2009

Differences in nitrate uptakeamong benthic algal assemblages in a mountain stream

Michelle A. Baker
Utah State University

G. de Guzman

Recommended Citation
Baker, M.A., G.deGuzman, and J. D. Ostermiller. 2009. Differences in nitrate uptake among benthic algal assemblages in a mountain stream. Journal of the North American Benthological Society 28: 24-33.

RRH: M. A. Baker et al.

LRH: Algal diversity and N uptake

Differences in nitrate uptake among benthic algal assemblages in a mountain stream

Michelle A. Baker[1]

Department of Biology and the Ecology Center, Utah State University, Logan, Utah 84322 USA

Glendell de Guzman[2]

Department of Biology, Utah State University, Logan, Utah 84322 USA

Jeffrey D. Ostermiller[3]

Utah Division of Water Quality, 288 North 1460 West, P.O. Box 14870, Salt Lake City, Utah 84114 USA

[1] E-mail address: michelle.baker@usu.edu

[2] Present address: Hershey Medical Center, Pennsylvania State University, 500 University Drive, Hershey, Pennsylvania 17033 USA. E-mail: deguzgs@yahoo.com

[3] E-mail address: jostermiller@utah.gov

Received: 12 October 2007

Accepted:

Abstract. We evaluated how benthic algal assemblages that vary in composition, richness, and other diversity metrics remove NO_3-N from the water column of a mountain stream. Ecological theory and empirical studies suggest that ecosystem process rates should increase as richness increases because of niche separation or activity of dominant taxa. Accordingly, we predicted that algal assemblages with highest richness would show the highest rates of NO_3-N uptake. To test this prediction, we transplanted 225 rocks representing 3 patch types (green, yellow, and brown) that differed macroscopically in algal composition from a lake outflow stream to a lake inflow stream where an experimental release of ^{15}N-NO_3 was ongoing. We measured ^{15}N uptake in each patch type during the stable isotope release. Benthic algal richness varied from 28 genera in the green patch type and 26 genera in the yellow patch type to 22 genera in the brown patch type. Without accounting for differences in chlorophyll *a* content, NO_3-N uptake ($2.1–3.3 \times 10^{-4}$/d) was highest in the green patch type, lowest ($0.3–0.6 \times 10^{-4}$/d) in the yellow patch type, and intermediate ($1.2–1.5 \times 10^{-4}$/d) in the brown patch type. NO_3-N uptake normalized to chlorophyll *a* increased in concert with algal richness in the 3 patch types. This result supports the hypothesis that increased assemblage diversity leads to higher rates of community processes. Aside from diversity differences per se, lower rates of NO_3-N uptake in the brown patch type might be the consequence of differences in functional characteristics of the taxa present. Approximately 29% of algal biovolume in the brown patch type consisted of taxa capable of N_2-fixation, a result that suggests that algae in this patch type might be capable of meeting N needs via N_2-fixation rather than via removal from the water column.

Key words: periphyton, nutrient immobilization, nitrogen-15, stable isotope, diversity.

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

(Vitousek et al. 1997), and stream benthic algal assemblages are no exception. Diatoms, in particular, are responsive to anthropogenic nutrient inputs (Pan et al. 2000) and are used as indicators of water quality (Stevenson 1998). Algal assemblages also are susceptible to invasions. For example, *Didymosphenia geminata,* a diatom native to oligotrophic waters of the Northern Hemisphere, has greatly expanded its range and caused nuisance blooms and changes to stream macroinvertebrate grazer assemblages in New Zealand and elsewhere (Kilroy 2004).

Little is known about the functional role of specific algal taxa or the effects of algal community structure (composition, richness, diversity) on nutrient uptake in streams (Covich et al. 2004). N_2-fixing taxa might increase in abundance as water-column N declines (Fisher et al. 1982). Mulholland et al. (1991) showed that nutrient reductions and herbivory by snails changed periphyton community composition and increased P cycling rates. Other studies have documented little change in algal assemblages but higher rates of production and nutrient cycling in response to nutrient enrichment (Miller et al. 1992, Peterson et al. 1993).

Strong inferences relating biodiversity to ecosystem functioning have been made using theoretical (Loreau 2000) and empirical approaches (Tilman 1999) and indicate that increased richness might lead to asymptotic increases in the magnitude of ecosystem processes over short time scales. Our objective was to examine how different benthic algal assemblages remove NO_3-N from the water column of a mountain stream during a stable isotope (^{15}N) tracer test. We hypothesized that assemblages with higher richness would exhibit higher rates of ^{15}N uptake because assemblages with higher diversity are more likely to include a dominant taxon (i.e., sampling effect, sensu Tilman 1997) responsible for much of the processing rate, or are more likely to include a group of taxa whose functional traits are complementary (sensu Loreau 2000) 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 2nd-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 µg/L in the inlet and
105 2.5 µg/L in the outlet. Total N typically shows the opposite pattern during baseflow with
106 concentrations on the order of 20 to 30 µg/L in the inlet and 90 to 100 µg/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 (84th 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,

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

Shannon–Weiner index (H'), and Bray–Curtis similarity in Systat V.5 (SPSS, Chicago, Illinois) for the genera identified. We chose to quantify diversity as richness, evenness, and H' because many studies relating diversity to ecosystem functioning have used these metrics, and we wanted our data to be comparable to those studies. We used Bray–Curtis similarity for pair-wise comparison of patch composition.

