautotrophic microbes prefer sediments where S oxidizers dominate and H_2S is present
79 in appreciable concentrations (Burgin and Hamilton 2007). While most of the denitrifying
80 microbes that use DNRA, are anaerobes (Tiedje 1988), recent evidence suggests they can also
81 tolerate low levels of O_2, while continuing to reduce NO_3^-, especially at high C:N
82 ratios.(Fazzolari et al. 1998, Silver et al. 2001).
83
84 The main factors believed to govern the balance between denitrification and DNRA in
85 freshwater sediments are the ambient O_2 concentration (Fazzolari et al. 1998, Silver et al. 2001),

86 the C:N ratio, (Tiedje 1988) and the presence of free sulfides (H_2S, S^{2-}) or elemental sulfur (S)
87 (Burgin and Hamilton 2007, Brunet and Garcia-Gil 1996). Other possible contributing factors
88 include the presence of macrophytes (Nijburg and Laanbroek 1997a,b) and ambient temperature
89 (Ogilvie et al. 1997, Scott et al. 2007, Nizzoli et al. 2010).

90 Spatial and temporal variations in the balance between denitrification and DNRA in
91 freshwater ecosystems have been studied by relatively few researchers, and studies seldom
92 quantify variation in both space (between different ecotypes) and time. Accordingly, we aimed to
93 elucidate NO_3^- losses due to potential DNRA and potential denitrification, across a stream lake
94 interaction zone of a sub-alpine watershed. We hypothesized that the relative importance of
95 DNRA compared to denitrification would vary significantly spatially and temporally, because of
96 variations in C:N ratios, presence/absence of highly reducing sediments and presence/absence of
97 aerenchymatous macrophytes.

98

99

100 # MATERIALS AND METHODS

101

102 **Sample sites**

103 The sampling area, consisting of Warm Springs creek and Bull Trout Lake, is an
104 oligotrophic stream–lake system in a sub-alpine watershed in the Sawtooth Mountains in Idaho,
105 USA. Four replicate cores were obtained from seven sites along with water samples (Fig 1.). We
106 sampled in June 2008, during snowmelt runoff (runoff), close to peak discharge, ~ 858 l s^{-1} at
107 site 1 (personal communication, K J Goodman).

Samples were again taken at baseflow in August 2008, with a discharge of about 154 l s^{-1} at site 1 (personal communication, K J Goodman). Peak discharge occurred on about the same date for all sites, as did the minimum.

Site 1 was 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, with 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 benthic sediment from 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 the stream, a hundred meters or so 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 each date (only 3 at site 7 at runoff and none 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 with the depth being recorded. Lake samples were taken using a Wildco® standard KB core sampler (Rickly Hydrological Company) at runoff and SCUBA diving at baseflow. Water samples were also taken at each site.

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

Stable isotope tracer (0.4 ml 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 at T_0. This protocol varied at baseflow when we added 0.8 ml of ^{15}N solution in order to ensure that the samples were adequately enriched. We estimate that addition of ^{15}N tracer enriched the nitrate pool to at least 70 atom percent. Nutrient solutions were added to alleviate nutrient limitations, thus all rates calculated in this study were potential not actual rates. Septa were re-sealed with Aquaseal (Urethane repair adhesive. McNett Corporation, 1411 Meador Ave Bellingham, WA, 98229-5845), incubated in the dark at 20°C for ~11 hours and then sampled once more for $^{15}N_2$, $^{15}N_2O$, $^{15}NH_4^+$, $^{14}NH_4^+$ and $^{14}NO_3^-$.

Chemistry

All $^{14}NO_3^-$ and $^{14}NH_4^+$ samples were run on an Astoria Pacific flow injection analyzer using methods adapted from the phenolhypochlorite method, by Solorzano (1969) for NH_4^+ and the cadmium reduction method by Grasshoff (1976) for NO_3^-. Dissolved organic carbon (DOC) samples (Personal communication, K J Goodman) were run on a OI Corporation model 700 TOC

