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Differences in nitrate uptakeamong benthic algal assemblages in a mountain stream

Michelle A. Baker *Utah State University*

G. de Guzman

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1	RRH: M. A. Baker et al.
2	LRH: Algal diversity and N uptake
3	
4	Differences in nitrate uptake among benthic algal assemblages in a mountain stream
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7	Michelle A. Baker ¹
8	Department of Biology and the Ecology Center, Utah State University, Logan, Utah 84322 USA
9	
10	Glendell de Guzman ²
11	Department of Biology, Utah State University, Logan, Utah 84322 USA
12	
13	Jeffrey D. Ostermiller ³
14	Utah Division of Water Quality, 288 North 1460 West, P.O. Box 14870, Salt Lake City, Utah
15	84114 USA
16	
17	¹ E-mail address: michelle.baker@usu.edu
18	² Present address: Hershey Medical Center, Pennsylvania State University, 500
19	University Drive, Hershey, Pennsylvania 17033 USA. E-mail: deguzgs@yahoo.com
20	³ E-mail address: jostermiller@utah.gov
21	
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25 Abstract. We evaluated how benthic algal assemblages that vary in composition, richness, and 26 other diversity metrics remove NO₃-N from the water column of a mountain stream. Ecological 27 theory and empirical studies suggest that ecosystem process rates should increase as richness 28 increases because of niche separation or activity of dominant taxa. Accordingly, we predicted 29 that algal assemblages with highest richness would show the highest rates of NO₃-N uptake. To 30 test this prediction, we transplanted 225 rocks representing 3 patch types (green, yellow, and 31 brown) that differed macroscopically in algal composition from a lake outflow stream to a lake inflow stream where an experimental release of ¹⁵N-NO₃ was ongoing. We measured ¹⁵N uptake 32 33 in each patch type during the stable isotope release. Benthic algal richness varied from 28 genera 34 in the green patch type and 26 genera in the yellow patch type to 22 genera in the brown patch 35 type. Without accounting for differences in chlorophyll a content, NO₃-N uptake $(2.1-3.3 \times 10^{-1})$ 4 /d) was highest in the green patch type, lowest (0.3–0.6 × 10⁻⁴/d) in the yellow patch type, and 36 intermediate $(1.2-1.5 \times 10^{-4}/d)$ in the brown patch type. NO₃-N uptake normalized to chlorophyll 37 38 *a* increased in concert with algal richness in the 3 patch types. This result supports the hypothesis 39 that increased assemblage diversity leads to higher rates of community processes. Aside from 40 diversity differences per se, lower rates of NO₃-N uptake in the brown patch type might be the 41 consequence of differences in functional characteristics of the taxa present. Approximately 29% 42 of algal biovolume in the brown patch type consisted of taxa capable of N₂-fixation, a result that 43 suggests that algae in this patch type might be capable of meeting N needs via N₂-fixation rather 44 than via removal from the water column.



47	Study of the relationship between biodiversity and ecosystem functioning has a long
48	tradition in ecology, and stems from work on diversity and stability in the context of animal
49	populations and energy flow through food webs (MacArthur 1955). Recent research and debate
50	have centered on relationships between productivity and diversity of terrestrial plant
51	communities (e.g., Tilman 1999), nutrient cycling and terrestrial plant diversity (e.g., Hooper and
52	Vitousek 1997), and decomposition and decomposer diversity in terrestrial (e.g., Naeem et al.
53	1995) and aquatic (e.g., Jonsson and Malmqvist 2000) ecosystems. A variety of patterns and
54	mechanisms relating biodiversity to ecosystem functioning have been reported, and the recent
55	consensus is that richness is important, but functional characteristics of taxa strongly influence
56	ecosystem properties (Hooper et al. 2005).
57	Benthic algae play a vital role in immobilization of nutrients from the water column of
58	streams (Fisher et al. 1982, Mulholland 1992, Covich et al. 2004) and contribute to stream self-
59	purification as an ecosystem service upon which human society depends (Hooper et al. 2005).
60	Much recent research in stream ecosystem ecology has focused on quantifying rates of stream
61	nutrient uptake across biomes (Peterson et al. 2001, Mulholland et al. 2008), across land uses
62	(Bernot et al. 2006, O'Brien et al. 2007), and across time (Simon et al. 2005, Hoellein et al.
63	2007). Physical factors such as groundwater-surface water exchange (Valett et al. 1996),
64	discharge (Peterson et al. 2001, Wollheim et al. 2001), hydrogeomorphology (Doyle et al. 2003),
65	temperature (Martí and Sabater 1996), and nutrient concentration (Bernot et al. 2006) control the
66	rate of nutrient uptake in streams. Fewer studies have examined biological factors that control
67	nutrient uptake, but nutrient uptake rates are positively correlated with rates of ecosystem
68	metabolism (Hall and Tank 2003, Gucker and Pusch 2006, Mulholland et al. 2006).
69	Human activities are altering the biological composition of communities world wide