Experimental design

We took advantage of a ^{15}N tracer study to evaluate how the algal assemblages from each patch type immobilize N from the water column. Briefly, a solution containing 75 g of ^{15}N (as 27% $Na^{15}NO_3$) was administered to Springs Creek at a rate of 10.4 mL/min for 14 d beginning 5 August 2003 at a site ~1895 m above Bull Trout Lake (Fig. 1). The tracer test elevated the isotopic signature of the NO_3^- pool to 11,670‰ above background on average, with higher values at the top of the reach and lower values at the bottom of the reach. Changes in ambient NO_3-N concentration as a result of the tracer test were not detectable (Hall, R. O., University of Wyoming, unpublished data).

We transplanted 75 rocks (median particle size [D_{50}]–D_{84} = 16–39 mm; Arp et al. 2007) from each patch type from Warm Springs Creek (lake outlet) to Springs Creek (lake inlet) 3 d before the isotope experiment commenced. We arranged 15 rocks from each patch type in plastic baskets (18 cm × 26 cm) and placed the buckets in riffles at 5 sites: 62, 120, 1069, 1560, and 1890 m downstream of the ^{15}N-drip site (Fig. 1).

We collected periphyton samples from each patch type at each location (pooled from 5 rocks) the day before the ^{15}N-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

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

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

We measured NO_3-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 ^{15}N-NO_3 in the water column from 3-L samples collected and

filtered (Whatman GF/F) at each stream location on each sample date. We processed these samples in the laboratory according to protocols outlined in Mulholland et al. (2004). Briefly, we elevated the pH of samples with MgO to drive off NH_3, 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 converted this NH_4^+ to NH_3 with MgO and collected the NH_3 on an acidified filter by diffusion (Mulholland et al. 2004). The ^{15}N content on the filter was measured using a Europa Hydra 20/20 mass spectrometer at the UC Davis Stable Isotope Facility as described above. The atomic ratio excess of ^{15}N in water samples (AR_{water}) reported by the isotope laboratory was used with measured NO_3-N concentration (µg/L) to calculate ^{15}N concentration in the water column as

$$^{15}N_{water}\ (\mu g/L) = [AR_{water} \times NO_3\text{-}N]_{day\ 8\ or\ 14} - [AR_{water} \times NO_3\text{-}N]_{background}. \quad [2]$$

Concentrations on day 8 or 14 are corrected for isotopic content in background samples.

Uptake calculations

We calculated immobilization (uptake) of ^{15}N tracer by each patch type as the background-corrected $^{15}N_{periphyton}$ (mg/m^2) incubated at each sample location divided by the background-corrected ^{15}N (AR_{water}) in the overlying water column at the same location and the number of days of exposure to the tracer (8 or 14 d) (Mulholland et al. 2000, 2004) as

$$^{15}N\text{-}NO_3 \text{ uptake } (mg\ m^{-2}\ d^{-1}) = {}^{15}N_{periphyton}/[AR_{water} \times \text{days of experiment}]. \quad [3]$$

This calculation assumes that the periphyton has reached equilibrium with tracer in the water 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 $^{15}N\text{-}NO_3$ uptake ($mg\ m^{-2}\ d^{-1}$) above by the areal N content (mg/m^2) of each patch type

(Dodds et al. 2004). Hereafter, we report results as N-specific uptake (NO_3-N uptake).

Hypothesis tests

We compared NO_3-N uptake rates with 2-way analysis of variance (ANOVA) with patch type and day of experiment as factors. We followed significant ANOVAs by Tukey–Kramer Honestly Significant Difference tests (SAS, version 8; SAS Institute, Cary, North Carolina). We considered rates significantly different at $p < 0.05$. We used linear regression to assess whether differences among rates were related to chlorophyll-*a* content (SAS version 8). Our study had only 3 levels of diversity, so we did not use statistics to test hypotheses for relationships between richness and NO_3-N uptake.

Results

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).

^{15}N-nitrate uptake rates

NO_3-N uptake rates differed significantly among patch types (ANOVA, df = 3, F = 36.09, $p < 0.001$; Fig. 3). NO_3-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). NO_3-N uptake rates did not differ between sample days, except in the green patch type, where NO_3-N uptake was significantly higher on day 14 than on day 8 (Fig. 3).