154 analyzer using the protocol outlined by Bernard (1984). ^{15}N (N_2, N_2O and NH_4^+) samples were
155 run at the UC Davis (on a continuous flow Isotope Ratio Mass Spectrometer - IRMS) and MBL
156 (Marine Biological Laboratory, using a Europa ANCA-SL elemental analyzer - gas
157 chromatograph preparation system attached to a continuous-flow Europa 20-20 gas source stable
158 isotope ratio mass spectrometer) stable isotope facilities.
159 Potential denitrification and DNRA rates were calculated as the change in $^{15}N_2$ and $^{15}NH_4^+$
160 nitrogen mass respectively over time per μg of ash free dry mass of sediment (given as μgN
161 $gAFDM^{-1}$ d^{-1} and corrected for initial ambient ^{15}N-NO_3 mass). Both microbial processes were
162 also calculated as % transformation of $^{15}NO_3$-N mass per day (to $^{15}NH_4$-N mass for DNRA and
163 $^{15}N_2$-N mass for denitrification) corrected for initial ambient ^{15}N-NO_3 mass. $^{15}N_2O$ production
164 was measured but not attributed to either of these two processes. DNRA was also measured as a
165 percentage of total dissimilatory nitrate removal, with the total being made up of denitrification
166 plus DNRA plus N_2O production. Note that we measured denitrification as production of ^{15}N-N_2
167 and our method did not distinguish between denitrification and anammox. In the rest of this
168 paper we refer to ^{15}N-N_2 production from as denitrification
169
170 Percent organics was measured as the percentage of the mass lost on combustion (sample
171 heated to 450°C in muffle furnace for 2 hours). Ash free dry mass (AFDM) was taken as the
172 mass of the pre-dried sample remaining after ashing.
173
174 **Statistical analysis**
175 For pairwise comparisons of data groups we used the multiple response permutation
176 procedure (MRPP) in the USGS statistical package Blossom (Cade and Richards 2005). This

non-parametric analysis accommodates data with heterogeneous variances, non-normal distributions and small sample sizes. One-sample, single tailed t-tests in R were used to evaluate whether the N transformations measured were significantly greater than zero.

RESULTS

Biogeochemistry

% organic matter was measured in the samples. 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 2339 µg and 2036 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

Rates of denitrification and DNRA varied spatially and temporally. Potential denitrification rate ranged from 0 to 0.14 $\pm$ 0.03 µgN $gAFDM^{-1}$ d^{-1} over the entire study, while potential DNRA rates ranged from 0 to 0.0051 $\pm$ 0.0008 µgN $gAFDM^{-1}$ d^{-1}. DNRA rate was always highest 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 ~ half of the samples (Fig. 2, asterisk denotes $p < 0.050$, with exception of August, site 2, $p = 0.057$). Denitrification rates were not significantly greater than zero at all sites during runoff but were greater than zero at more than half of the sites during baseflow (Fig. 2., asterisk denotes $p < 0.050$, with the following exceptions; site 1 $p = 0.058$, site 5 $p = 0.060$). 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}, MRPP, $p = 0.033$, Fig. 2). The maximum DNRA rate was 0.0051 ± 0.0008 μgN $gAFDM^{-1}$ d^{-1} (MRPP, $p < 0.050$) and was measured at site 6. Denitrification rate exceeded DNRA rate at site 6 in June by an order of magnitude (MRPP, $p = 0.050$). All other pairwise comparisons between denitrification and DNRA were not statistically significant ($p > 0.050$).

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

To gain more insight into spatial variation, the data were grouped by ecotype: wetland, inflow stream, lake and outflow stream (Fig. 3). The mean % transformation of NO_3-N due to

223 denitrification was lowest in the wetland ecotype (12.70 $\pm$ 4.37 %, Fig. 3) and highest in the
224 stream ecotypes (36.10 $\pm$ 8.01 %, inflow stream, Fig 3). However, the only statistically
225 significant difference between denitrification values was between the wetland and inflow
226 ecotypes (MRPP, p= 0.028), so there was no statistically significant spatial trend.
227 Percent NO_3-N transformation per day due to DNRA, averaged over both seasons,
228 increased downstream from the wetland ecotype (0.51 $\pm$ 0.23 %) to peak at the lake ecotype,
229 (3.57 $\pm$ 0.72 %, Fig. 3). MRPP analysis showed the lake maximum to be significantly different to
230 all other ecotypes ($p < 0.050$, with exception of comparison to outflow - only marginal
231 significance, $p = 0.086$)
232 Percent transformation of NO_3-N per day (calculated by mass) was also measured in the
233 sample microcosms as production of N_2O gas, (Fig. 3) and arranged by ecotype. The rate of N_2O
234 production was considerably lower than that of DNRA per ecotype, (MRPP, $p < 0.001$) with the
235 exception of the wetland ecotype, which had approximately equal transformations of N due to
236 DNRA and N_2O production (wetland DNRA = 0.51 $\pm$ 0.23 %, wetland N_2O = 0.48 $\pm$ 0.24 %.
237 MRPP, $p = 0.641$).
238 We calculated DNRA as a percentage of total dissimilatory nitrate removal (with the total
239 being defined as denitrification plus DNRA plus N_2O production) to evaluate the relative
240 importance of this process as a NO_3-N removal pathway. Nitrogen transformations due to DNRA
241 were greatest at the lake site (34.42 % $\pm$ 21.92 %. Fig. 4) and lowest at the inflow stream site
242 (3.69 % $\pm$ 2.78 % Fig. 4). Ecotypes were not significantly different to each other except for
243 comparisons between the inflow and lake (MRPP $p = 0.043$) and between the inflow and outflow
244 (MRPP $p = 0.075$, only marginal significance) DNRA seems to be a potentially more important
245 pathway for NO_3-N removal in the lake, than in any of the other ecotypes in our study.