70 (Vitousek et al. 1997), and stream benthic algal assemblages are no exception. Diatoms, in 71 particular, are responsive to anthropogenic nutrient inputs (Pan et al. 2000) and are used as 72 indicators of water quality (Stevenson 1998). Algal assemblages also are susceptible to 73 invasions. For example, Didymosphenia geminata, a diatom native to oligotrophic waters of the 74 Northern Hemisphere, has greatly expanded its range and caused nuisance blooms and changes 75 to stream macroinvertebrate grazer assemblages in New Zealand and elsewhere (Kilroy 2004). 76 Little is known about the functional role of specific algal taxa or the effects of algal 77 community structure (composition, richness, diversity) on nutrient uptake in streams (Covich et 78 al. 2004). N₂-fixing taxa might increase in abundance as water-column N declines (Fisher et al. 79 1982). Mulholland et al. (1991) showed that nutrient reductions and herbivory by snails changed 80 periphyton community composition and increased P cycling rates. Other studies have 81 documented little change in algal assemblages but higher rates of production and nutrient cycling 82 in response to nutrient enrichment (Miller et al. 1992, Peterson et al. 1993). 83 Strong inferences relating biodiversity to ecosystem functioning have been made using 84 theoretical (Loreau 2000) and empirical approaches (Tilman 1999) and indicate that increased 85 richness might lead to asymptotic increases in the magnitude of ecosystem processes over short 86 time scales. Our objective was to examine how different benthic algal assemblages remove NO₃-N from the water column of a mountain stream during a stable isotope (^{15}N) tracer test. We 87 hypothesized that assemblages with higher richness would exhibit higher rates of ¹⁵N uptake 88 89 because assemblages with higher diversity are more likely to include a dominant taxon (i.e., 90 sampling effect, sensu Tilman 1997) responsible for much of the processing rate, or are more 91 likely to include a group of taxa whose functional traits are complementary (sensu Loreau 2000) 92 and reduce interspecific competition, thereby leading to higher processing rates.

93	
94	Methods
95	Study site
96	We worked in the Bull Trout Lake watershed in the Boise National Forest in central
97	Idaho, USA. Bull Trout Lake (44° N, 115° W) is a 0.28-km ² dimictic lake formed by a moraine
98	dam. Spring Creek and Warm Springs Creek are the 2 nd -order streams that flow into and out of
99	the lake, respectively (Fig. 1). Catchment area at Warm Springs Creek is 11.7 km ² and elevation
100	is 2118 m above sea level. Both streams have an open canopy with vegetation dominated by
101	willows (Salix sp.), sedges (Carex sp.), and grasses along the stream banks and lodgepole pine
102	(Pinus contorta) in forested uplands (Arp et al. 2006).
103	Physicochemical variables differ greatly in Spring Creek (lake inlet) and Warm Springs
104	Creek (lake outlet). During our study (August 2003), NO ₃ -N averaged 6.9 μ g/L in the inlet and
105	$2.5 \ \mu g/L$ in the outlet. Total N typically shows the opposite pattern during baseflow with
106	concentrations on the order of 20 to 30 μ g/L in the inlet and 90 to 100 μ g/L in the outlet (Arp and
107	Baker 2007, Marcarelli and Wurtsbaugh 2007). Outlet water temperatures are, on average, 10°C
108	warmer than inlet water temperatures (Arp and Baker 2007, Marcarelli and Wurtsbaugh 2007).
109	Rock substrates in the lake outlet are larger (84^{th} percentile particle diameter [D_{84}] = 39
110	mm) compared to those in the lake inlet (D_{84} = 18 mm) because of sediment trapping by the lake
111	and absence of tributaries below the lake to supply new sediment (Arp et al. 2007). Periphyton
112	biomass in the outlet stream often is colimited by N and P during summer months (Marcarelli
113	and Wurtsbaugh 2007). Distinct patch types that differ macroscopically in benthic algal
114	composition are observed in Warm Springs Creek (the outlet), but not in Spring Creek (the inlet).
115	Hereafter, we refer to these patch types as green—characterized by green filamentous algae,

brown—characterized by brown flocs of periphyton, and *yellow*—characterized by a yellow, less developed (thin) periphyton. Rocks from each patch type were transplanted to the inflow stream before the experiment (see *Experimental design* below). At the time of the experiment, stream flow in the inlet at the station above the lake ranged from 120 to 150 L/s with a mean velocity of 0.26 m/s (Arp et al. 2006, 2007).

121

122 Algal assemblage analysis

123 We collected 5 rocks from each patch type for analysis of algal assemblage composition. 124 We scrubbed the periphyton from rock surfaces with a soft-bristled brush, collected it 125 volumetrically, and pooled it by patch type. We measured rock surface area by covering the rock 126 surfaces with aluminum foil and relating the mass of foil to known surface area (Bergey and 127 Getty 2006). We preserved a 60-mL homogenized aliquot of slurry from each patch type with 128 Lugol's solution (final concentration 1%) and sent the aliquots to PhycoTech (St. Joseph, 129 Michigan) for periphyton analysis. There, samples were mounted in triplicate using 2-130 hydroxypropyl methacrylate (Crumpton 1987) and examined by epifluorescence microscopy to 131 identify and enumerate algal taxa to genus. A minimum of 300 natural units and 15 fields at 132 200× magnification were counted except when samples were dominated by diatoms, in which 133 case a minimum of 400 natural units and 15 fields at 1000× magnification were counted. 134 Biovolume was calculated using Phycotech's proprietary software, Aquatic Sample Analysis, 135 from measurements of greatest axial length dimension, length, depth, and width of cells for 10 to 136 30 natural units of each taxon. Biovolume provides a better estimate of algal biomass than do 137 total counts, so we report only biovolume data; we observed similar patterns with cell counts. 138 We quantified algal diversity of each patch type by calculating richness, evenness,

139	Shannon–Weiner index (H'), and Bray–Curtis similarity in Systat V.5 (SPSS, Chicago, Illinois)
140	for the genera identified. We chose to quantify diversity as richness, evenness, and H' because
141	many studies relating diversity to ecosystem functioning have used these metrics, and we wanted
142	our data to be comparable to those studies. We used Bray-Curtis similarity for pair-wise
143	comparison of patch composition.
144	
145	Experimental design
146	We took advantage of a ¹⁵ N tracer study to evaluate how the algal assemblages from each
147	patch type immobilize N from the water column. Briefly, a solution containing 75 g of 15 N (as
148	27% $Na^{15}NO_3$) was administered to Springs Creek at a rate of 10.4 mL/min for 14 d beginning 5
149	August 2003 at a site ~1895 m above Bull Trout Lake (Fig. 1). The tracer test elevated the
150	isotopic signature of the NO_3^- pool to 11,670‰ above background on average, with higher
151	values at the top of the reach and lower values at the bottom of the reach. Changes in ambient
152	NO ₃ -N concentration as a result of the tracer test were not detectable (Hall, R. O., University of
153	Wyoming, unpublished data).
154	We transplanted 75 rocks (median particle size $[D_{50}]-D_{84} = 16-39$ mm; Arp et al. 2007)
155	from each patch type from Warm Springs Creek (lake outlet) to Springs Creek (lake inlet) 3 d
156	before the isotope experiment commenced. We arranged 15 rocks from each patch type in plastic
157	baskets (18 cm \times 26 cm) and placed the buckets in riffles at 5 sites: 62, 120, 1069, 1560, and

158 1890 m downstream of the ¹⁵N-drip site (Fig. 1).