NO_3-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/m^2) than did algae from the brown and green patch types (~60 mg/m^2) (ANOVA, df = 3, $F = 36.09$, $p < 0.01$), a result that explained some portion of the difference in NO_3-N uptake by the yellow patch.

NO_3-N uptake rates did not increase in concert with algal richness, as predicted from the diversity–function hypothesis. NO_3-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, NO_3-N uptake rate increased in concert with richness, as predicted by the diversity–function hypothesis (Fig. 5). Algal composition also was related to NO_3-N uptake rate, which was negatively related

to Cyanophyta biovolume and positively related to Chlorophyta biovolume (Fig. 5).

Discussion

Relationship between diversity and ecosystem functioning

Our study is the 1st attempt to use a stable isotope experiment to relate algal diversity to ecosystem functioning in streams. As average richness increased, chlorophyll-corrected NO_3-N uptake appeared to increase in concert. However, our results should be interpreted with great caution because we had only 3 levels of richness that differed by only 6 genera. We chose to identify taxa to the genus level because of financial constraints, but positive correlation between genus richness and species richness has been reported for stream periphyton (Hill et al. 2001). We also did not separate algal from bacterial and fungal components of the periphyton, and this decision might bias our results and interpretation. Nevertheless, the pattern we observed is consistent with that seen in studies from a variety of ecosystems, including grasslands (Tilman 1999) and other terrestrial ecosystems (Hooper et al. 2005), estuaries (Zedler et al. 2001), and stream filter-feeding guilds (Cardinale et al. 2002).

Our results oppose predictions from the diversity–function hypothesis. The algal assemblage in the brown patch type had the highest values of H' and evenness and was most different in composition from the algal assemblage in the other patch types as measured by Bray-Curtis distance, yet the algal assemblage in the brown patch type had the lowest NO_3-N uptake rate when normalized to chlorophyll *a*. Low NO_3-N uptake in the brown patch type might be related to differences in functional characteristics of the dominant taxa in each patch, rather than to diversity per se. Algae in the brown patch included a relatively high proportion of taxa potentially capable of N_2-fixation (Table 1). These taxa included Cyanophyta (Gallon 2004),

277 which accounted for ~15% of biovolume, and diatoms (*Epithemia* and *Rhopalodia*) with N_2-
278 fixing endosymbionts (Prechtl et al. 2004), which accounted for another 14% of biovolume. In
279 contrast, taxa potentially capable of N_2-fixation accounted for ~5% of biovolume in the yellow
280 patch type and <0.03% of biovolume in the green patch type. The negative relationship between
281 NO_3-N uptake and biovolume of Cyanophya supports this interpretation. Algae from brown
282 patches might be able to meet its N demands via N_2-fixation rather than via assimilation of N
283 from the water column. Rates of N_2-fixation on the order of 8.7 µg N m^{-2} h^{-1} have been
284 measured in this system (Marcarelli et al. 2008), and N_2-fixation rates are correlated with
285 presence of N_2-fixing taxa (Marcarelli and Wurtsbaugh 2006).

286 Stable isotopes have been used in terrestrial ecosystems to examine relationships between
287 diversity and ecosystem functioning. For example, Hooper and Vitousek (1997) used ^{15}N-tracers
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
295 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
298 patch type (122 cm^2), and greatest for the yellow patch type (187 cm^2). Chlorophyll *a* was
299 highest in the green patch type and lowest in the yellow patch type.

Physiognomic differences among algae can affect nutrient immobilization because growth form and stature affect resource (light and nutrient) supply to algal mats (Stevenson 1996). Competitively dominant diatoms in algal mats are those that produce mucilaginous stalks, which enable better access to light and nutrients (McCormick and Stevenson 1991). Mucilaginous stalks were visible in the brown patch type, but increased resource supply does not explain low NO_3-N uptake by periphyton in this patch type. If nutrient delivery rates were higher, this patch should have assimilated more N than we measured.

That NO_3-N uptake was not linearly correlated with chlorophyll *a* is interesting because the pigment is rich in N and usually is a good estimate of N requirements of algae (Graham and Wilcox 2000). However, the relationship probably does not hold in situations in which algal assemblages are distinctly different because algae in the brown patch type had low NO_3-N uptake relative to its internal store of chlorophyll *a*.

We do not know the degree to which each genus contributed to NO_3-N uptake. Some taxa (*Oocystis*, *Tetraedron*, and *Pediastrum*) are more typical of lake phytoplankton than of stream periphyton (Wehr and Sheath 2003) and might be cells that were trapped in the periphyton when the rocks were in the lake outflow. These taxa were present in low numbers and probably did not contribute significantly to NO_3-N uptake. Dominant taxa, such as *Spirogyra* in the green and yellow patch types, probably contributed significantly to NO_3-N uptake. *Spirogyra fluviatum* has high N requirements, particularly at high flows (Borchardt 1994).

