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FOOD WEB OF A TROPICAL HIGH MOUNTAIN STREAM: EFFECTS OF NUTRIENT ADDITION

Red trófica de un arroyo de montaña tropical: efectos de la adición de nutrientes

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ABSTRACT

In order to define the effect of nutrient enrichment on trophic webs in an Andean mountain stream we performed an experiment using stable isotope ratios ($\delta^{15}N$ and $\delta^{13}C$) to analyze different trophic compartments: 1) basal level: CPOM and biofilm; 2) primary consumers - macroinvertebrates: collector-gatherers (*Heterelmis* sp., *Thraulodes* sp. and *Trichorythodes* sp.), and collector-filterers (*Simulium* sp.); 3) predators - fish (*Oncorhynchus mykiss* and *Trichomycterus bogotensis*). The average fractionation of nitrogen among the primary consumers with respect to CPOM was 4.7 ‰, and 1.7 ‰ with respect to biofilm. Predators incremented their $\delta^{15}N$ signal by 5.9 % with respect to primary consumers. A depletion of $\delta^{15}N$ was observed in Impact with respect to Control reach after fertilization in different compartments (biofilm, *Heterelmis*, *Simulium* and *Tricorythodes*), while depletion was not significant for top predators. In most cases, the $\delta^{13}C$ signal of biofilm overlapped with that of primary consumers, but a clear enrichment was observed with respect to CPOM. Macroinvertebrate gut contents showed fine detritus to be their most abundant food, and that in general there were no changes in diet as a consequence of nutrient enrichment. The only exception was *Heterelmis*, who increased its consumption in the Impact reach.

Keywords: food webs, gut content, isotope ratios, nutrient enrichment, trophic compartments.

RESUMEN

Con el fin de determinar el efecto del incremento de nutrientes sobre la red trófica de un río de montaña andino, se realizó un experimento en donde se analizaron las proporciones de isotopos estables ($\delta^{15}N$ y $\delta^{13}C$) para analizar los siguientes compartimientos tróficos: 1) Nivel basal: CPMO y Biofilm; 2) Consumidores primarios - macroinvertebrados: colectores - recolectores (*Heterelmis* sp., *Thraulodes* sp., y*Trichorythodes* sp.) y colectores - filtradores (*Simulium* sp.); 3) Depredadores - peces (*Oncorhynchus mykiss* y *Trichomycterus bogotensis*). La fracción promedio de nitrógeno entre los consumidores primarios con respecto a CPOM fue de 4.7 ‰, y de 1.7 ‰ con respecto al biofilm. Los depredadores incrementaron en un 5.9 % la señal $\delta^{15}N$ con respecto a los consumidores primarios. Después de la fertilización, se observó en diferentes compartimientos (biofilm, *Heterelmis*, *Simulium* y *Tricorythodes*) del tramo impacto un agotamiento de $\delta^{15}N$ con respecto al control, mientras que el agotamiento no fue significativo para los depredadores superiores. En la mayoría de los casos la señal $\delta^{13}C$ del biofilm se sobrepuso con la de los consumidores primarios pero un claro enriquecimiento fue observado con respecto a CPOM. Los

macroinvertebrados referidos fueron seleccionados para analizar su contenido estomacal y los resultados nos mostraron que el detritus fino es el alimento más abundante para los invertebrados, y únicamente, *Heterelmis* mostró, después de la adición de nutrientes, diferencias significativas en el detritus fino y el material vegetal entre el tramo control y el impactado.

Palabras clave: compartimientos tróficos, contenido estomacal, enriquecimiento de nutrientes, proporción de isotopos, redes tróficas.

INTRODUCTION

Food webs are complex trophic connections among interacting organisms in ecosystems (Elser and Hessen, 2005), and their structure influences population dynamics, community structure and ecosystem function (Polis *et al.*, 1997). Knowledge of the food web in freshwater systems is essential to integrate the dynamics of organic matter with organism interactions.

The maximum food-chain length is an important food-web property that is correlated with resource availability, ecosystem size, environmental stability and colonization history (Doi, 2011). Some of these correlations may result from environmental effects on predator-prey mass ratios (Jennings, 2005). In streams, the structure of food webs is affected by numerous factors, such as biogeography, stream order, disturbance, temperature, resource type and anthropogenic activities (Hershey *et al.*, 2007). Light availability and nutrient levels are often regarded as the most important factors influencing primary production in streams. Moreover, nutrient excess is one of the most common disturbances affecting river ecosystems, through "bottom-up" effects to the whole community structure (Biggs and Smith, 2002).