We collected periphyton samples from each patch type at each location (pooled from 5 rocks) the day before the ¹⁵N-release and 8 and 14 d after the release began. We collected periphyton and measured rock area as described above. We filtered homogenized aliquots of the

162 slurry onto 4 precombusted glass-fiber filters (Whatman GF/F, Whatman International Ltd., 163 Maidstone, England UK). We folded 2 of the filters in aluminum foil and froze them before 164 extraction with 95% ethanol for analysis of chlorophyll *a* (Welschmeyer 1994). We dried the 165 other 2 filters at 60°C and encapsulated them in tin capsules (Elemental Micoranalysis Ltd., 166 Manchester, Massachussets) for measurement of isotopic composition and N content 167 (Mulholland et al. 2000). N content and isotopic enrichment require a target mass of 100 µg N 168 (D. Harris, University of California, Davis, personal communication), so for samples with large 169 biomass, we cut the periphyton filters in half prior to encapsulation. We sent all samples to the 170 University of California (UC) Davis Stable Isotope Facility (Davis, California) for analysis. 171 There, N content and isotopic composition were measured using a PDZ Europa ANCA-GSL 172 elemental analyzer connected to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon 173 Ltd., Cheshire, UK). These instruments combust the samples at 1020°C in a reactor filled with 174 Cr and Co oxides. N is separated from other gases using a Carbosieve GC column (Supelco, 175 Bellefonte, Pennsylvania) before quantification (D. Harris, University of California, Davis, 176 personal communication). Data are reported by this laboratory as the atomic ratio excess $(AR_{periphyton}; {}^{15}N/{}^{14}N + {}^{15}N)$ and N mass $(N_{periphyton}; \mu g)$. Values of N and chlorophyll a in 177 178 periphyton were expressed in units per area of rock sampled (Bergey and Getty 2006). Isotopic content of periphyton biomass (¹⁵N_{periphyton}) was calculated from AR_{periphyton} (Mulholland et al. 179 180 2000, 2004) as

181
$${}^{15}N_{\text{periphyton}} (\text{mg/m}^2) = AR_{\text{periphyton}} \times N_{\text{periphyton}} (\text{mg/m}^2).$$
 [1]

We measured NO₃-N concentration using ion chromatography (DIONEX, Sunnyvale,
California) from filtered (Whatman GF/F) samples collected at each location on each sample
date. We measured dissolved ¹⁵N-NO₃ in the water column from 3-L samples collected and

185 filtered (Whatman GF/F) at each stream location on each sample date. We processed these 186 samples in the laboratory according to protocols outlined in Mulholland et al. (2004). Briefly, we 187 elevated the pH of samples with MgO to drive off NH₃, concentrated samples to ~100 mL by boiling, after which we converted the NO_3^- in the sample to NH_4^+ using Devarda's alloy. We 188 converted this NH₄⁺ to NH₃ with MgO and collected the NH₃ on an acidified filter by diffusion 189 (Mulholland et al. 2004). The ¹⁵N content on the filter was measured using a Europa Hydra 20/20 190 191 mass spectrometer at the UC Davis Stable Isotope Facility as described above. The atomic ratio excess of ¹⁵N in water samples (AR_{water}) reported by the isotope laboratory was used with 192 measured NO₃-N concentration (μ g/L) to calculate ¹⁵N concentration in the water column as 193 ¹⁵N_{water} (μ g/L) = [AR_{water} × NO₃-N]_{dav 8 or 14} - [AR_{water} × NO₃-N]_{background}. 194 [2] 195 Concentrations on day 8 or 14 are corrected for isotopic content in background samples. 196 197 *Uptake calculations* We calculated immobilization (uptake) of ¹⁵N tracer by each patch type as the 198 background-corrected ${}^{15}N_{periphyton}$ (mg/m²) incubated at each sample location divided by the 199 background-corrected ¹⁵N (AR_{water}) in the overlying water column at the same location and the 200 201 number of days of exposure to the tracer (8 or 14 d) (Mulholland et al. 2000, 2004) as ¹⁵N-NO₃ uptake (mg m⁻² d⁻¹) = ${}^{15}N_{\text{periphyton}}/[AR_{\text{water}} \times \text{days of experiment}]$. 202 [3] 203 This calculation assumes that the periphyton has reached equilibrium with tracer in the water 204 column at the time of sampling (Mulholland et al. 2000). We averaged values from each site to

estimate mean uptake rates by each patch type for each sample date. To account for differences in N content of each patch type, we calculated N-specific uptake (units are 1/d) by dividing the areal 15 N-NO₃ uptake (mg m⁻² d⁻¹) above by the areal N content (mg/m²) of each patch type

208 (Dodds et al. 2004). Hereafter, we report results as N-specific uptake (NO₃-N uptake).