Taxa differ in their requirements for N and their affinity for NO_3-N, but NO_3-N uptake probably was not saturated in our study because ambient NO_3-N concentration was <10 µgN/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

323 tolerance values for total N between 26 and 140 µgN/L (Lowe and Pan 1996). Concentrations
324 <100 µgN/L are growth limiting for benthic algae grown in artificial streams (Lohman et al.
325 1991). NO_3-N uptake by *Cladophera glomerata*, a species common in nutrient-rich water,
326 saturates at concentrations ~330 µgN/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

346 benefit from including an autecological approach with the synecological approach already
347 inherent in the research. The stable isotope approach we present here might be one way to do so.
348

Acknowledgements

The authors thank J. Rothlisberger, B. Hall, C. Arp, A. Marcarelli, and A. Chartier for help in the field and the laboratory. Dr. Ann St. Amand at PhycoTech identified and enumerated periphyton samples. D. Harris and the UC Davis Stable Istotope Facility analyzed ^{15}N content of samples. K. Grover-Weir of the Boise National Forest allowed access to the study site. Reviews from C. Crenshaw and A. Marcarelli improved the quality of this manuscript. This research was funded by a grant from National Science Foundation to DEB-0132983 awarded to W. Wurtsbaugh, R. Hall, M. Baker, and J. Haefner. G. de Guzman was supported by a Research Experiences for Undergraduates supplemental grant.

Literature Cited

Arp, C. D., and M. A. Baker. 2007. Discontinuities in stream nutrient uptake below lakes in mountain drainage networks. Limnology and Oceanography 52:1978–1990.

Arp, C. D., M. N. Gooseff, M. A. Baker, and W. Wurtsbaugh. 2006. Surface-water hydrodynamics and regimes of a small mountain stream-lake ecosystem. Journal of Hydrology 329:500–513.

Arp, C. D., J. C. Schmidt, M. A. Baker, and A. K. Myers. 2007. Stream geomorphology in a mountain lake district: sediment sources and sinks, lake-modified hydraulics, and downstream lake effects. Earth Surface Processes and Landforms 32:525–543.

Bergey, E. A., and G. M. Getty. 2006. A review of methods for measuring the surface area of stream substrates. Hydrobiologia 556:7–16.

Bernot, M. J., J. L. Tank, and T. V. Royer. 2006. Nutrient uptake in streams draining agricultural catchments of the midwestern United States. Freshwater Biology 51:499–509.

Biggs, B. J. F. 1996. Patterns in benthic algae of streams. Pages 31–56 *in* R. J. Stevenson, M. L. Bothwell, and R. L. Lowe (editors). Algal ecology: freshwater benthic ecosystems. Academic Press, San Diego, California.

Borchardt, M. A. 1994. Effects of flowing water on nitrogen- and phosphorus-limited photosynthesis and optimum N:P ratios by *Spirogyra fluviatilis* (Charophyceae). Journal of Phycology 30:418–430.

Cardinale, B. J., M. A. Palmer, and S. L. Collins. 2002. Species diversity enhances ecosystem functioning through interspecific facilitation. Nature 415:46–429.

Covich, A. P., M. C. Austen, F. Bärlocher, E. Chauvet, B. J. Cardinale, C. L. Biles, P. Inchausti, 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.

Crumpton, W. G. 1987. A simple and reliable method for making permanent mounts of phytoplankton for light and fluorescence microscopy. Limnology and Oceanography 32:1154–1159.

Dodds, W. K., E. Martí, J. L. Tank, J. Pontius, S. K. Hamilton, N. B. Grimm, W. B. Bowden, W. H. McDowell, B. J. Peterson, H. M. Valett, J. R. Webster, and S. Gregory. 2004. Carbon and nitrogen stoichiometry and nitrogen cycling rates in streams. Oecologia 140:458-467.

Doyle, M. W., E. H. Stanley, and J. M. Harbor. 2003. Hydrogeomorphic controls on phosphorus retention in streams. Water Resources Research 39. doi:10.1029/2003WR002038.

Earl, S. R., H. M. Valett, and J. R. Webster. 2006. Nitrogen saturation in streams. Ecology 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.

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 nitrogen fixation in free-living bacteria. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Graham, L. E., and L. W. Wilcox. 2000. Algae. Prentice Hall, Upper Saddle River, New Jersey.

Gucker, B., and M. T. Pusch. 2006. Regulation of nutrient uptake in eutrophic lowland streams. Limnology and Oceanography 51:1443–1453.

Hall, R. O., and J. L. Tank. 2003. Ecosystem metabolism controls nitrogen uptake in streams in 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.

