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RESEARCH ARTICLE

Moderate nutrient enrichment affects algal and detritus pathways differently in a temperate rainforest stream

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Abstract We manipulated nutrient concentrations in 14 channels adjacent to a forested headwater stream to examine the influence of nutrient enrichment on detrital and algal pathways. Our fertilization experiment increased the average water concentration of N up to a maximum of ~2 times and of P up to ~7 times relative to control channels, levels considered as moderate enrichment. We measured algal biomass and leaf mass loss as a proxy measure of primary production and leaf decomposition, respectively. We determined the effects of nutrients on the quantity and quality of food resources and tested whether these effects influenced biotic structure and stoichiometry. Our results indicate that algal pathways showed significant and consistent responses across treatments by increasing epilithon quantity and quality. Moreover, despite an increase in quality of leaves, its quantity and loss rate were unaltered. Importantly, changes to detritivore densities were subtle, but they showed a hump-shaped response along the induced nutrient gradient. This trend suggests the existence of nutrient limitation at low nutrient concentrations and the existence of negative biotic interactions and/or sublethal toxic effects at higher concentrations, while enhancing detritivore densities at intermediated enriched conditions (threshold at ~10 µg/l of $P–PO_4$ in water and 0.10% of leaf-P). This study reveals the complexity of connections between algal and detritus pathways with implications in the study of transfer of matter and energy in oligotrophic, forested headwater streams.

Keywords Detritus pathway · Ecosystem functioning · Epilithic biofilm · Fertilizer addition · Invertebrate dynamics

Introduction

Food webs of small forested streams are principally heterotrophic because they are based primarily on cross-boundary flows of terrestrial plant litter. In many of these streams, autochthonous primary production is strongly limited by light and nutrients (Wallace et al. 1997; Hill et al. 2009). However, some studies in forested streams have demonstrated the importance of autochthonous resources for macroinvertebrates (Mulholland et al. 2000; Delong and Thorp 2006; March and Pringle 2003), highlighting the importance of both detrital and algal pathways for food webs in these systems. Algal production and decomposition are two complementary ecosystem processes that promote organic matter turnover, nutrient cycling, and the provisioning of many ecosystem services (Hooper et al. 2012). At relatively low concentrations, nutrient enrichment often stimulates microbial conditioning, algal growth, and organic C

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mineralization (Rosemond et al. 2015). At much higher concentrations, nutrients may negatively influence these processes as conditions shift to non-optimal or even toxic (Odum et al. 1979; Woodward et al. 2012).

Most knowledge about the influence of nutrient enrichment in streams is based on empirical (short or long-term enrichments) and theoretical studies of specific detritus—(Woodward et al. 2012; Ferreira et al. 2015; Rosemond et al. 2015) or algal-based pathways (Elser et al. 2007; Francoeur 2001). From these studies, we know that the response of detrital pathways to nutrients can differ from those based on living plants, because algal productivity tends to increase with increasing concentrations of limiting nutrients while leaf decomposition depends on microbial decomposers and detritivores (Rosemond et al. 2015). A general pattern of nutrient pollution observed for streams at the continental-scale is a hump-shaped relationship between leaf breakdown rates and invertebrate densities (Woodward et al. 2012), which suggests strong nutrient limitation in unpolluted systems, potential for strong stimulation in moderately altered systems, and inhibition in highly polluted streams.

In addition to such responses, nutrient enrichment or higher nutrient availability can also influence body size of primary consumers by increasing growth and body mass during a period of time. Body size variation results from a combination of top–down and bottom–up effects, which simultaneously increase the quantity and/or the quality of food resources (Finlay et al. 2007; Greenwood et al. 2007; Davis et al. 2010). The supply of nutrients, among other substances, determines the growth and performance of consumers (Sterner and Elser 2002), but responses are specific and may vary depending on functional feeding group or ecosystem (Elser et al. 2000). Other factors such as the identity of nutrients and resources, background nutrient availability, and the magnitude of the enrichment may also influence these responses. Therefore, the mixture of inter-dependent relationships among the abiotic environment, resources and consumers that occur in stream ecosystems reflects their complexity and calls for integrative approaches (see Danger et al. 2013; Dunck et al. 2015).

Differentiating algal vs. detrital responses to nutrient addition is critical for understanding how nutrients may influence or control stream ecosystem structure and function. The present study used an integrative approach to assess effects of nutrient enrichment in algal and detritus pathways of temperate, rainforest stream in south-western British Columbia by examining resources and consumers in tandem. Traditional measurements of biomass and densities were combined with quantification of stoichiometric patterns to examine these effects. We measured algal biomass and leaf mass loss as a proxy measure of primary production and leaf decomposition, respectively. We also tested whether nutrient enrichment affected dominant consumers by increasing their densities and/or influencing their elemental content.

We specifically examined (1) the effect of nutrient addition on algal and leaf litter resources, and (2) whether responses of food resources to increased nutrient supply propagate upwards in food webs via effects on consumers. We predicted that nutrient addition would stimulate growth of autotrophs and heterotrophs and thus, colonization of scraper and shredder invertebrates from low to moderate levels of enrichment, and we anticipated an asymptote in responses as space, nutrients and other resources become co-limiting at higher nutrient concentrations.