209

210 Hypothesis tests

211 We compared NO₃-N uptake rates with 2-way analysis of variance (ANOVA) with patch 212 type and day of experiment as factors. We followed significant ANOVAs by Tukey-Kramer 213 Honestly Significant Difference tests (SAS, version 8; SAS Institute, Cary, North Carolina). We 214 considered rates significantly different at p < 0.05. We used linear regression to assess whether 215 differences among rates were related to chlorophyll-*a* content (SAS version 8). Our study had 216 only 3 levels of diversity, so we did not use statistics to test hypotheses for relationships between 217 richness and NO₃-N uptake. 218 219 Results 220 Algal assemblage composition

Algal assemblage richness differed among patch types, with 28 genera in the green patch type, 26 genera in the yellow patch type, and 22 genera in the brown patch type (Table 1, Fig. 2). Six taxa were unique to the green patch, whereas 4 and 3 taxa were unique to the yellow and brown patches, respectively (Table 1).

The algal assemblage from the green patch type was dominated by the Chlorophyta *Spirogyra* and *Rhizoclonium*, which represented >90% of total biovolume. The algal assemblage
from the yellow patch type was also dominated by Chlorophyta (85% of biovolume); *Spirogyra*accounted for 61% of biovolume and *Bulbochaete* accounted for 25% of biovolume. In contrast,
the periphyton assemblage from brown patches was dominated by Bacillariophyta (>82% of
biovolume); *Synedra, Cymbella, Fragilaria*, and *Epithemia* each accounted for ~10% of

biovolume. Cyanophyta also were important (15% of biovolume) in the brown patch type.

The green and yellow patch types had higher algal richness than did the brown patch type, but the brown patch type had higher evenness (Table 2, Fig. 2) and higher H' than the green or yellow patch types. Bray–Curtis distances indicated that the brown patch type had the most unique algal composition, whereas the yellow and green patch types were most similar to each other (Table 2).

237

238 ¹⁵*N*-nitrate uptake rates

239 NO₃-N uptake rates differed significantly among patch types (ANOVA, df = 3, F =

240 36.09, p < 0.001; Fig. 3). NO₃-N uptake rates were highest in the green patch type, intermediate

in the brown patch type, and lowest in the yellow patch type (Fig. 3). NO₃-N uptake rates did not

242 differ between sample days, except in the green patch type, where NO₃-N uptake was

significantly higher on day 14 than on day 8 (Fig. 3).

NO₃-N uptake rate was not significantly correlated with chlorophyll *a* content ($R^2 = 0.40$, p > 0.05; Fig. 4). Algae from the yellow patch type had 3× lower chlorophyll *a* concentrations (~ 20 mg/m²) than did algae from the brown and green patch types (~60 mg/m²) (ANOVA, df = 3, F = 36.09, p < 0.01), a result that explained some portion of the difference in NO₃-N uptake by the yellow patch.

NO₃-N uptake rates did not increase in concert with algal richness, as predicted from the diversity–function hypothesis. NO₃-N uptake was greatest for the green patch type and lowest for the yellow patch type (Figs 3, 4). However, when normalized to chlorophyll *a* content, NO₃-N uptake rate increased in concert with richness, as predicted by the diversity–function hypothesis (Fig. 5). Algal composition also was related to NO₃-N uptake rate, which was negatively related to Cyanophyta biovolume and positively related to Chlorophyta biovolume (Fig. 5).

255

256

Discussion

257 Relationship between diversity and ecosystem functioning

Our study is the 1st attempt to use a stable isotope experiment to relate algal diversity to 258 259 ecosystem functioning in streams. As average richness increased, chlorophyll-corrected NO₃-N 260 uptake appeared to increase in concert. However, our results should be interpreted with great 261 caution because we had only 3 levels of richness that differed by only 6 genera. We chose to 262 identify taxa to the genus level because of financial constraints, but positive correlation between 263 genus richness and species richness has been reported for stream periphyton (Hill et al. 2001). 264 We also did not separate algal from bacterial and fungal components of the periphyton, and this 265 decision might bias our results and interpretation. Nevertheless, the pattern we observed is 266 consistent with that seen in studies from a variety of ecosystems, including grasslands (Tilman 267 1999) and other terrestrial ecosystems (Hooper et al. 2005), estuaries (Zedler et al. 2001), and 268 stream filter-feeding guilds (Cardinale et al. 2002).

269 Our results oppose predictions from the diversity-function hypothesis. The algal 270 assemblage in the brown patch type had the highest values of H' and evenness and was most 271 different in composition from the algal assemblage in the other patch types as measured by Bray-272 Curtis distance, yet the algal assemblage in the brown patch type had the lowest NO₃-N uptake 273 rate when normalized to chlorophyll a. Low NO_3 -N uptake in the brown patch type might be 274 related to differences in functional characteristics of the dominant taxa in each patch, rather than 275 to diversity per se. Algae in the brown patch included a relatively high proportion of taxa 276 potentially capable of N₂-fixation (Table 1). These taxa included Cyanophyta (Gallon 2004),

277 which accounted for $\sim 15\%$ of biovolume, and diatoms (*Epithemia* and *Rhopalodia*) with N₂-278 fixing endosymbionts (Prechtl et al. 2004), which accounted for another 14% of biovolume. In 279 contrast, taxa potentially capable of N₂-fixation accounted for ~5% of biovolume in the vellow 280 patch type and <0.03% of biovolume in the green patch type. The negative relationship between 281 NO₃-N uptake and biovolume of Cyanophya supports this interpretation. Algae from brown 282 patches might be able to meet its N demands via N₂-fixation rather than via assimilation of N from the water column. Rates of N₂-fixation on the order of 8.7 μ g N m⁻² h⁻¹ have been 283 284 measured in this system (Marcarelli et al. 2008), and N₂-fixation rates are correlated with 285 presence of N₂-fixing taxa (Marcarelli and Wurtsbaugh 2006). 286 Stable isotopes have been used in terrestrial ecosystems to examine relationships between diversity and ecosystem functioning. For example, Hooper and Vitousek (1997) used ¹⁵N-tracers 287 288 to show that productivity and N retention in a Mediterranean grassland were best explained by 289 community composition rather than richness. Hooper and Vitousek (1997) also explained their 290 results by suggesting that functional characteristics of the taxa better explained ecosystem 291 process rates than did richness or other measures of diversity. 292 293 Autecological factors influencing our results 294 Substratum size can influence periphyton biomass and assemblage composition directly if

large rocks are more stable and less susceptible to disturbance than are small rocks (Biggs 1996).