Lohman, K., and J. C. Priscu. 1992. Physiological indicators of nutrient deficiency in *Cladophora* (Chlorophyta) in the Clark Fork of the Columbia River, Montana. Journal of Phycology 28:443–448.

Loreau, M. 2000. Biodiversity and ecosystem functioning: recent theoretical advances. Oikos 91:3–17.

Lowe, R. L., and Y. Pan. 1996. Benthic algal communitites as biological monitors. Pages 705–739 *in* R. J. Stevenson, M. L. Bothwell, and R. L. Lowe (editors) Algal ecology: freshwater benthic ecosystems. Academic Press, San Diego, California.

MacArthur, R. H. 1955. Fluctuations of animal populations and a measure of community stability. Ecology 36:533–536.

Marcarelli, A. M., M. A. Baker, and W. A. Wurtsbaugh. 2008. Is in-stream nitrogen fixation an important nitrogen source for benthic communities and stream ecosystems? Journal of the North American Benthological Society 27:186–211.

Marcarelli, A. M., and W. A. Wurtsbaugh. 2006. Temperature and nutrient supply interact to control nitrogen fixation in oligotrophic streams: an experimental examination. Limnology and Oceanography 51:2278–2289.

Marcarelli, A. M., and W. A. Wurtsbaugh. 2007. Effects of upstream lakes and nutrient limitation on periphytic biomass and nitrogen fixation in oligotrophic, subalpine streams. Freshwater Biology 52:2211–2225.

Martí, E., and F. Sabater. 1996. High variability in temporal and spatial nutrient retention in Mediterranean streams. Ecology 77:854–869.

McCormick, P. V., and R. J. Stevenson. 1991. Mechanisms of benthic algal succession in lotic 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 NO_3^- 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: ^{15}N 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.
486 Peterson, B., B. Fry, L. Deegan, and A. Hershey. 1993. The trophic significance of epilithic algal
487 production in a fertilized tundra river ecosystem. Limnology and Oceanography 38:872–
488 878.
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.
491 K. Hamilton, S. Gregory, and D. D. Morrall. 2001. Control of nitrogen export from
492 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 $^{15}N\text{-}NO_3$ 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 $^{15}N\text{-}NO_3$ 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 *a* content and mean (±1 SE)
536 NO_3-N uptake for each patch type on days 8 and 14 of $^{15}N\text{-}NO_3$ release.

537 Fig. 5. Relationship between N-uptake (normalized to chlorophyll *a*) and % Cyanophyta
538 biovolume (closed symbols) and % Chlorophyta biovolume (open symbols) in 3 patch
539 types with different algal richness.

540

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*	*Oscillatoria*[a]
Gomphonema	*Rhopalodia*[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**	*Oscillatoria*[a]	Chlorococcaceae[c]
Cosmarium	*Cosmarium*	*Protoderma*
Protoderma	*Nitzschia*	
Eunotia	***Aphanocapsa***[a]	
Tetraedron	*Aulacoseira*	
Nitzschia	*Scenedesmus*	
Oscillatoria[a]		
Oocystis		