Resources in streams are usually represented by detritus and primary producers that are always associated with fungi, bacteria and micro- and meio-fauna. Invertebrates can thus be both primary and secondary consumers. Predators are usually omnivorous with a mixed diet of prey, detritus and algae. These characteristics produce confusing results that make it difficult to clearly determinate the trophic position of animals.

Stable isotope analysis has proved to be a useful tool in reconstructing diets, elucidating patterns of resource allocation, characterizing trophic relationships and constructing food webs (Boecklen *et al.*, 2011), thus providing a measurement of trophic position that integrates the assimilation of energy or mass flow through all the different trophic pathways leading to an organism (Post, 2002). Carbon and nitrogen stable isotopes are frequently used to study energy sources and food web structure in ecosystems (Bergfur *et al.*, 2009), as well as to show which processes or components are more sensitive to perturbation (Peterson and Fry, 1987).

Nitrogen isotopes are useful for differentiating trophic levels and food-web dynamics (Bergfur *et al.*, 2009) because $\delta^{15}N$ isotopic fractioning increases with each trophic level (Finlay, 2001) and a consumer is typically enriched by 3-4 ‰ relative to its diet (Jardine *et al.*, 2012). In contrast, the ratio of carbon isotope ($\delta^{13}C$) changes little (0.3-0.5 ‰ on average) as carbon moves through food webs (Peterson and Fry, 1987), what makes it is an effective diet tracer (Finlay *et al.*, 2002). Analysis of $\delta^{13}C$ signature has an advantage over gut-content analysis because it measures the amount of carbon assimilated from each food source as opposed to that ingested (March and Pringle, 2003). However, as mentioned, due to high overlapping in the diets of stream organisms, gut contents provide basic and complementary information about food sources.

The aim of this study was to assess the effect of nutrient enrichment on trophic webs and identify the links between resources and consumers in an Andean mountain stream. We analyze the stable isotope ratios ($\delta^{15}N$ and $\delta^{13}C$) of different trophic compartments in two reaches, one of which was subjected to a nutrient enrichment experiment. We hypothesized that nutrient enrichment would increase nitrogen and phosphorous content of the basal compartments, mainly algae, thus improving their quality for consumers. This better quality would favor basal resource consumption for all trophic levels in the enriched reach, and thus, lead to lower $\delta^{15}N$ values.

MATERIALS AND METHODS

Experimental Design

The two study reaches were located in the Tota stream, within the area of the municipality of Cuítiva, department of Boyacá (Colombia).

In order to define the effect of nutrient enrichment on trophic webs, we chose two 50 m reaches that were geo-morphologically and hydrologically similar: Control (C) and Impact (I). Both reaches were studied for 12 months prior to the enrichment and ten months after. The C reach was located 700 m upstream from the I reach, where the nutrients were added. The continuous addition of nutrients was performed using a drop system over a 10-month period. Two commercial grain fertilizers (Nitron 26 (26-0-0) and Abocol (NPK) (10-30-10)) were diluted in a 500 L tank in order to at least double the average basal (natural) phosphate and ammonium concentrations in the stream. Nutrient addition was adjusted bi-weekly, and natural N:P proportions were maintained as well.

Hydrological, Physical and Chemical Variables

Measurements of hydrological, physical and chemical variables were taken bi-monthly. Current velocity and flow (Q) were taken with a digital flow-meter SCHILTKNECHT (MiniAir 20). Temperature and dissolved oxygen (were registered with a HACH LDO HQ30d oxygen sensor). Conductivity was measured with a YSI model 556 MPS multi-parametric probe. The pH was measured with a SCHOTT pH 11/SET sensor. The ammonium, nitrate, nitrite and phosphate were all measured spectrophotometrically by following the techniques described by APHA-AWWA-WEF (2005).

Biological Sampling

Two samplings were carried out in each stream reach (C, I), one before (B, April 2008) and one after (A, January 2009) the enrichment. Three replicates of each trophic compartment (Coarse Particulate Organic Matter (CPOM), biofilm, macroinvertebrates and fish) were taken on each occasion. Samples were collected and processed according to the indications of Muñoz *et al.* (2009) and Hershey *et al.* (2007). The samples were refrigerated between sampling and lab processing.

Coarse Particulate Organic Matter. We collected three fractions of accumulated leaves from the stream bed in the field using a surber net of 900 cm^2.