Materials and methods

Study site and experimental channels

The experiment was carried out in 14 experimental channels (each 14 m long, 25 cm wide, and 0.03 in gradient) constructed adjacent to Mayfly Creek in the Malcolm Knapp Research Forest (MKRF) of the University of British Columbia, near Maple Ridge, British Columbia, Canada (49°16′N, 122°34′W). Mayfly Creek is an oligotrophic second-order stream that flows through a steep and narrow valley dominated by western hemlock (*Tsuga heterophylla* (Raf.) Sarg.), Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco), and western redcedar (*Thuja plicata* Donn), with deciduous trees along the riparian area. The stream is generally shaded by riparian vegetation so that stream temperatures vary at most 3 °C daily. Forest harvesting activities have been ongoing in the MKRF since the 1920s, and the riparian zones of recently logged streams were dominated by stands of red alder (*Alnus rubra* Bong). Streams within the MKRF are primarily light limited, but once light levels are high enough to saturate photosynthesis, phosphorus potentially limits periphyton production (Kiffney and Bull 2000). See Kiffney et al. (2004) for further details about the climate, water chemistry, geology, and the set-up of the experimental channels.

The experimental channels are closely situated (~0.3 m gap), parallel, and fed with stream water from the nearby Mayfly Creek and thus, they are appropriate to mimic the natural ecosystem, as previously demonstrated in several studies (e.g., Lecerf and Richardson 2011; Atwood et al. 2014). The forest and light conditions around the channels is closely similar to the main stream. The experimental set-up allows natural communities of bacteria, fungi, and invertebrates to freely migrate into and from the channels but prevents fish from entering (García et al. 2012). One month before the experiment, channels were flushed and substrates cleaned to allow colonization. Discharge

estimates in the channels ranged from 0.68 to 1.60 L s during the experiment (three measures per week and channel throughout the experiment), but without significant differences among channels (GLMM, $p > 0.05$). Stream water pH, specific conductivity (at 25 °C), temperature, and dissolved oxygen were measured in-situ using standard meters (YSI 63 and YSI 550A, YSI Environmental Incorporated, Ohio, USA) at the beginning and at the end of the experimental period.

Nutrient addition

Experimental channels were randomly designated as controls and treatments. The experiment consisted of seven treatment levels differing in the amount of fertilizer applied (0, 0.5, 1, 2, 3, 5 and 7 kg), each level replicated twice. These concentrations were chosen to produce a gradient of nutrient concentrations. On May 4, 2015 (day 0), nutrients in the form of slow-release fertilizer pellets were inserted into small 1-mm mesh bags, and placed in the upstream section of the channels for the 30-days experiment. Fertilizer (15-5-15 N-P-K) consisted of slow-release, polymer coated-nutrient pellets manufactured by Florikan ESA Corporation, that are frequently used in horticulture, agriculture, golf and turf, and professional landscape activities. Fertilizer contained 15.0% nitrogen as nitrate ammonium (7.2%) and ammoniacal nitrogen (7.8%), available phosphate (5.0%), soluble potash (15.0%), and magnesium (1.2%). The company determines a longevity of the fertilizer pellets of ~150 days (at temperatures <15.6 °C). Analysis of fertilizer by central laboratory facilities at the University of British Columbia (UBC Forestry Stable Isotope Facility) showed that pellets contained 18.0% nitrogen and 4.3% phosphorus at the beginning of the experiment while 12.0% nitrogen and 3.2% phosphorus at the end of the experiment, confirming the slow-release rates stated by the company.

Water samples were collected at the beginning and at the end of the experimental period, filtered through pre-ashed glass fiber filters (Whatman GF/C 47-mm diameter, 1.2-µm pore size, VWR, Ontario, Canada), kept on ice for transport to the laboratory, and frozen until further analysis of dissolved nutrients. Following standard methods (APHA 2005), water samples were analysed for nitrates, nitrites, ammonium and phosphates using an automated continuous-flow colorimetric analyzer (OI-Analytical "Alpkem" Flow Solution IV, B.C. Ministry of Environment, Victoria, Canada).

Epilithic biofilm

To ensure a similar and natural colonization of biofilm in all experimental channels, nine unglazed ceramic tiles (7.5 × 7.5 cm) were placed in each channel 1 month prior to the beginning of the experiment. At 30 days after the fertilizer addition, tiles were randomly removed for quantification of algal biomass (n = 3 for AFDM, n = 3 for chlorophyll *a*) and elemental content (n = 3). Biofilm colonizing the tiles was brushed and filtered onto a glass fiber filter (Whatman GF/C 47-mm diameter, 1.2-µm pore size, VWR, Ontario, Canada). Samples for AFDM were oven-dried at 60 °C for 48 h, and weighed. Then, filters were placed in a muffle furnace at 500 °C for 1.5 h, kept in a desiccator until constant mass, and reweighed to determine AFDM. Samples for chlorophyll *a* (Chl *a*) were extracted in acetone (90%) for 24 h at 4 °C under dark conditions. Absorbance of the extract was measured using spectrophotometry (Cary 5000, Agilent Technologies, Ontario, Canada). Algal biomass was estimated based on Chl *a* concentration in each sample, corrected by substrate area brushed (µg cm^{-2}). Finally, samples for analysis of elemental content were oven-dried at 60 °C for 48 h, and dried filters were stored until further analysis (see "Elemental content" section).

Leaf decomposition

Alder leaves were collected after abscission in autumn 2014, and stored in the laboratory until the experiment. Eighty-four leaf bags (10-mm mesh size; 25 × 15 cm) were filled with 4 g (±0.001 g) of air-dried leaves and were randomly immersed in each channel (n = 6) at day 0. Six extra bags were prepared in a similar way and kept in the laboratory as controls to determine initial dry mass and organic matter content.

Leaf bags were randomly collected at 30 days, individually placed in plastic zip-lock bags, and transported to the laboratory in coolers. Once in the laboratory, leaves were washed under tap water through sieves to retain the invertebrates (see "Macroinvertebrates colonization" section). The leaf material was used for quantification of biomass (n = 3 for AFDM remaining) following the same procedure as above for epilithon. Remaining leaf dry mass was calculated as the ratio between final and initial dry mass (after correcting for humidity and handling) and expressed as a percentage. Leaf decomposition rates were calculated as the rate coefficient (k) by regressing the natural logarithm of remaining leaf AFDM (%) against time in the channels (days). The other three leaf bags from each channel were used for elemental content. These leaves were ground into a fine powder using a grinder and stored in Eppendorf vials until further analysis (see "Elemental content" section).