246

247 **Temporal trends**

248 Transformation of N due to denitrification was potentially more important during
249 baseflow, 31.17 % ± 4.87 %, compared to runoff, 19.93 % ± 6.02 %, when averaged across sites
250 (MRPP, $p = 0.011$; Fig. 5). In contrast, NO_3-N transformation due to DNRA was higher at
251 runoff, 2.93 % ± 0.72 %, than at baseflow, 1.30 % ± 0.41 %, (MRPP, $p = 0.027$; Fig. 5).
252 Similarly N_2O production was higher at runoff, 0.23 % ± 0.10 %, than at baseflow, 0.03 % ±
253 0.02 %, (MRPP, $p = 0.037$; Fig 5).

254

255

256 **DISCUSSION**

257

258 **Spatial patterns in NO_3^- losses by dissimilatory pathways**

259 The lake sediments were relatively productive in the littoral zone (compared to the other
260 ecotypes) where the samples were taken, as confirmed by the calculated % organics. The wetland
261 and lake ecotype sediments contained considerably more organic matter, than the inflow and
262 outflow ecotype sediments. Additionally, during sampling, the top 5 – 6 cm of each lake core
263 (site 5) were visibly green, and site 6 samples were noted as smelling strongly of sulfides. Owing
264 to high organic matter content, sediments from lake and wetland ecotypes were relatively highly
265 reducing as they all went anoxic within 30 minutes of being sealed in the dark, whereas
266 microcosms from the other ecotypes took close to 11 hours. Highly reducing sediments,
267 containing free sulfides (S^{2-} or H_2S) are known to enable the chemolithoautotrophic DNRA
268 process (Buresh and Patrick 1981, Burgin and Hamilton 2007) while at the same time, free

sulfides also inhibit the enzymes that sustain the final steps of the denitrification process (Burgin and Hamilton 2007, Brunet and Garcia-Gil 1996). So 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. It has been speculated that 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 and Laanbroek 1997b). Aerenchymatous plants release O_2 into the root zone when healthy, (Nijburg et al. 1997), and this 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). Species of *Potamogeton praelongus* and *Elodea Canadensis* (identified as aerenchymatous macrophytes. Personal communication, M Barkworth), 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, denitrification : DNRA ratio was 25-fold lower at runoff (0.6) compared with at baseflow, suggesting that NO_3^- removal was significantly higher during baseflow conditions. However water temperatures (from piezometers)

were ± 2°C between seasons and were therefore unlikely to account for this trend (Tobias et al. 2001.)

It is generally accepted that denitrification and DNRA are carried out by different competing species of microbes, and that certain ambient conditions select for or against denitrifiers (Megonigal et al. 2004, Tiedje 1988). The relative increase in denitrification and relative decrease in DNRA from runoff to baseflow could be explained by this competition, possibly due to a shift in the balance of available nutrients in the system, amongst other potential factors. Denitrification is generally thought to be favored by more C limited conditions, and DNRA by sediments more enriched with available C, specifically with high C:N ratios, (Tiedje 1988, Kelso et al. 1997, Omnes et al. 1996). 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 dissolved C:N ratios (DOC:DIN) in our microcosms of 46 at runoff and 23 at baseflow on average. The higher ratio at runoff is expected in this system, due to increased DOC inputs with snowmelt from the watershed. McGlynn (personal communication) found C:N ratios (NPOC:TDN) 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} (Personal communication, K J Goodman).

Temperature is another factor that influences the balance of denitrification and DNRA. Conclusions vary in the literature, but mounting evidence points towards a summer DNRA maximum. Ogilvie et al. (1997) found that denitrifying bacteria were better than fermentative nitrate-ammonifiers at scavenging NO_3^- at low temperatures and vice versa, (5°C and 20°C

