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Contrasting food web linkages for the grazing pathway in three temperate forested streams using ^{15}N as a tracer

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Introduction

Nitrogen is a critical element controlling the productivity and dynamics of stream ecosystems and many streams are limited by the supply of biologically available nitrogen (e.g. GRIMM & FISHER 1986, LOHMAN et al. 1991). We are learning more about the fate of inorganic nitrogen entering streams through ^{15}N tracer additions (PETERSON et al. 1997). The Lotic Intersite Nitrogen eXperiment (LINX) is studying the uptake, cycling, and fate of ^{15}N-NH_4 in the stream food web of 10 streams draining different biomes. Using the ^{15}N tracer method and data from three sites in the study, we can differentiate patterns in the cycling of nitrogen through the grazing pathway (N from the epilithon to grazing macroinvertebrates) for three temperate forested streams. Here, we quantify the relationship between the dominant grazer and its proposed food resource, the epilithon, by comparing ^{15}N levels of grazers with those of the epilithon, as well as the biomass, nitrogen content, and chlorophyll *a* standing stocks of the epilithon in three streams.

Study sites

Upper Ball Creek (UBC) is a second-order stream located at Coweeta Hydrologic Laboratory in the southern Appalachian mountains of North Carolina, USA. Walker Branch (WB) is a first-order stream located at Oak Ridge National Lab in the Ridge and Valley province of eastern Tennessee, USA. Bear Brook (BB) is a second-order stream located in the Hubbard Brook Experimental Forest in the White Mountains of New Hampshire, USA. All three streams are narrow (2–3 m), shallow (5–15 cm), low discharge (3–51 L/s), groundwater fed streams draining forested catchments (Table 1). The streams contain relatively low levels of dissolved nutrients: ammonia concentrations range from <2 to 10 µg N/L, nitrate ranges from 1 to 90 µg N/L, and soluble reactive phosphorus (SRP) ranges from 1 to 8 µg P/L and are considered to be relatively undisturbed.

Table 1. Study stream parameters measured during the ^{15}N tracer addition.

Parameter	UBC	WB	BB
Discharge (L/s)	51	9.8	3.1
Width (m)	2.7	3.1	2.1
Depth (cm)	15	4.6	9.8
Temperature (°C)	7.2	12.4	15
GPP (g $O_2/m^2/day$)	0.10	1.3	0.27
R (g $O_2/m^2/day$)	30	6.4	11.2

Methods

In each stream we continuously added $^{15}NH_4$ as 10% $^{15}NH_4Cl$ for 42 days at a rate that was intended to increase the ^{15}N level of the streamwater ammonium pool by approximately 50% at the addition site while raising the background concentration of ammonia by <1%. Slight variations in discharge and ammonium concentration resulted in actual ^{15}N levels ± a factor of 3. The ^{15}N release in UBC was conducted in late autumn 1996, the WB release was conducted in early spring 1997, and BB release occurred in early summer 1997. Although all of these streams had a complete forest canopy, the timing of the studies resulted in differences in light regime and presumed primary production with WB having the highest light levels and BB the lowest.

As part of the larger sampling regime for the LINX project, we sampled the epilithon and the dominant grazing macroinvertebrate on a weekly basis during the ^{15}N release. Sampling was conducted at one station 10 m upstream of the ^{15}N addition site (hereafter noted as the dripper) to determine background ^{15}N levels and seven sampling stations along a 150–250-m reach downstream from the dripper to examine ^{15}N labeling both spatially and temporally during the release. Epilithon was sampled by scraping three randomly collected rocks at each station, pooling the rinsed scrubbate, and filtering it onto a

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25-mm pre-ashed glass fiber filter (Whatman GFF) which was later dried. The grazers were collected using a combination of kick net sampling and hand-picking from rocks (5–10 individuals per site), stored in streamwater overnight to allow gut clearance, dried, and ground. In UBC and WB, the mayfly *Stenonema* sp. was chosen as the representative grazer, while the mayfly *Epeorus* sp. was chosen in BB. Samples were analyzed for ^{15}N by mass spectrometry at The Ecosystems Center, Marine Biological Lab, Woods Hole, MA using an automated sample combustion system and a Finnigan Delta S isotope ratio mass spectrometer. All ^{15}N values are expressed as $\delta^{15}N$ calculated from the following equation:

$$\delta^{15}N = [(R_{sample}/R_{standard} - 1] \times 1000$$

Where R represents the ^{15}N:^{14}N ratio and the N isotope standard is air (EHLERINGER et al. 1986). All ^{15}N data are reported as background-corrected tracer $\delta^{15}N$ values (i.e. we subtracted the $\delta^{15}N$ of upstream samples from the $\delta^{15}N$ of samples collected downstream of the dripper).