Macroinvertebrate colonization

Invertebrates inhabiting the experimental channels were collected using a Surber sampler (225 cm^2; 250-µm mesh

size) by hand scrubbing the substratum within the sampler (n = 6). Additionally, invertebrates colonizing leaf bags (n = 6) were rinsed from leaf surfaces under tap water through sieves (250-µm mesh size). Half of the collected samples (three Surbers + three leaf bags) were stored frozen for analyses of elemental content, but only two common shredders: *Brillia retifinis* (Saether 1969) and *Zapada cinctipes* (Banks 1897) were abundant enough in all samples and treatments for elemental content analyses. The other half of the collected samples was stored in 70% ethanol before identification and counting. All macroinvertebrates were hand-picked using a dissecting microscope. Each organism was identified to the lowest possible taxonomic level, although some chironomids were identified to the subfamily level, and non-insects to the order level or higher (e.g. oligochaetes, nematodes, copepods). All macroinvertebrates were classified into functional feeding groups (FFGs) following Merritt et al. (2008). Because of the large number of individuals in some samples, subsampling was used to obtain a representative fraction of the total community when needed (Wrona et al. 1982).

In addition, the size of *B. retifinis* larvae inhabiting both leaf bags and stream channel was estimated. A maximum of 100 individuals per channel were photographed using an Olympus DP10 digital colour camera attached to an Olympus SZX9 binocular microscope (at 40×). Photos were used to determine total body length and area (Olympus Micro-Image Software, v. 4.0 for Windows, Media Cybernetics, Silver Spring, MD, USA).

Elemental content

We measured carbon (C) and nitrogen (N) content of epilithic biofilm, leaves and dominant shredders. All samples were analyzed by high-temperature flash combustion with a Vario EL Cube elemental analyzer (Elementar, Germany) at the University of British Columbia (UBC Forestry Stable Isotope Facility). Preserved epilithon filters were divided into subsamples of approximately 6 mg, and weighed into 9 × 10-mm tin capsules. Approximately 10 mg of leaf powder, and 1 mg of larvae, were weighed into 8 × 5-mm tin capsules, respectively. A certified organic standard (orchard leaves, Elemental Microanalysis, United Kingdom) was analyzed with the samples. Repeated analyses of the standard yielded a 1-sigma relative standard deviation of 0.5% for C and 2.5% for N.

For P analysis, all samples (i.e. epilithic biofilm, leaves, larvae) were placed into acid-washed borosilicate tubes, and ashed at 500 °C for 2 h. P content was quantified using persulfate digestion and the ascorbic acid method (APHA 2005). P samples were processed using a spectrophotometer (Shimadzu UV-1800). Bovine muscle was used as an external standard for P analysis (average recovery 97%). All elemental content data were presented either as percentages and/or as molar ratios.

Statistical analysis

Linear mixed-effects models (GLMM) were fitted with the statistical software R (Development Core Team 2014, version 3.1.0) using the *lmer* function in the *lme4* package to initially test for differences in water chemistry among channels during the experimental period that could influence the ecosystem responses. Channel was used as a main factor, the amount of nutrients placed at each channel (i.e. controls and treatments) was used as a random nested factor, and sampling time (i.e. repeated measures) was used as a random factor. Normality was tested on model residuals with the Shapiro test and data were normalized by transformation with the Box–cox function when necessary. Regression analyses were used to analyze the relationships between nutrient concentration and algal biomass, leaf mass loss, invertebrate colonization and elemental content (as proxies for different stream ecosystem responses). The overall effect was assessed in terms of P (average concentrations during the experiment), because it is the most limiting nutrient in the study area. We compared linear and second-order polynomial regressions with the independent variable ($P–PO_4$), as different studies have shown non-linear relationships with nutrient enrichment (Niyogi et al. 2007; Woodward et al. 2012). Finally, an ANOVA was fitted to test for differences in the size of *Brillia* individuals among habitats (Surbers vs. leaf bags), and regression analyses were used to analyze the relationships between the size of *B. retifinis* larvae inhabiting the experimental channels, both in Surber samples and leaf bags, and the dissolved nutrient concentrations in water. Homoscedasticity and normality of variable distributions were examined prior to analysis, and variables were transformed as necessary to meet assumptions of parametric tests.

Results

Effects of nutrient enrichment on water chemistry

During the experimental period, water temperature increased from 6.6 to 11.1 °C (~4.5 °C in 30 days), but these changes were similar among channels (GLMM, $p > 0.05$). Other parameters such as pH, electric conductivity and dissolved oxygen varied during the experimental period, but were also similar among channels (GLMM, $p > 0.05$).

Nutrient concentration, primarily phosphate-P, increased across the channels following the nutrient enrichment (Fig. 1). Average DIN and phosphate-P concentrations in treated channels were elevated up to ~2 times and ~7