314 respectively). Scott et al. (2008) were only able to measure DNRA during the summer months
315 when temperatures averaged 28.6°C, (winter average = 8.4°C) and Nizzoli et al. (2010) found
316 that DNRA was appreciably higher in lake Verde in the summer samples (13°C compared with
317 5°C in the winter). However, Kelly-Gerreyn et al. (2001), suggested that DNRA is favored in
318 more extreme temperatures (< 14 to > 17°C) whereas denitrifying microbes prefer a narrow
319 range of 14 - 17°C. Although our microcosms were all incubated at 20˚C, different ambient
320 temperatures between seasons may have selected for different microbial populations at the time
321 of sample collection.
322
323 **Data limitations**
324 All rates and % transformations mentioned in this study refer to potential values,
325 although the nutrient concentrations we employed were not outside the realms of natural
326 variation at this study site (Hall et al. 2009, Marcarelli and Wurtsbaugh 2009). The addition of N,
327 C and P to the microcosms in order to remove low-level nutrient limitation, (and ^{15}N as a tracer),
328 altered the available nutrient pool and influenced the rates of localized microbial processes
329 (Burgin and Hamilton 2008). Therefore it was not possible to measure actual in-situ rates of
330 denitrification and DNRA for our sites in this experiment.
331
332 N_2O production represented a small transformation of NO_3 compared to the processes of
333 DNRA and denitrification. N_2O could be attributed to either DNRA or denitrification as it is
334 believed to be an intermediate in both pathways (Tiedje 1988, Welsh et al. 2001, Burgin and
335 Hamilton 2008).

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

341 Anammox, the combination of NO_2^- (from reduction of NO_3^-) and NH_4^+ to form N_2 gas
342 under anaerobic conditions (Dalsgaard et al. 2005) has not been addressed in this study. This
343 process is mainly of interest in marine systems, contributing up to 67% of total N_2 production in
344 continental shelf sediments (Thamdrup and Dalsgaard 2002.) In one freshwater system that it has
345 been studied in, anammox accounted for 7-13% of the total production of N_2 but this was only
346 measured in the water column (Schubert et al. 2006) Since we have not attempted to measure
347 anammox in this study it is therefore possible that our denitrification figures could be
348 overestimated by approximately 10%. However, since anammox is believed to prefer eutrophic
349 sediment conditions (Megonigal 2004) with relatively high NO_3^- concentrations (Rysgard et al.
350 2004) and low labile carbon concentrations (Jetten et al. 1999) it would seem probable that this
351 process would be minimal in our system.
352
353 **Global comparisons**
354 Measured as % of the total dissimilatory nitrate removal at each ecotype, our DNRA
355 results can be compared to global data as reviewed by Burgin and Hamilton (2007). Our results
356 range from 0-12 % at the inflow stream ecotype to 6-99 % at the lake ecotype and overlap with
357 global freshwater data, (Freshwater lakes; Nijburg and Laanbroek 1997b, Nizzoli et al. 2010.
358 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 and Hamilton (2007), wetland and lake ecotypes in general have higher % 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 also was most variable, (34.42 % $\pm$ 21.92 % Fig. 4).

From data compiled in Fig. 4, we infer that denitrification accounts for the main proportion of dissimilatory nitrate removal in each ecotype. 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.

CONCLUSIONS

In conclusion, DNRA was measured in each ecotype and season and whilst not as prevalent as denitrification, was still 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 here. DNRA was significantly higher during runoff compared to baseflow conditions although temperature was kept constant between the two seasonal experiments and so did not contribute directly. Therefore DNRA may be more important during runoff conditions compared to baseflow, with the opposite being true for denitrification.

ACKNOWLEDGEMENTS

We would like to thank Jason Reed, Caleb Izdepski and Wayne Wurtsbaugh for help with field sampling, Keli Goodman and Natalie Day for help in the laboratory, Alexey Kalinin and Brian McGlynn for consultations over data, Susan Durham for statistical advice, Dave Epstein for macrophyte classification and Mary Barkworth (director of the USU Intermountain Herbarium), for identification of aerenchyma. Map courtesy of Tim Covino. Funding was provided by NSF DEB 05-19327.

Figure Captions

Fig. 1 Map of the field sites at Bull Trout lake in the Sawtooth Mountains in southern Idaho.

Fig. 2 Potential denitrification and dissimilatory nitrate reduction to ammonium (DNRA) rates, (ugN $gAFDM^{-1}$ d^{-1}), for each site sampled at runoff and baseflow $\pm$ SE. Asterisks denote statistical significance versus zero.

Fig. 3 Dissimilatory nitrate reduction to ammonium (DNRA), denitrification and N_2O production measured per ecotype down the watershed (left to right). Measured as % NO_3-N transformation per day (calculated by mass). Data are means $\pm$ SE.

Fig. 4 Mean dissimilatory nitrate reduction to ammonium (DNRA, $\pm$ SE) as a percentage of total dissimilatory nitrate removal per ecotype.

Fig. 5 Meandissimilatory nitrate reduction to ammonium (DNRA), denitrification and N_2O values measured as % transformation of NO_3-N per day (calculated by mass), at runoff, (June samples) and at baseflow, (August samples) $\pm$ SE. MRPP analysis ran for DNRA gave a p value of 0.0270, for denitrification a p value of 0.0114 and for N_2O a p value of 0.0369. Number of observations 'n' is indicated above each bar.