296 The differences we present here probably are not a result of substratum size because we used

297 particles in the D_{50} - D_{84} range. Average surface area of rocks sampled was smallest for the green

patch type (122 cm²), and greatest for the yellow patch type (187 cm²). Chlorophyll *a* was

299 highest in the green patch type and lowest in the yellow patch type.

300 Physiognomic differences among algae can affect nutrient immobilization because 301 growth form and stature affect resource (light and nutrient) supply to algal mats (Stevenson 302 1996). Competitively dominant diatoms in algal mats are those that produce mucilaginous stalks, 303 which enable better access to light and nutrients (McCormick and Stevenson 1991). 304 Mucilaginous stalks were visible in the brown patch type, but increased resource supply does not 305 explain low NO₃-N uptake by periphyton in this patch type. If nutrient delivery rates were 306 higher, this patch should have assimilated more N than we measured. 307 That NO₃-N uptake was not linearly correlated with chlorophyll *a* is interesting because 308 the pigment is rich in N and usually is a good estimate of N requirements of algae (Graham and 309 Wilcox 2000). However, the relationship probably does not hold in situations in which algal 310 assemblages are distinctly different because algae in the brown patch type had low NO₃-N 311 uptake relative to its internal store of chlorophyll a. 312 We do not know the degree to which each genus contributed to NO₃-N uptake. Some taxa 313 (Oocystis, Tetraedron, and Pediastrum) are more typical of lake phytoplankton than of stream 314 periphyton (Wehr and Sheath 2003) and might be cells that were trapped in the periphyton when

contribute significantly to NO₃-N uptake. Dominant taxa, such as Spirogyra in the green and

the rocks were in the lake outflow. These taxa were present in low numbers and probably did not

315

316

317 yellow patch types, probably contributed significantly to NO₃-N uptake. *Spirogyra fluviatum* has
318 high N requirements, particularly at high flows (Borchardt 1994).

Taxa differ in their requirements for N and their affinity for NO₃-N, but NO₃-N uptake probably was not saturated in our study because ambient NO₃-N concentration was $<10 \mu gN/L$ and did not change as a result of the tracer test. Many of the genera we found (e.g., *Mougeotia*, *Consmarium*, *Teilingia*) are common in oligotrophic waters (Wehr and Sheath 2003) and have

324	$<100 \ \mu gN/L$ are growth limiting for benthic algae grown in artificial streams (Lohman et al.
325	1991). NO ₃ -N uptake by <i>Cladophera glomerata</i> , a species common in nutrient-rich water,
326	saturates at concentrations ~330 μ gN/L (Lohman and Priscu 1992).
327	
328	Implications for studies of nutrient uptake
329	Our results have interesting implications for studies of nutrient cycling in streams. First,
330	nutrient uptake rates vary widely among patch types, even when they are in close proximity (cm)
331	to each other. This result reinforces the notion that care should be taken during sampling to
332	stratify for habitat or patch type to capture the variability inherent within the stream reach being
333	studied (Mulholland et al. 2000).
334	Second, nutrient enrichment decreases algal diversity in benthic (e.g., Whitton et al.
335	1991) and pelagic (e.g., Schindler 1977) systems. Our study might provide some insight to why
336	nutrient uptake rates decline as nutrient availability increases. This pattern often is attributed to
337	nutrient saturation (e.g., Earl et al. 2006), which might be the case when nutrient uptake is
338	measured experimentally with sequential releases of nutrient over short time scales (h).
339	However, when this pattern is observed across streams that differ in nutrient availability (e.g.,
340	O'Brien et al. 2007), changes in assemblage structure and diversity should not be ruled out as
341	possible causes.
342	Streams are important landscape features in the transport and storage of nutrients
343	(Peterson et al. 2001), but little is known about how benthic algal assemblages contribute to
344	nutrient transport and storage. Our results illustrate that algal assemblage composition might
345	affect nutrient uptake rate. Future studies of factors regulating nutrient uptake in streams would

tolerance values for total N between 26 and 140 $\mu g N/L$ (Lowe and Pan 1996). Concentrations

323

- 346 benefit from including an autecological approach with the synecological approach already
- inherent in the research. The stable isotope approach we present here might be one way to do so.

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358	

359	Literature Cited
360	Arp, C. D., and M. A. Baker. 2007. Discontinuities in stream nutrient uptake below lakes in
361	mountain drainage networks. Limnology and Oceanography 52:1978–1990.
362	Arp, C. D., M. N. Gooseff, M. A. Baker, and W. Wurtsbaugh. 2006. Surface-water
363	hydrodynamics and regimes of a small mountain stream-lake ecosystem. Journal of
364	Hydrology 329:500–513.
365	Arp, C. D., J. C. Schmidt, M. A. Baker, and A. K. Myers. 2007. Stream geomorphology in a
366	mountain lake district: sediment sources and sinks, lake-modified hydraulics, and
367	downstream lake effects. Earth Surface Processes and Landforms 32:525-543.
368	Bergey, E. A., and G. M. Getty. 2006. A review of methods for measuring the surface area of
369	stream substrates. Hydrobiologia 556:7–16.
370	Bernot, M. J., J. L. Tank, and T. V. Royer. 2006. Nutrient uptake in streams draining agricultural
371	catchments of the midwestern United States. Freshwater Biology 51:499-509.
372	Biggs, B. J. F. 1996. Patterns in benthic algae of streams. Pages 31-56 in R. J. Stevenson, M. L.
373	Bothwell, and R. L. Lowe (editors). Algal ecology: freshwater benthic ecosystems.
374	Academic Press, San Diego, California.
375	Borchardt, M. A. 1994. Effects of flowing water on nitrogen- and phosphorus-limited
376	photosynthesis and optimum N:P ratios by Spirogyra fluviatilis (Charophyceae). Journal
377	of Phycology 30:418–430.
378	Cardinale, B. J., M. A. Palmer, and S. L. Collins. 2002. Species diversity enhances ecosystem
379	functioning through interspecific facilitation. Nature 415:46–429.
380	Covich, A. P., M. C. Austen, F. Bärlocher, E. Chauvet, B. J. Cardinale, C. L. Biles, P. Inchausti,
381	O. Dangles, M. Solan, M. O. Gessner, B. Stazner, and B. Moss. 2004. The role of