543 [a] Division Cyanophyta

544 [b] Diatoms with endosymbiont Cyanobacteria

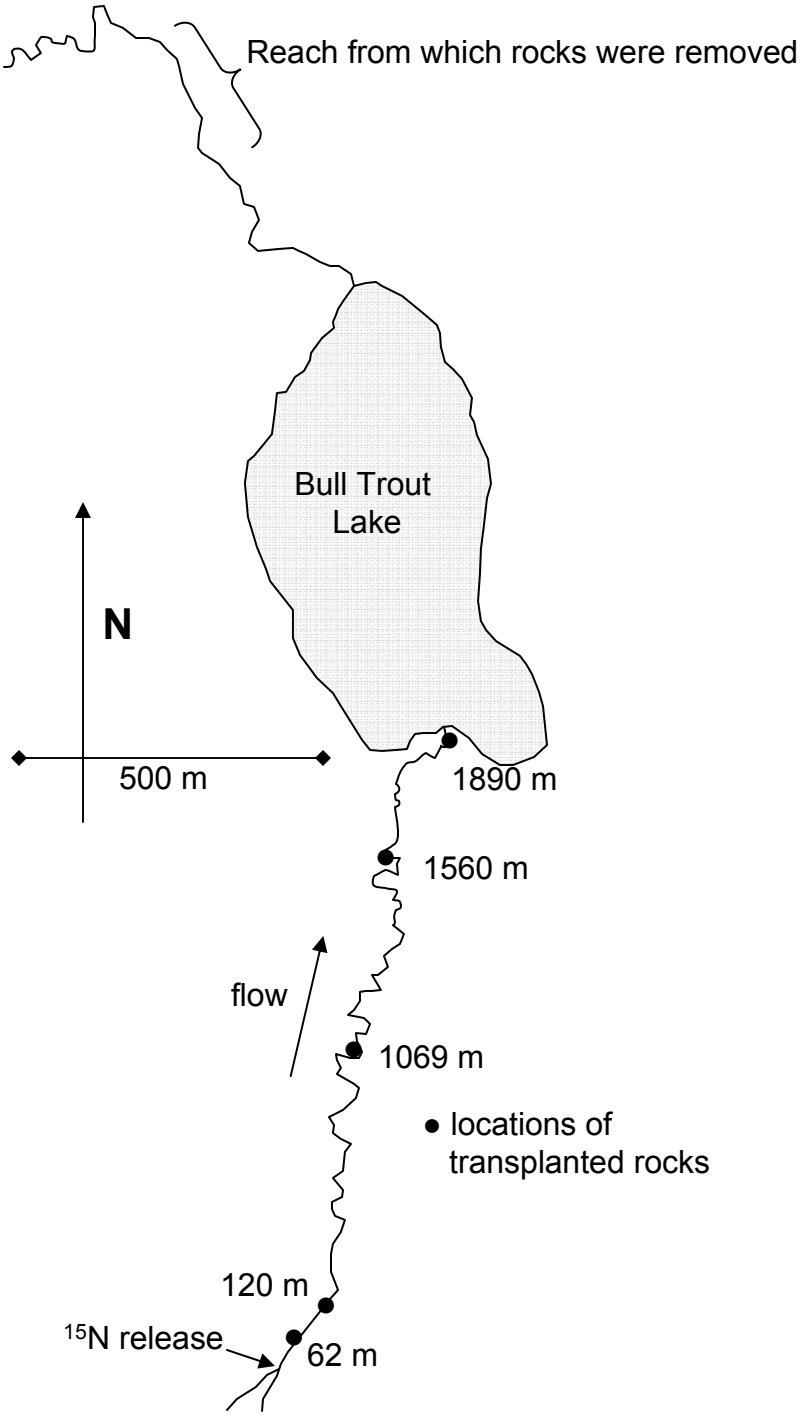
545 [c] Identification to family

546

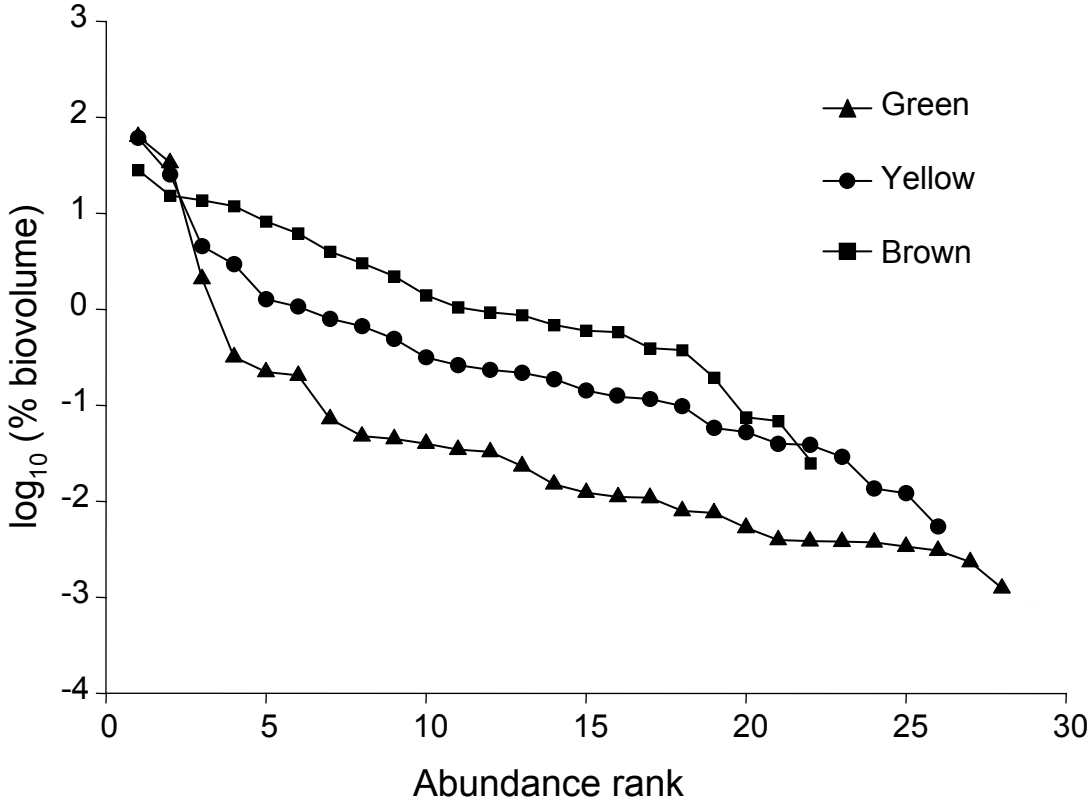
547 Table 2. Algal diversity metrics calculated for each patch type.
548