Fig. 6 Ranges of dissimilatory nitrate reduction to ammonium (DNRA) as a % of total dissimilatory nitrate removal. Means from Fig 4. are displayed in white lines for the Bull Trout Lake data. Data were obtained from the following sources, left to right: Kelso et al. 1997, Bengtsson and Annadotter 1989, Buresh and Patrick 1978, Yin et al. 2002, Nizzoli et al. 2010, Nijburg and Laanbroek 1997b, Scott et al. 2008, Matheson et al. 2005, and, Ambus et al. 1992. BTL prefix signifies ranges from Bull Trout Lake measured in this study.

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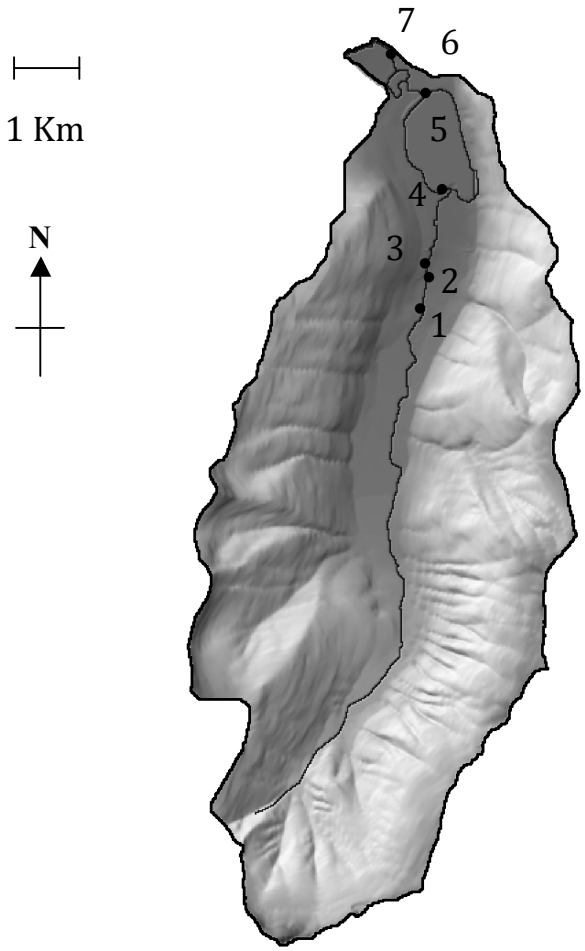

Fig. 1. Map of field sites at Bull Trout lake watershed.

1. Inflow 1.5
2. Sulfur marsh
3. Iron marsh
4. Inflow delta
5. Lake
6. Outflow interface
7. Outflow culvert

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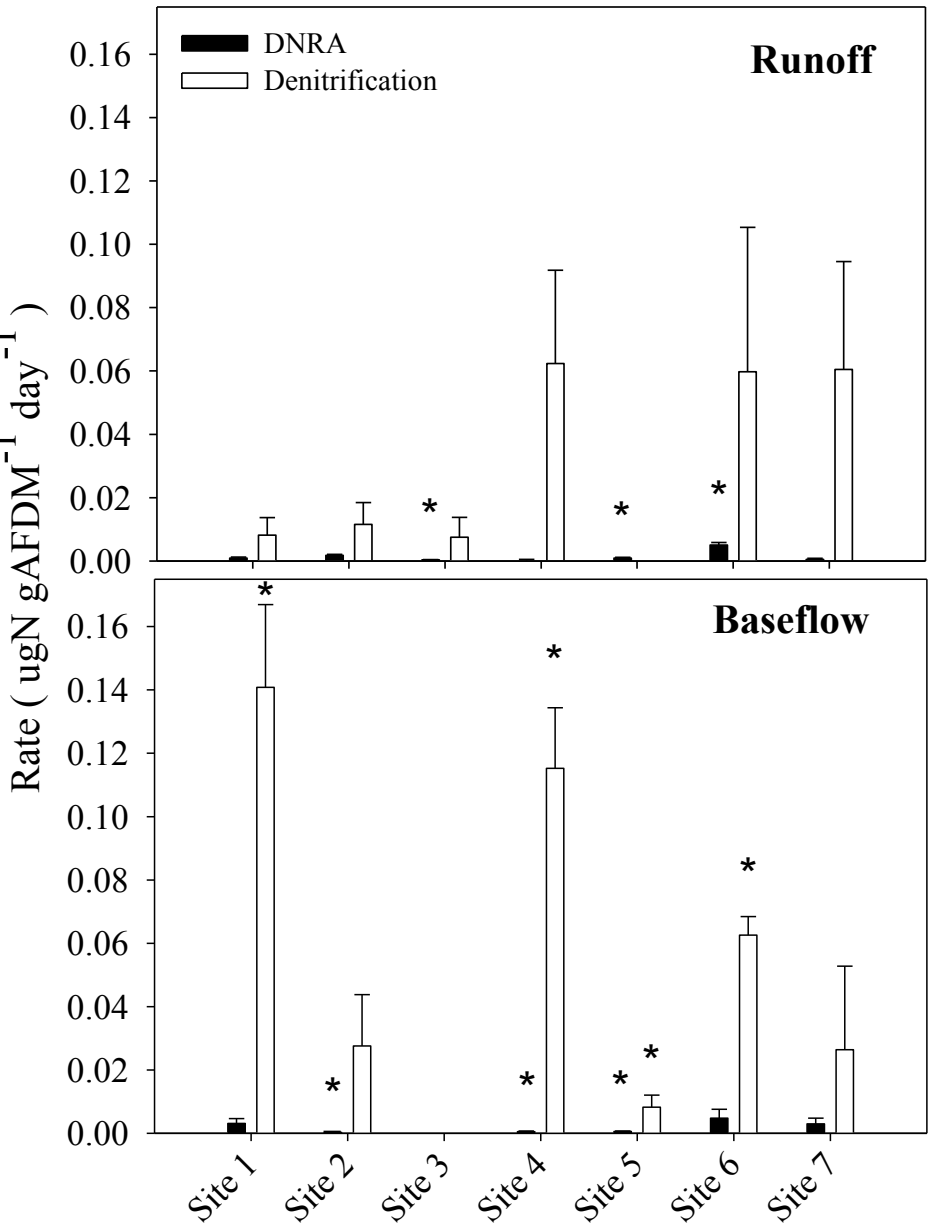
424 Fig. 1