- biodiversity in the functioning of freshwater and marine benthic ecosystems. BioScience
 54:767–775.
- 384 Crumpton, W. G. 1987. A simple and reliable method for making permanent mounts of
- 385 phytoplankton for light and fluorescence microscopy. Limnology and Oceanography
 386 32:1154–1159.
- 387 Dodds, W. K., E. Martí, J. L. Tank, J. Pontius, S. K. Hamilton, N. B. Grimm, W. B. Bowden, W.
 388 H. McDowell, B. J. Peterson, H. M. Valett, J. R. Webster, and S. Gregory. 2004. Carbon
 389 and nitrogen stoichiometry and nitrogen cycling rates in streams. Oecologia 140:458-467.
- 390 Doyle, M. W., E. H. Stanley, and J. M. Harbor. 2003. Hydrogeomorphic controls on phosphorus
- 391 retention in streams. Water Resources Research 39. doi:10.1029/2003WR002038.
- 392 Earl, S. R., H. M. Valett, and J. R. Webster. 2006. Nitrogen saturation in streams. Ecology
 393 87:3140–3151.
- Fisher, S. G., L. J. Gray, N. B. Grimm, and D. E. Busch. 1982. Temporal succession in a desert
 stream ecosystem following flash flooding. Ecological Monographs 52:93–130.
- 396 Gallon, J. R. 2004. N2 fixation by non-heterocystous cyanobacteria. Pages 111–139 in W. Klipp,
- B. Masepohl, J. R. Gallon, and W. E. Newton (editors). Genetics and regulation of
- 398 nitrogen fixation in free-living bacteria. Kluwer Academic Publishers, Dordrecht, The399 Netherlands.
- 400 Graham, L. E., and L. W. Wilcox. 2000. Algae. Prentice Hall, Upper Saddle River, New Jersey.
- 401 Gucker, B., and M. T. Pusch. 2006. Regulation of nutrient uptake in eutrophic lowland streams.
 402 Limnology and Oceanography 51:1443–1453.
- 403 Hall, R. O., and J. L. Tank. 2003. Ecosystem metabolism controls nitrogen uptake in streams in
- 404 Grand Teton National Park, Wyoming. Limnology and Oceanography 48:1120–1128.

405	Hill, B. H., R. J. Stevenson, Y. Pan, A. T. Herlihy, P. R. Kaufmann, and C. B. Johnson. 2001.
406	Comparison of correlations between environmental characteristics and stream diatom
407	assemblages characterized at genus and species levels. Journal of the North American
408	Benthological Society 20:299–310.
409	Hoellein, T. J, J. L. Tank, E. J. Rosi-Marshall, S. A. Entrekin, and G. A. Lamberti. 2007.
410	Controls on spatial and temporal variation of nutrient uptake in three Michigan headwater
411	streams. Limnology and Oceanography 52:1964–1977.
412	Hooper, D. U., and P. M. Vitousek. 1997. The effects of plant composition and diversity on
413	ecosystem processes. Science 277:1302–1305.
414	Hooper, D. U., F. S. Chapin, J. J. Ewel, A. Hector, P. Inchausti, S. Lavorel, J. H. Lawton, D. M.
415	Lodge, M. Loreau, S. Naeem, B. Schmid, H. Setala, A. J. Symstad, J. Vandermeer, and
416	D. A. Wardle. 2005. Effects of biodiversity on ecosystem functioning: a consensus of
417	current knowledge. Ecological Monographs 75:3-35.
418	Jonsson, M., and B. Malmqvist. 2000. Ecosystem process rate increases with animal species
419	richness: evidence from leaf-eating aquatic insects. Oikos 89:519-523.
420	Kilroy, C. 2004. A new alien diatom, Didymosphenia geminata (Lyngbye) Schmidt: its biology,
421	distribution, effects and potential risks for New Zealand fresh waters. NIWA Client
422	Report: CHC2004-128. National Institute of Water and Atmospheric Research,
423	Christchurch, New Zealand. (Available from: http://www.biosecurity.govt.nz/files/pests/
424	didymo/didymo-preliminary-org-ia-nov-04.pdf)
425	Lohman, K., J. R. Jones, and C. Baysinger-Daniel. 1991. Experimental evidence for nitrogen
426	limitation in a northern Ozark stream. Journal of the North American Benthological
427	Society 19:14–23.