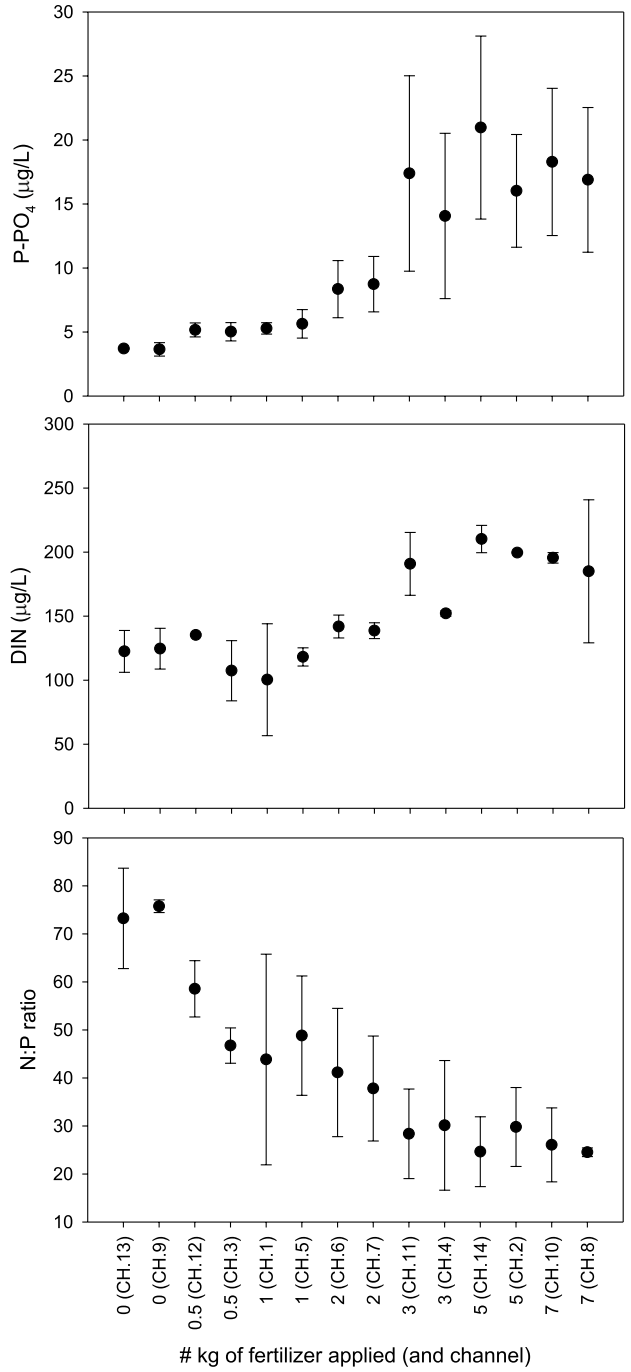

Fig. 1 Average measurements of nutrient concentrations ($P–PO_4$ in µg/L; DIN in µg/L and N:P ratio) between 2 and 30 days across the experimental channels. The x-axis shows the #kg of fertilizer applied and the channel between brackets

times higher than control channels, respectively (Fig. 1). Although the fertilization represented a moderate nutrient increase, N:P ratios decreased approximately three times (from 74.5 down to 25.3, Fig. 1). Nutrient concentrations in the control channels were extremely low during the experiment period (mean ± SD of 3.7 ± 0.2 µg $P–PO_4$/L, 123.5 ± 9.3 µg DIN/L, and 74.5 ± 4.4 N:P ratio), and suggested likely P limitation in these oligotrophic streams.

Effects of nutrient enrichment on epilithon and leaves

Nutrient enrichment resulted in higher epilithic biomass (primarily influencing Chl *a*) and P content (Fig. 2). Chlorophyll *a* was low in the control channels (0.78 ± 0.01 µg/cm^2) and increased to a maximum of ten times in channels with the highest nutrient addition. AFDM in the control channels was also low (0.26 ± 0.12 mg/cm^2), and increased to a maximum of seven times the control channels. Linear regressions showed a significant positive relationship between epilithic biomass and dissolved P concentration, with 54% of the variation explained by the model for Chl *a*, and little variation explained for AFDM (Table 1; Fig. 2). Dissolved P concentration also explained 38% of the variation observed in epilithon-P (Table 1). Epilithon-P increased from an average of 0.08–0.37% along the enrichment gradient (Fig. 2). Epilithon-C and N also varied (C content from 2.92 to 10.53%; N content from 0.31 to 1.16%), but variation was not related to nutrient enrichment (Table 1; Fig. 2). Regarding molar ratios, epilithon-C:N decreased from an average of 13.3–8.8 along the enrichment gradient, while C:P and N:P ratios of epilithon were not related to nutrient enrichment (Table 1; Fig. 2).

Nutrient enrichment had no significant effect on alder leaf mass loss (neither on loss rates), as we observed leaves lost ~50% of their initial mass in all channels, but led to elevated nutrient content of litter (Table 1; Fig. 2). Leaf litter displayed substantial variation in their elemental content as a response to the nutrient enrichment (Table 1). N content increased only slightly with nutrient enrichment (between 2.3 and 3.1%), while P content more than doubled (between 0.07 and 0.15%). The strongest relationship was found between leaf-P and water-P (adjusted $R^2 = 0.88$, Fig. 2). All leaf-molar ratios decreased significantly along the enrichment gradient (Table 1; Fig. 2).

Effects of nutrient enrichment on invertebrates

All experimental channels supported a similar invertebrate community, consisting of mostly Orthocladiinae midges, nemourid stoneflies, oligochaetes and mayflies in both leaf bags and Surber samples, though with different relative abundance between samples collected in leaf bags and Surbers (Table 2). Shredders were the primary feeding group represented in these streams; the nemourid *Z. cinctipes* numerically dominated Surber samples, while the chironomid *B. retifinis* was most abundant in leaf bags (Table 2).