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427 Fig. 2

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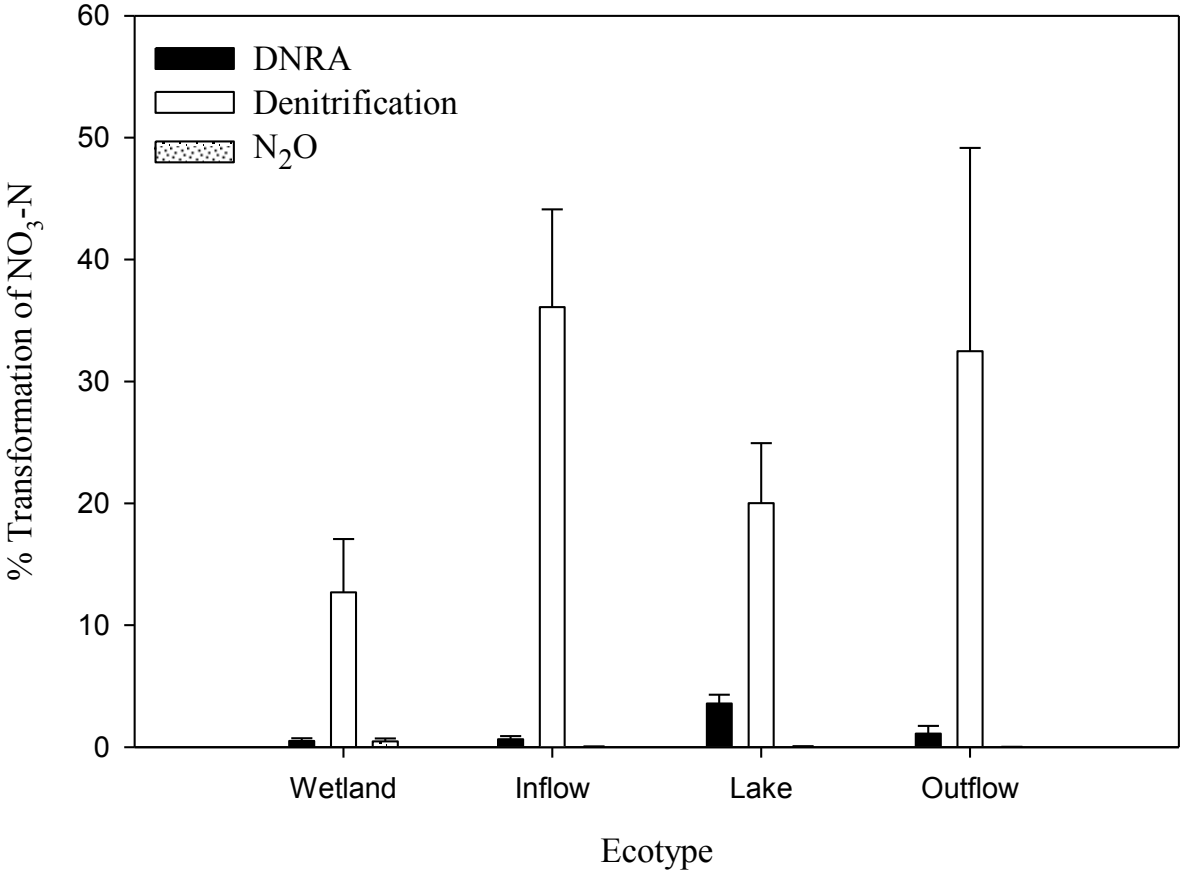
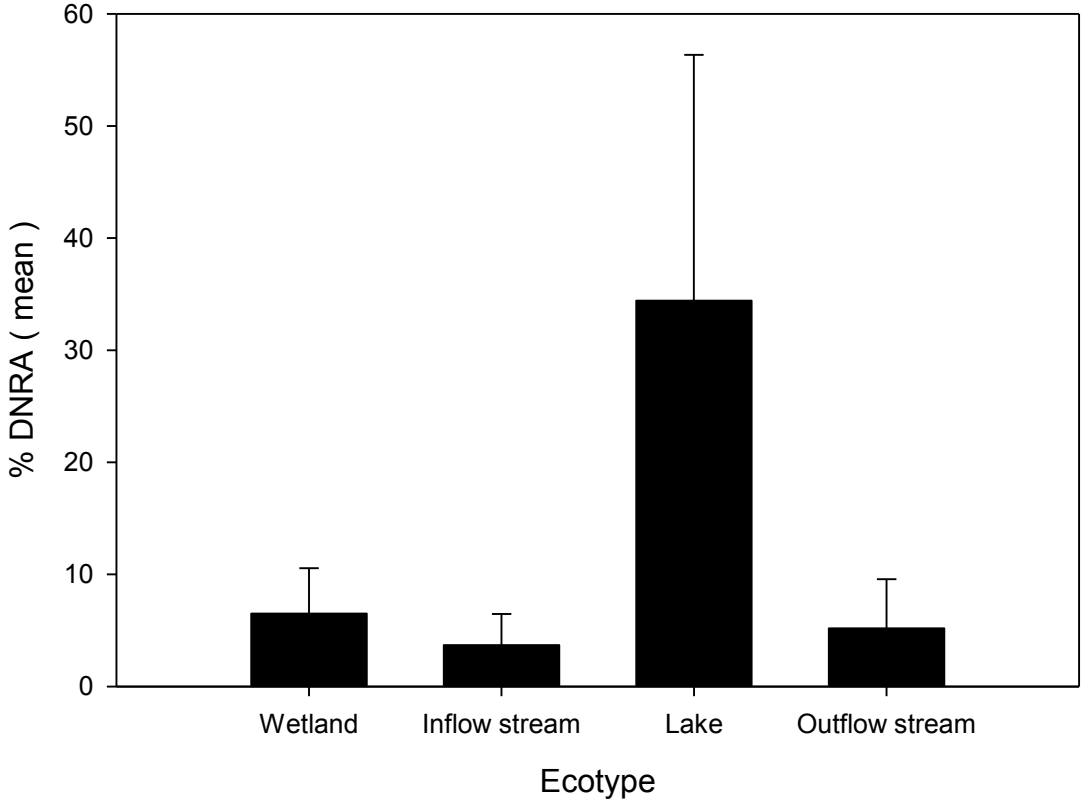