428	Lohman, K., and J. C. Priscu. 1992. Physiological indicators of nutrient deficiency in
429	Cladophora (Chlorophyta) in the Clark Fork of the Columbia River, Montana. Journal of
430	Phycology 28:443–448.
431	Loreau, M. 2000. Biodiversity and ecosystem functioning: recent theoretical advances. Oikos
432	91:3–17.
433	Lowe, R. L., and Y. Pan. 1996. Benthic algal communitites as biological monitors. Pages 705-
434	739 in R. J. Stevenson, M. L. Bothwell, and R. L. Lowe (editors) Algal ecology:
435	freshwater benthic ecosystems. Academic Press, San Diego, California.
436	MacArthur, R. H. 1955. Fluctuations of animal populations and a measure of community
437	stability. Ecology 36:533–536.
438	Marcarelli, A. M., M. A. Baker, and W. A. Wurtsbaugh. 2008. Is in-stream nitrogen fixation an
439	important nitrogen source for benthic communities and stream ecosystems? Journal of the
440	North American Benthological Society 27:186–211.
441	Marcarelli, A. M., and W. A. Wurtsbaugh. 2006. Temperature and nutrient supply interact to
442	control nitrogen fixation in oligotrophic streams: an experimental examination.
443	Limnology and Oceanography 51:2278–2289.
444	Marcarelli, A. M., and W. A. Wurtsbaugh. 2007. Effects of upstream lakes and nutrient
445	limitation on periphytic biomass and nitrogen fixation in oligotrophic, subalpine streams.
446	Freshwater Biology 52:2211–2225.
447	Martí, E., and F. Sabater. 1996. High variability in temporal and spatial nutrient retention in
448	Mediterranean streams. Ecology 77:854–869.
449	McCormick, P. V., and R. J. Stevenson. 1991. Mechanisms of benthic algal succession in lotic
450	environments. Ecology 72:1835–1848.

451	Miller, M. C., P. DeOliveria, and G. G. Gibeau. 1992. Epilithic diatom community response to
452	years of PO ₄ fertilization: Kuparuk River, Alaska (68 N lat.). Hydrobiologia 240:103-
453	119.
454	Mulholland, P. J. 1992. Regulation of nutrient concentration in a temperate forest stream: roles
455	of upland, riparian, and instream processes. Limnology and Oceanography 37:1512-
456	1526.
457	Mulholland, P. J., A. D. Steinman, A. V. Palumbo, J. W. Elwood, and D. B. Kirschtel. 1991.
458	Role of nutrient cycling and herbivory in regulating periphyton communities in
459	laboratory streams. Ecology 72:966–982.
460	Mulholland, P. J., J. L. Tank, D. M. Sanzone, W. M. Wollheim, B. J. Peterson, J. R. Webster,
461	and J. L. Meyer. 2000. Nitrogen cycling in a forest stream determined by a ¹⁵ N tracer
462	addition. Ecological Monographs 70:471–493.
463	Mulholland P. J., S. A. Thomas, H. M. Valett, J. R. Webster, and J. Beaulieu. 2006. Effects of
464	light on NO3 ⁻ uptake in small forested streams: diurnal and day-to-day variations. Journal
465	of the North American Benthological Society 25:583-595.
466	Mulholland, P. J., H. M. Valett, J. R. Webster, S. A. Thomas, L. W. Cooper, S. K. Hamilton, and
467	B. J. Peterson. 2004. Stream denitrification and total nitrate uptake rates measured using
468	a field ¹⁵ N tracer addition approach. Limnology and Oceanography 49:800–820.
469	Mulholland, P. J., A. M. Helton, G. C. Poole, R. O. Hall, S. K. Hamilton, B. J. Peterson, J. L.
470	Tank, L. R. Ashkenas, L. W. Cooper, C. N. Dahm, W. K. Dodds, S. G. Findlay, S. V.
471	Gregory, N. B. Grimm, S. L. Johnson, W. H. McDowell, J. L. Meyer, H. M. Valett, J. R.
472	Webster, C. P. Arango, J. J. Beaulieu, M. J. Bernot, A. J. Burin, C. L. Crenshaw, L. T.
473	Johnson, R. R.Neiderlehner, J. M. O'Brien, J. D. Potter, R. W. Sheibley, D. J. Sobota,

- 474 and S. M. Thomas. 2008. Stream denitrification across biomes and its response to
 475 anthropogenic nitrate loading. Nature. 452:202-206.
- 476 Naeem, S. L., L. J. Thompson, S. P. Lawler, J. H. Lawton, and R. M. Woodfin. 1995. Empirical
- 477 evidence that declining species diversity may alter the performance of terrestrial
- 478 ecosystems. Philosophical Transactions of the Royal Society of London B: Biological
- 479 Sciences. 347:249–262.
- 480 O'Brien, J. M., W. K. Dodds, K. C. Wilson, J. N. Murdock, and J. Eichmiller. 2007. The
- 481 saturation of N cycling in Central Plains streams: ¹⁵N experiments across a broad gradient
 482 of nitrate concentrations. Biogeochemistry 84:31–49.
- 483 Pan, Y., R. J. Stevenson, P. Vaithiyanathan, J. Slate, and C. J. Richardson. 2000. Changes in
- 484 algal assemblages along observed and experimental phosphorus gradients in a subtropical
 485 wetland, U.S.A. Freshwater Biology 43:1–15.
- Peterson, B., B. Fry, L. Deegan, and A. Hershey. 1993. The trophic significance of epilithic algal
 production in a fertilized tundra river ecosystem. Limnology and Oceanography 38:872–
 878.
- 489 Peterson, B. J., W. M. Wollheim, P. J. Mulholland, J. R. Webster, J. L. Meyer, J. L. Tank, E.
- 490 Martí, W. B. Bowden, H. M. Valett, A. E. Hershey, W. H. McDowell, W. K. Dodds, S.
- K. Hamilton, S. Gregory, and D. D. Morrall. 2001. Control of nitrogen export from
 watersheds by headwater streams. Science 292:86–90.
- 493 Prechtl, J., C. Kneip, P. Lockhart, K. Wenderoth, and U.-G. Maier. 2004. Intracellular spheroid
- 494 bodies of *Rhopalodia gibba* have nitrogen-fixing apparatus of cyanobacterial origin.
- 495 Molecular Biology and Evolution 21:1477–1481.
- 496 Schindler, D. W. 1977. Evolution of phosphorus limitation in lakes. Science 195:260–262.