Total densities of benthic macroinvertebrates, of EPT (*Ephemeroptera, Plecoptera* and *Trichoptera*) taxa and

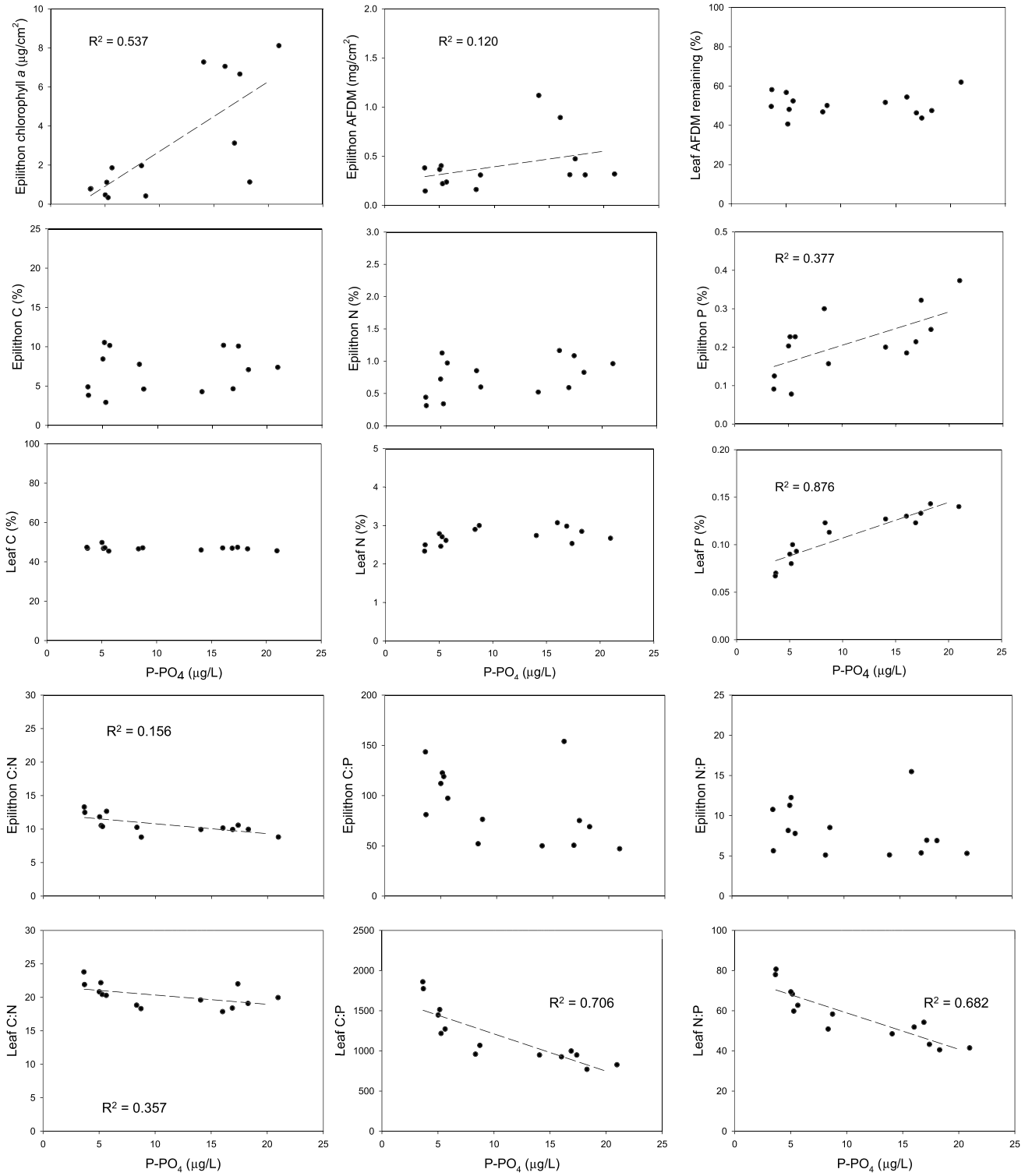

Fig. 2 Epilithon and leaf litter biomass and stoichiometry in relation to dissolved P–PO_4 concentration. *Regression lines* and their respective R-square are shown in the *plots* when significant relationships were found

of dominant shredders inhabiting the leaf bags were significantly related, in a non-linear manner, to the nutrient enrichment (Table 3; Fig. 3a). Densities were low at the extremes, but highest at intermediate levels (~10 µg/l of P–PO_4 and 0.10% leaf-P) of nutrient enrichment, suggesting the highest potential colonization. The total density of

Table 1 Linear relationships between response variables measured from algal- and detritus-pathways vs. $P–PO_4$ concentration in water

Component	Response variable	$F_{1,12}$	R^2	Significance
Periphyton	AFDM (mg/cm^2)	2.83	0.12	n.s
	Chl *a* (µg/cm^2)	16.06	0.54	**
	C (%)	0.34	−0.05	n.s
	N (%)	2.56	0.11	n.s
	P (%)	8.84	0.38	**
	C:N ratio	8.60	0.16	**
	C:P ratio	1.76	0.02	n.s
	N:P ratio	0.44	−0.01	n.s
Leaf litter	AFDM remaining (%)	0.06	−0.08	n.s
	k (d^{-1})	0.16	−0.07	n.s
	C (%)	1.40	0.03	n.s
	N (%)	2.66	0.11	n.s
	P (%)	100.40	0.88	**
	C:N ratio	8.48	0.15	**
	C:P ratio	62.04	0.60	**
	N:P ratio	75.20	0.64	**

Significance *p < 0.05, **p < 0.001, *n.s.* non-significant

Table 2 Relative abundance of invertebrate taxa inhabiting the experimental channels

Taxa name	Functional feeding group	Relative abundance in leaf bags	Relative abundance in Surber samples
Brillia retifinis	SH	52.8	14.0
Orthocladiinae spp.[a]	CG	15.8	5.4
Zapada cinctipes	SH	8.7	46.6
Corynoneura spp.	CG	7.2	4.6
Orthocladiinae spp. (pupae and adults)		3.9	2.1
Oligochaeta Gen. sp.	CG	3.4	10.4
Tanytarsini spp.	CG	2.8	3.8
Thienemanniella sp.	CG	1.5	1.0
Tanypodinae spp.	P	1.3	2.9
Baetis spp.	SC/CG	0.0	1.7
Leuctridae spp.[a]	CG	0.0	1.6
Paraleptophlebia spp.	CG	0.0	1.5
Malenka spp.	SH	0.0	1.1