60
50
40
30
20
10
0
DNRA
Denitrification
N_2O
% Transformation of NO_3-N
Wetland
Inflow
Lake
Outflow
Ecotype

Fig. 3



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448 Fig. 4

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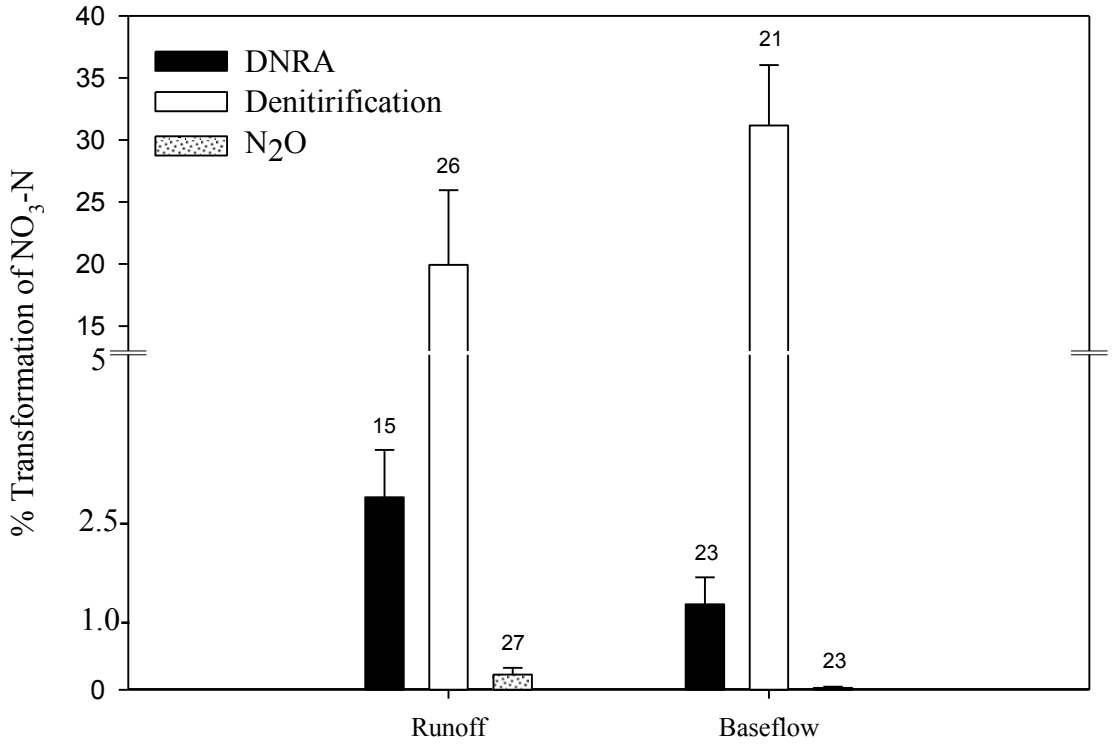
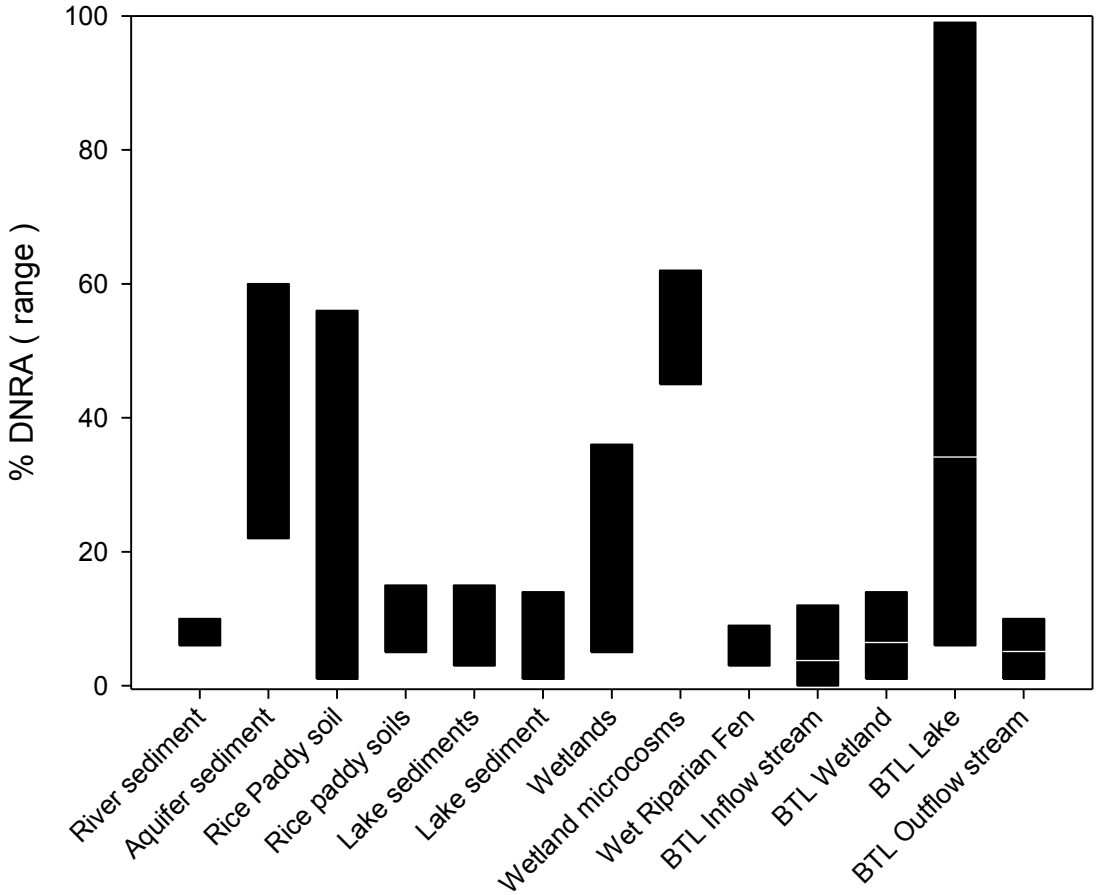

40
35
30
25
20
15
5
2.5
1.0
0
% Transformation of NO_3-N
DNRA
Denitirification
N_2O
15
26
27
23
21
23
Runoff
Baseflow

Fig. 5



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471 Fig. 6
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