497	Simon, K. S., C. R. Townsend, B. J. F. Biggs, and W. B. Bowden. 2005. Seasonal patterns of N
498	and P uptake in 2 New Zealand streams. Journal of the North American Benthological
499	Society 24:1–18.
500	Stevenson, R. J. 1996. An introduction to algal ecology in freshwater benthic habitats. Pages 3–
501	30 in R. J. Stevenson, M. L. Bothwell, and R. L. Lowe (editors). Algal ecology:
502	freshwater benthic ecosystems. Academic Press, San Diego, California.
503	Stevenson, R. J. 1998. Diatom indicators of stream and wetland stressors in a risk management
504	framework. Environmental Monitoring and Assessment 51:107–118.
505	Tilman, D. 1997. Distinguishing the effects of species diversity and species composition. Oikos
506	80:185.
507	Tilman, D. 1999. The ecological consequences of changes in biodiversity: a search for general
508	principles. Ecology 80:1455–1474.
509	Valett, H. M., J. A. Morrice, C. N. Dahm, and M. E. Campana. 1996. Parent lithology, surface-
510	groundwater exchange, and nitrate retention in headwater streams. Limnology and
511	Oceanography 41:333–345.
512	Vitousek, P. M., H. A. Mooney, J. Lubchenco, and J. M. Melillo. 1997. Human domination of
513	earth's ecosystems. Science 277:494–499.
514	Wehr, J. D., and R. G. Sheath. 2003. Freshwater algae of North America. Elsevier Science, San
515	Diego, California.
516	Welschmeyer, N. A. 1994. Fluorometric analysis of chlorophyll a in the presence of chlorophyll
517	b and phaeopigments. Limnology and Oceanography 39:1985–1992.
518	Whitton, B. A., E. Rott, and G. Friedrich. 1991. Use of algae for monitoring rivers. E. Rott
519	Publishers, Innsbruck, Austria.

520	Wollheim, W. M., B. J. Peterson, L. A. Deegan, J. E. Hobbie, B. Hooker, W. B. Bowden, K. J.
521	Arscott, A. E. Hershey, and J. C. Finlay. 2001. Influence of stream size on ammonium
522	and suspended particulate nitrogen processing. Limnology and Oceanography 46:1-13.
523	Zedler, J. B., J. C. Callaway, and G. Sullivan. 2001. Declining biodiversity: why species matter
524	and how their functions might be restored in California tidal marshes. BioScience
525	51:1005–1017.
526	

527	Figure Captions
528	Fig. 1. Study site in central Idaho, USA, showing original location of rocks and locations to
529	which they were transplanted and during the ¹⁵ N-NO ₃ experiment.
530	Fig. 2. Rank abundance curves (abundance rank 1 is highest by biovolume) for periphyton
531	assemblages from 3 patch types.
532	Fig. 3. Mean (±1 SE) NO ₃ -N uptake rates for periphyton assemblages from 3 patch types on days
533	8 and 14 of ¹⁵ N-NO ₃ release. Bars with the same letter are not significantly different
534	(analysis of variance followed by Tukey-Kramer post hoc comparisons $p < 0.05$).
535	Fig. 4. Relationship between mean (± 1 SE) periphyton chlorophyll <i>a</i> content and mean (± 1 SE)
536	NO ₃ -N uptake for each patch type on days 8 and 14 of ¹⁵ N-NO ₃ release.
537	Fig. 5. Relationship between N-uptake (normalized to chlorophyll <i>a</i>) and % Cyanophyta
538	biovolume (closed symbols) and % Chlorophyta biovolume (open symbols) in 3 patch
539	types with different algal richness.
- 10	

541 Table 1. Periphyton taxa list by patch type in order of abundance by biovolume. Taxa unique to

542 each patch type are indicated in bold font.

Green patch	Yellow patch	Brown patch
Spirogyra	Spirogyra	Synedra
Rhizoclonium	Bulbochaete	Cymbella
Synedra	$Calothrix^+$	Fragilaria
Fragilaria	Protoderma	Epithemia ^b
Cymbella	Achnanthes	Calothrix ^a
Achnanthes	Synedra	<i>Oscillatoria</i> ^a
Gomphonema	<i>Rhopalodia</i> ^b	Achnanthes
Navicula	Fragilaria	Eunotia
Stigeoclonium	Gomphonema	Rhopalodia ^b
Mougeotia	Cymbella	Navicula
Aulacoseira	Closterium	Teilingia
Bulbochaete	Synechocystis ^a	Gomphonema
Cocconeis	Navicula	Anomoeneis
Pseudanabaena ^a	Cocconeis	Nitzschia
Synechocystis ^a	Stigeoclonium	Oocystis
Tabellaria	Eunotia	Cocconeis
Oedogonium	Denticula	Euastrum
Anomoeneis	Anomoeneis	Synechocystis ^a
Chlorococcum	Chlorococcum	Pediastrum
Scenedesmus	Diploneis	Scenedesmus

	Epithemia*	<i>Oscillatoria</i> ^a	Chlorococcaceae ^c	
	Cosmarium	Cosmarium	Protoderma	
	Protoderma	Nitzschia		
	Eunotia	<i>Aphanocapsa</i> ^a		
	Tetraedron	Aulacoseira		
	Nitzschia	Scenedesmus		
	Oscillatoria ^a			
	Oocystis			
543	^a Division Cyanophyta			
544	^b Diatoms with endosymbiont Cyanobacteria			
545	^c Identification to family	I		
510				

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	Green patch	Yellow patch	Brown patch
Richness	28	26	22
Shannon-Weiner	0.814	1.33	2.25
Shannon evenness	0.244	0.375	0.718
Bray–Curtis distance	Green vs yellow	Yellow vs brown	Brown vs green
	0.496	0.611	0.621