Only those taxa with relative abundance >1% are shown. Functional feeding groups for each taxon are also shown (*SH* shredders, *CG* collector-gatherers, *SC* scrapers, *P* predators)

[a]Early instar individuals

invertebrates and EPT taxa on leaf bags were significantly correlated with the densities of shredders *B. retifinis* and *Z. cinctipes* (Pearson $r = 0.74$ and 0.98, respectively, p value <0.001, n = 14), evidencing that these two taxa represented the greatest relative abundance of total and EPT densities. Therefore, densities of these dominant shredders showed similar hump-shape responses (Fig. 3a). However, the density of invertebrates (and of EPT taxa and dominant shredders) inhabiting the Surbers was not linearly related to level of nutrient enrichment (Table 3; Fig. 3b). Neither the increase in epilithon biomass or P content influenced the density of *Baetis* larvae, the most abundant scraper/collector-gatherer species ($F_{1,12} = 1.21$, $p = 0.29$ and $F_{1,12} = 0.35$, $p = 0.56$ for epilithon Chl *a* and quality, respectively). None of the response variables observed for both leaves and Surbers was influenced by DIN concentration or N:P ratio (Online resource 1).

Elemental content of the dominant shredders, *B. retifinis* and *Z. cinctipes*, was not related to the elemental contents of leaves, except for a significant relationship between the N content of *B. retifinis* larvae and that of the leaves ($F_{1,12} = 6.15$, $R^2 = 0.28$, $p < 0.05$). Larvae of *B. retifinis* colonizing leaf bags were always larger than those collected in Surbers (mean length ± SE: 3.04 ± 0.07 and 3.68 ± 0.18 in Surbers and leaf bags, respectively). Interestingly, the size of *B. retifinis* larvae collected in Surber samples was related to nutrient enrichment (Fig. 4). There was no relationship between body size and nutrient concentration in those larvae collected from leaf bags.

Discussion

The fertilization supplied in the present study represented a moderate nutrient increase, primarily of P, which is the most limiting nutrient in these streams. Our data confirmed that Mayfly Creek is P-limited under natural conditions and, even after the enrichment applied, as at the greatest fertilizer addition the N:P ratio was 25:1 (close to the classical Redfield ratio ~16:1). Our moderate enrichment caused a linear increase of basal resources quality and a hump-shape response in the density of invertebrate shredders. This unexpected and interesting response of invertebrate consumers may respond to a combination of bottom–up and top–down forces as discussed below.

Nutrient enrichment resulted in significant but varied responses in the quantity and quality of epilithon and leaves, mostly related to increases in P concentrations, the most limiting nutrient in these streams. Most likely due to released nutrient limitation in these oligotrophic streams as initially predicted, as growth and nutrient content of primary producers tend to respond to and reflect the nutrient ratios of their environment (Malzahn et al. 2010). The

Table 3 Relationships between invertebrate densities (i.e., total invertebrates, EPT taxa and dominant shredders *B. retifinis* and *Z. cinctipes*) inhabiting experimental channels vs. P concentration in water ($P–PO_4$), leaves (Leaf-P, for those invertebrates collected from the leaf bags) or epilithon (Epilithon-P, for those collected from the Surber samples)

Habitat	Independent variable	Response variable	$F_{2,11}$	Adjusted-R^2	Significance
Surbers	$P–PO_4$ (µg/L)	Total invertebrates (#/cm^2)	0.52	−0.08	n.s
		EPT taxa (#/cm^2)	0.86	−0.02	n.s
		Brillia retifinis (#/cm^2)	1.61	0.09	n.s
		Zapada cinctipes (#/cm^2)	0.56	−0.07	n.s
	Epilithon-P (%)	Total invertebrates (#/cm^2)	0.55	−0.08	n.s
		EPT taxa (#/cm^2)	0.55	−0.07	n.s
		Brillia retifinis (#/cm^2)	0.81	−0.03	n.s
		Zapada cinctipes (#/cm^2)	0.52	−0.08	n.s
Leaf bags	$P–PO_4$ (µg/L)	Total invertebrates (#/g leaf)	4.02	0.32	*
		EPT taxa (#/ g leaf)	4.71	0.36	*
		Brillia retifinis (#/g leaf)	2.43	0.18	n.s
		Zapada cinctipes (#/g leaf)	4.31	0.34	*
	Leaf-P (%)	Total invertebrates (#/g leaf)	12.14	0.63	**
		EPT taxa (#/g leaf)	9.97	0.58	**
		Brillia retifinis (#/g leaf)	4.03	0.32	0.05
		Zapada cinctipes (#/g leaf)	7.22	0.49	**

Significance *$p < 0.05$, **$p < 0.001$, *n.s.* non-significant

effects of nutrient enrichment on leaf litter were somewhat less pronounced, with significant increases in litter quality but not in mass loss, contrary to our predictions. Increases in leaf quality may respond to an increase of nutrient availability for the microbial community colonizing leaves, while we argue that the lack of response in leaf mass loss was primarily due to the nutrient-rich leaf type used in the experiment. Alder leaves represent an accessible, labile, and a high quality substrate for microbial decomposers and detritivore invertebrates in comparison to other species (Lecerf et al. 2007; Hoover et al. 2011). Other studies found that the magnitude of response to nutrient enrichment is driven in part by C:N ratios (Stelzer et al. 2003; Gulis et al. 2004), demonstrating that leaf decomposition of nutrient-rich plant species is less responsive to nutrient enrichment than that of nutrient-poor species (Greenwood et al. 2007; Ferreira et al. 2015). Secondly, the limited response of the leaf mass loss may be also attributed to the moderate nutrient gradient supplied here in comparison with other studies that showed hump-shaped responses at much higher enrichment levels (~100–1000 µg/L SRP; Woodward et al. 2012; Rosemond et al. 2015). Thirdly, colder water temperatures during the period of the study may have also influence the limited response on leaf mass loss. Previous studies performed in these channels during summer time and without nutrient enrichment (temperature range ~10–14 °C) showed more than 50% of leaf mass loss in less than 30 days (García et al. 2012). Leaves and algae are often viewed as distinct resources that support separate but parallel food webs, but they are actually co-dependent suggesting priming effects that go in both directions: heterotrophic decomposers provide inorganic nutrients to producers (Danger et al. 2013), while producers provide organic nutrients to decomposers (Naeem et al. 2000).