	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

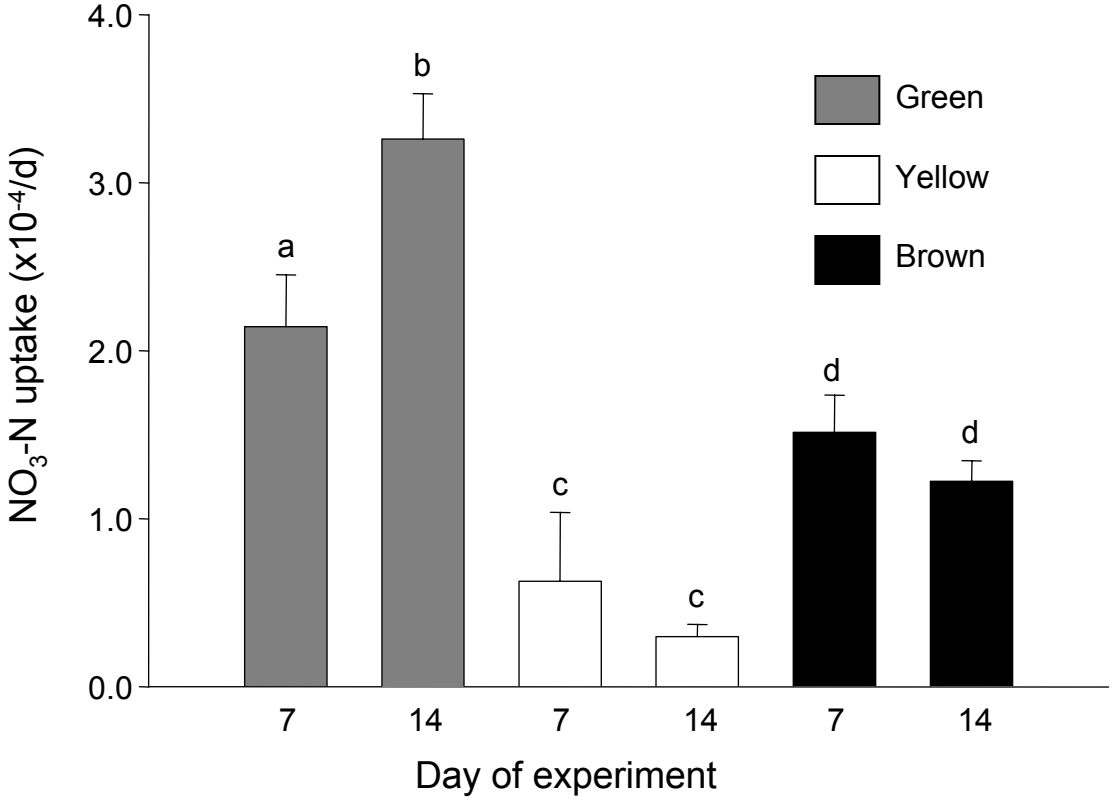
549



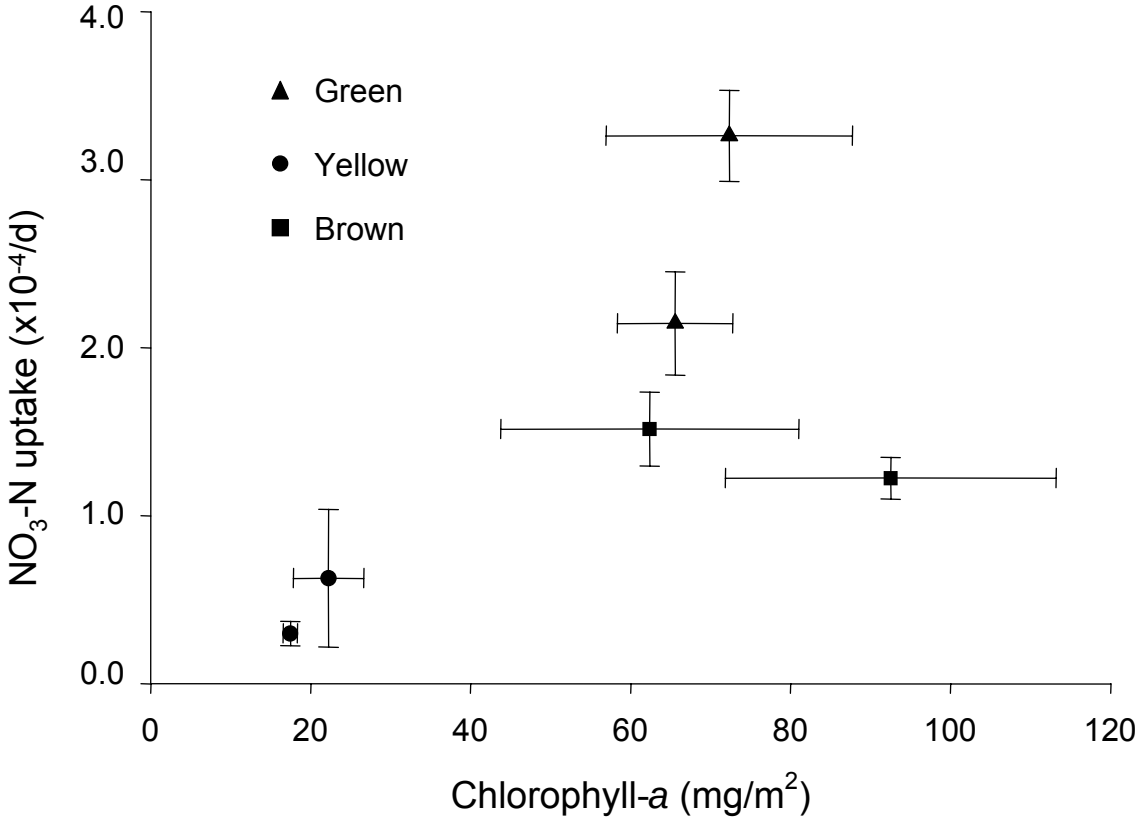