To further characterize the food resource for grazers, we also measured epilithon chlorophyll *a*, biomass, and nitrogen content. We measured chlorophyll *a* by placing a 5-cm diameter PVC cylinder sealed to the rock surface with a neoprene cuff and scrubbing the rock surface with a stiff brush. Scrubbate was suctioned into a container, filtered onto ashed GFF filters, extracted for 24 h at 4 °C, in the dark in 20 mL of 90% acetone. Extracts were analyzed on a spectrophotometer at 664 nm and 750 nm, before and after acidification (APHA 1989). Biomass of epilithon was also sampled with the PVC cylinder, except that scrubbate was filtered onto a pre-weighed GFF, dried at 55 °C, weighed, ashed, dried, and reweighed to determine g AFDM/m². Total N in epilithon was derived from C:N analysis on epilithon samples using a CHN analyzer (Carlo Erba Model 1500).

Results and discussion

Tracer $\delta^{15}N$ values (background-corrected) for epilithon and grazers collected on day 42 of the ^{15}N release were plotted against distance from the dripper for all three streams: UBC, WB, and BB (Fig. 1). In WB, the mayfly *Stenonema* appears to track the $\delta^{15}N$ value of epilithon quite closely. In contrast, for UBC and BB, the grazers (*Stenonema* and *Epeorus*, respectively) were more highly ^{15}N-labeled than the epilithon. To quantify the relative ^{15}N labeling of grazers and epilithon, we divided the $\delta^{15}N$ of grazers by the $\delta^{15}N$ of epilithon for all sampling stations, on day 21 and day 42. In WB, the grazer:epilithon $\delta^{15}N$ ratio was very close to 1 indicating that the grazer *Stenonema* is tracking the ^{15}N of its food resource very closely (Fig. 2). In contrast, in UBC and BB, the grazer:epilithon $\delta^{15}N$ ratios were greater than 1 and significantly different than those in WB (ANOVA followed by LSM, $P < 0.05$) indicating selective assimilation of epilithon by grazers. Ratios were not different between day 21 and day 42 indicating that grazers were in approximate isotopic equilibrium with their food by day 21 in all three streams (ANOVA, $P > 0.05$).

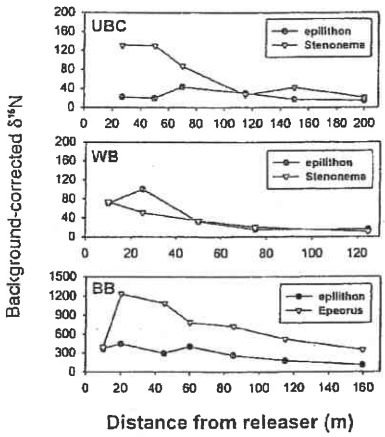

Fig. 1. Comparison of background-corrected $\delta^{15}N$ values in bulk epilithon and grazing macroinvertebrates plotted over distance on day 42 for UBC, WB, and BB.

Epilithon N content and biomass were highest in WB, followed by BB and UBC (Fig. 3A and B). A similar pattern was found for chlorophyll *a* concentrations, with WB having 2.5 times as much chlorophyll *a* per unit area as BB and 25 times as much as UBC (Fig. 3C). Although epilithon biomass in BB was nearly as great as in WB, chlorophyll *a* was considerably lower in BB indicating that algae made up a lower proportion of epilithon in BB compared with WB. In UBC, biomass and chlorophyll *a*

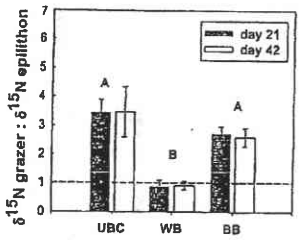

Fig. 2. Ratio of δ^{15}N grazer to δ^{15}N epilithon in UBC, WB, and BB for day 21 and day 42. There were no significant differences between day 21 and day 42 ratios in any stream (ANOVA, $P > 0.05$). Letters indicate significant differences between streams (ANOVA followed by LSM, $P < 0.05$).