It has been previously shown that effects of nutrient limitation can travel up the food chain via effects on invertebrate colonization and the nutritional condition and growth of consumers (Rosemond et al. 2001; Cross et al. 2003, 2006; Boersma et al. 2009). Importantly, our nutrient fertilization altered the detrital pathways by influencing the colonization dynamics of invertebrates, even under conditions of moderate enrichment without changes in leaf litter quantity. Benthic invertebrates colonizing the leaf bags, primarily represented by the shredders *B. retifinis* and *Z. cinctipes*, showed a hump-shaped response to the nutrient enrichment. The highest densities observed at intermediate levels of nutrient enrichment demonstrated the positive response of consumers, indicating that their limitation by nutrients under natural low nutrient concentrations. Meanwhile at the highest nutrient concentrations attained, we considered that other factors beyond nutrients, such as density-dependent intra- or interspecific interactions (competition and predation), might negatively influence consumer densities. The shredders *B. retifinis* and *Z. cinctipes*, known to rely primarily on allochthonous detritus for energy, though *Brillia* can also be considered as a collector taxa (Merritt et al. 2008), were the dominant feeding group inhabiting the experimental channels, reflecting the detritus-based nature of the source stream. In particular, the chironomid *B. retifinis* (Diptera) was the dominant taxon inhabiting the leaf bags, comprising 53% of total invertebrate abundance, as shown in other studies (García et al. 2012, 2014). Chironomids have some of the fastest growth rates and shortest life histories among aquatic insect larvae (Wallace and

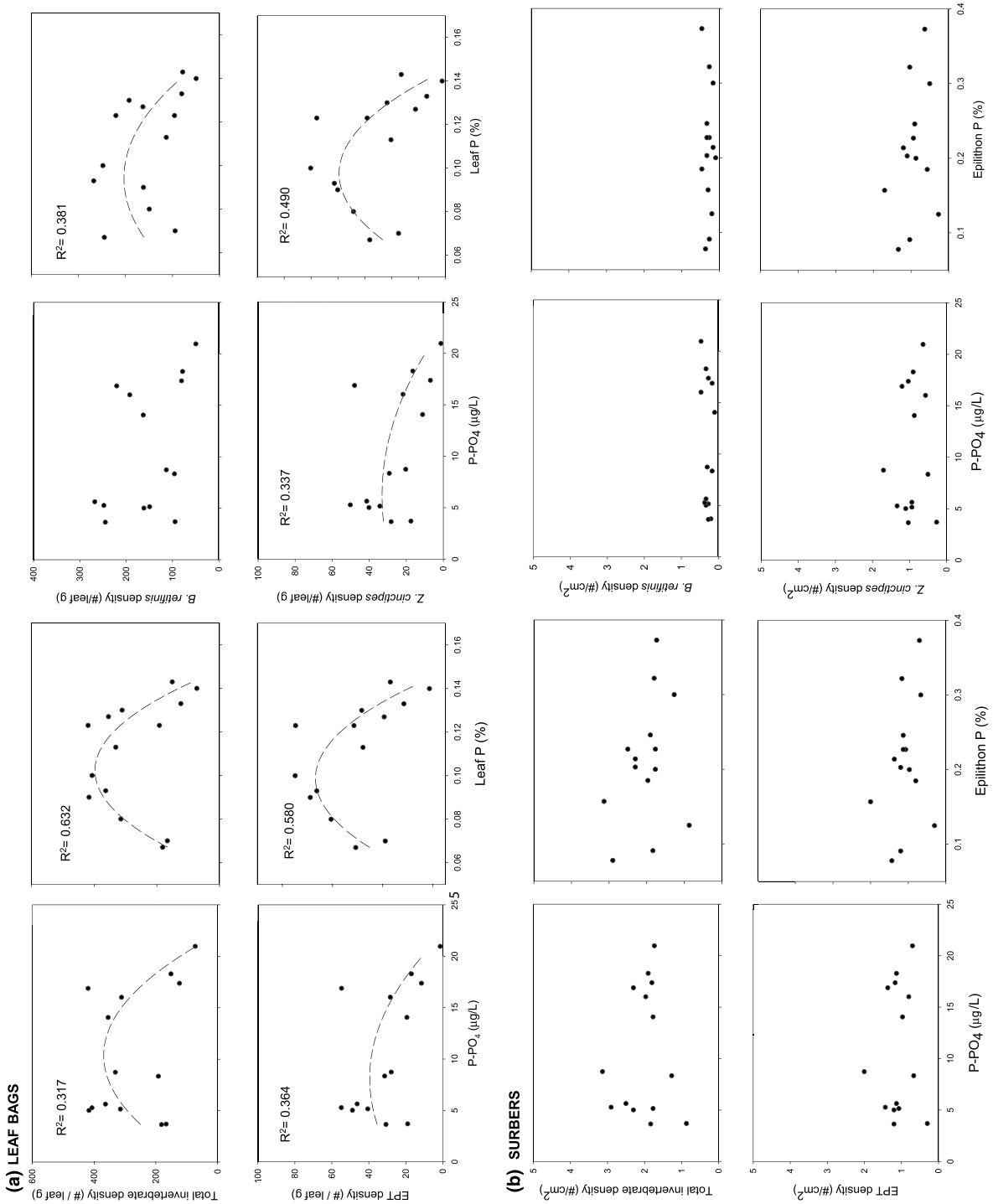

Fig. 3 Invertebrate densities colonizing the leaf bags (**a**) and the Surber samples (**b**) in relation to dissolved P–PO_4 concentration, P content of leaves (Leaf-P) and/or P content of epilithon (Epilithon-P) during the experiment. *Regression lines* and their respective R-square are shown in the *plots* when significant relationships were found