Baker et al. Fig 1



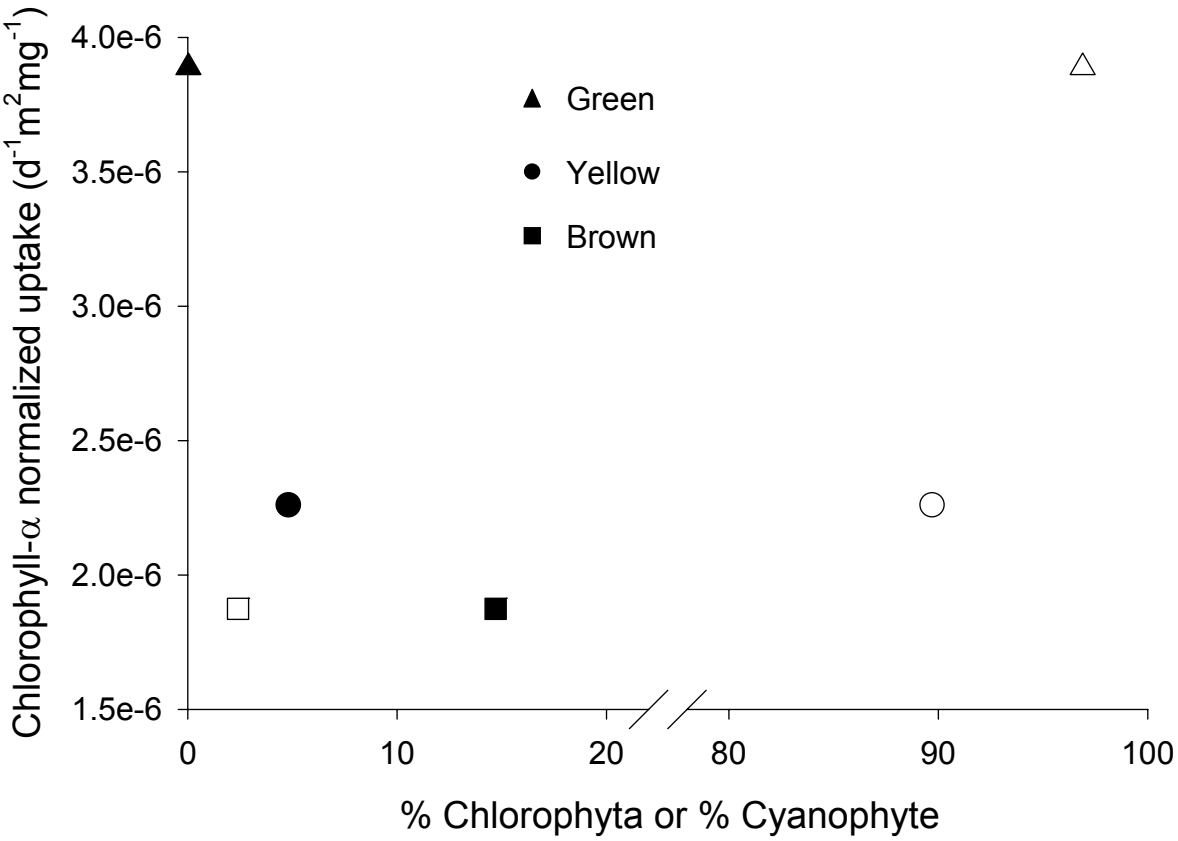
Baker et al. Fig 2



Baker et al. Fig 3



Baker et al. Fig 4



Baker et al. Fig 5