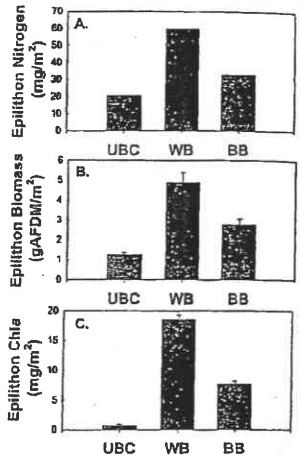

Fig. 3. (A) Mean epilithon nitrogen (mg/m²), (B) mean epilithon biomass ± SE (g AFDM/m²) and (C) mean epilithon chlorophyll *a* ± SE (mg/m²) for UBC, WB, and BB at the beginning of the ^{15}N tracer addition.

were both low (Fig. 3A). The differences in epilithon were likely, at least in part, a result of the ^{15}N tracer additions being conducted during different seasons: early spring in WB, just prior to leaf out when light levels are high, late autumn in UBC, after leaffall, and summer in BB, under dense canopy shading.

In WB, the amount of ^{15}N tracer in *Stenonema* was nearly the same as its food resource indicating that the bulk epilithon was assimilated by the grazer. This non-selective assimilation of epilithon may reflect its uniformly high quality. WB also has a very high density of snail grazers (*Elimia clavaeformis*) and experimental studies have shown that *Elimia*-grazed epilithic communities were higher in nutrients (%N and %C) and chlorophyll *a* for a given biomass than ungrazed communities (Rosemond 1993). Snails in similar Tennessee streams have been found to prevent the accumulation of particulate detrital matter in the loosely attached layer of periphyton, and maintain high rates of primary productivity (Hill & Harvey 1990). In fact, higher biomass-specific production and algal turnover rates with grazing have been reported numerous times (e.g. Lamberti & Resh 1983, Mulholland et al. 1991, Lamberti et al. 1995).

In contrast, in our low-light streams, BB and UBC, the insect grazer was more highly labeled with ^{15}N than the epilithon. In these streams, the epilithon may consist of a greater proportion of bacteria than in WB, and may also contain considerable amounts of detrital particles and bacterially-produced mucilage which would likely not be highly labeled with ^{15}N. Grazers may assimilate bacterial and algal cells to a much greater degree, thus acquiring a higher concentration of ^{15}N than the bulk epilithon. Therefore, our results suggest that only a portion of the epilithic biofilm in these low-light streams is actively playing a role in nitrogen cycling.

In an Alaskan stream, previous ^{15}N tracer studies have also found that the insect grazer *Baetis* became more highly labeled than the bulk epilithon (Peterson et al. 1997, Wollheim et al. 1999). They concluded that the epilithon ^{15}N signal was diluted by a detrital component that acquires streamwater ammonia to a much lesser extent than algae and heterotrophic bacteria, and the mode of epilithon collection pools all of it into one heterogeneous sample. Based on their model, 37% of *Baetis* N was attributed to feeding on epilithic detritus and 63% to diatoms (Wollheim et al. 1999).

Surprisingly, in another ^{15}N tracer study conducted in the same drainage basin as UBC,

HALL et al. (1998) did not report higher ^{15}N levels in *Stenonema* relative to epilithon, although *Chironomids* (collector-gatherers) were more highly labeled than their food source (FBON). Again, higher ^{15}N labeling of the consumer was attributed to preferential assimilation of the microbial N fraction of the FBOM–microbe complex. HALL et al. (1998) attributed the close isotopic tracking of the epilithon by *Stenonema* to a higher fraction of labile N in the Hugh White Creek epilithon. Hugh White Creek epilithon in summer had a biomass of 1.9 g AFDM/m^2, about twice that of UBC epilithon in winter, suggesting a greater algal component.

In tracing nitrogen transfer through stream food webs, it cannot be assumed that all of the material ingested by a consumer is assimilated, and in most cases, this is probably not true (MIHUC & MINSHALL 1995). Invertebrates can assimilate N from both the detritus and its associated microbes (FINDLAY & TENORE 1982) but what fraction of N is derived from which source remains unknown. Our ^{15}N tracer study has demonstrated that in shaded streams, the bulk epilithon is not of uniform quality and the food resource for grazing macroinvertebrates is not the bulk material, but only a portion of it. Lower light availability resulted in a larger non-living component in the epilithon resulting from heterotrophic processes, and thus detrital N diluted the ^{15}N label in the bulk epilithon. In contrast, in a higher-light, grazed stream, we saw that the invertebrate grazer was uniformly assimilating the high-quality bulk epilithon composed primarily of algae.

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