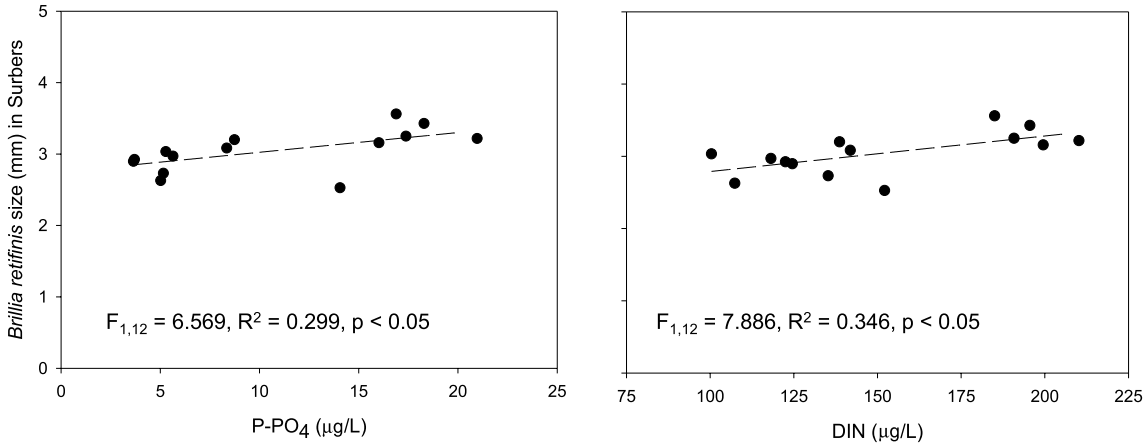

Fig. 4 Relationships between the size of *B. retifinis* larvae inhabiting the Surber samples and the dissolved nutrient concentrations in water. *Regression lines* and their respective adjusted-R^2 are shown in the *plots* when significant relationships were found

Anderson 1996; Richardson 2001), and their ability as "early colonizing" species allows for a faster colonization of the experimental channels in comparison with other taxa (García et al. 2012). Regarding the algal consumers, our results showed that scrapers such as *Baetis* spp. were not influenced by algal quantity or quality, likely because our levels of enrichment were moderate. In addition, this species has longer life cycles than *B. retifinis* and showed relatively low densities in the present study, potentially limiting our ability to detect responses.

Our fertilizer addition did not result in changes to the invertebrate assemblage composition or the elemental content of dominant shredders, except for a weak relationship between the N content of *B. retifinis* larvae and that of leaves. This lack of response may have similar explanations as above (i.e., the short period of the experiment and/or to the moderate nutrient enrichment), but it may also be due to the limited taxonomic pool of invertebrates, their life cycles and/or specific nutrient requirements (Sterner and Elser 2002; Frost et al. 2005). Whether organisms or communities respond to changing conditions is strongly regulated by their species-specific capacity for nutrient storage, relative growth rate, physiological plasticity, and the degree of environmental resource availability relative to organismal demand (Sistla et al. 2015). Although considerable flexibility has been demonstrated in some studies (Cross et al. 2003; Small and Pringle 2010), consumers typically maintain their elemental content regardless of food quality (Sterner and Elser 2002) and our results suggest this response for shredders along the fertilization gradient.

Interestingly, the size of *B. retifinis* larvae collected from the Surber samples were larger after nutrient enrichment, whereas those larvae colonizing the leaf bags did not vary in size. These results may indicate that larvae colonizing the stream channels are indirectly benefited, likely by feeding on either leaves and fine particulate organic matter resulting from leaf decomposition, while the size of those individuals feeding directly on leaves was not directly related to the nutrient enrichment, likely because the already rich-quality of the food resource they are feeding up on. In a previous study performed in the same experimental channels during summer (García et al. 2012), maximum densities of *B. retifinis* on alder leaf bags (~1200 individuals per g leaf) were reached before >50% of their initial leaf mass was lost (maximum values achieved after 28 days of incubation and at 344 degree-days). In our study, maximum densities observed (~300 individuals per leaf gram) were considerably lower, likely due to colder temperatures (maximum values achieved after 30 days of incubation and at 239 degree-days). Thus, both food type (both quantity and quality) and temperature are critical factors regulating the *B. retifinis* dynamics in temperate, forested streams as previously demonstrated for *B. bifida* (García and Pardo 2015).

In summary, our results suggest that epilithic and detrital pathways in highly oligotrophic, forested streams, may respond differently than eutrophic, unforested streams. Nutrient fertilization significantly affected algal pathways by increasing epilithon quantity and quality, though this resource enhancement did not result in higher scrapers densities. Moreover, nutrient enrichment increased leaf quality likely via microbial assimilation, and promoted a hump-shaped response of shredder densities along the induced nutrient gradient and also indirectly affected shredders by increasing their size. This study reveals the complexity of connections between algal and detritus pathways with implications in the study of transfer of matter and energy in streams.

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Compliance with ethical standards

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Conflict of interest The authors declare that they have no conflict of interest.

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