Biofilm. Ten ceramic tiles (1 cm^2) glued onto three rock slab that were located at both reaches and kept at a depth of 10 to 20 cm in riffle zones for biofilm colonization (60 days). This colonization time ensured a complete development of biofilm similar to natural substrates (Donato-Rondón *et al.*, 2010). In the lab, we cleaned the ten tiles with a toothbrush and added 10 ml of Milli-Q water. Each sample was sonicated in an ultrasonic bath (ELMAULTRASONIC Elma E 15) for five minutes.

Macroinvertebrates. Samples were taken in rock substrata using a Surber sampler with a 900 cm^2 surface area and 500 µm net mesh size. In the lab we then sorted the animals and left them in filtered river water under temperature-regulated conditions for 12 hours to clean their stomach content. For isotope analysis, we selected the most abundant species of insecta: *Heterelmis* sp. (adult stage), *Simulium* sp., *Tricorythodes* sp. and *Thraulodes* sp.

Fish. Were sampled using an electric fishing device (ELT 60II GI) Individuals from the only two species found in the river (*Oncorhynchus mykiss* and *Trichomycterus bogotensis*) were collected, from which we obtained a subsample of 1 g of muscle tissue.

Sample Analyses

The extract of samples for analysis of $\delta^{13}C$ and $\delta^{15}N$ were dried at 60 °C for three days and were then crushed with a mortar to obtain a homogeneous sample of 0.1 mm particle size. For liquid extract samples of biofilm, we added 1 ml of the extract concentrate onto the pre-weighed tin capsule, and then dried and reweighed it. All samples were subsequently packed into tin capsules and stored in dry conditions. Samples were analyzed in a Thermo Elemental Analyzer 1108 associated to a mass spectrometer. Standards specified by the International Atomic Energy Agency (IAEA)were used to calibrate the isotopic signal: sucrose, polyethylene and graphite for carbon; ammonium sulphate and potassium nitrate for nitrogen. The standard test was run repeatedly to ensure linearity. The results were compared with the isotopic composition of atmospheric nitrogen for nitrogen, and PeeDee belemnite carbonate rock (PDB) for carbon as reference.

We analyzed gut contents of invertebrates, not of fish. After organism collection, the animals were deposited in plastic bags before being frozen. Five individuals of each species were analyzed. The individuals were placed in vials containing rose Bengal for 24 hours. Afterwards, the digestive tract of each individual was extracted and the anterior part removed under a stereomicroscope. This material was then placed on a slide to be studied under a microscope (400x). Ten visual fields were selected at random and photographed. The photos were quantified using the Coral Point (CpCe 3.4) program. Five categories of food sources were identified: coarse detritus (CD), fine detritus (FD), diatom algae (DA), filamentous algae (FA) and plant matter (PM).

Isotopic signals were represented in a bi-plot figure using the SIGMAPLOT 10 program. Differences in isotopic signals and in category percentages in gut content were tested with Student's t-test to find differences between reaches and time separately.

RESULTS

In general terms, physical and hydrological variables presented similar conditions in the different reaches (C, I) and enrichment time (B, A) (Table 1). However, during nutrient addition, maximum discharge values were lower and the

Table 1. Maximum (Max) and Minimum (Min) values of the physical, chemical and hydrological variables for the Control (C) and Impact (I) reaches Before (B) and After (A) nutrient enrichment in Tota stream.

	B				A			
	C		I		C		I	
Parameter	Max	Min	Max	Min	Max	Min	Max	Min
Discharge (m^3s^{-1})	1.77	0.04	1.47	0.04	0.8	0.15	1.34	0.12
Temperature (T°)	15.9	12-4	17.51	11.9	14.57	13.03	15.47	13.19
Dissolved Oxygen (mg l^{-1} O_2)	8.01	7.08	8.11	6.84	7.89	7.54	7.87	7.22
Conductivity (µm cm^{-1})	1.75	39.67	1.97	4.2	133.67	52.67	152.67	51.67
pH	7.7	6.96	8.17	6.9	7.86	6.47	7.56	6.9
Ammonium (µg l^{-1} NH_4^+)	35.2	6.38	51.88	8.34	23.6	4.8	327.96	23.14
Phosphate (µg l^{-1} PO_4^{3-})	56.58	11.59	47.79	7.21	69.9	38.5	159.3	66.93
Nitrite (µg $l^{-1}NO_2^-$)	9.5	0.92	8.7	0.46	19.5	5.91	17.7	5.59
Nitrate (µg $l^{-1}NO_3^-$)	130.68	5.79	123.22	4.27	287.6	3.72	233.2	28

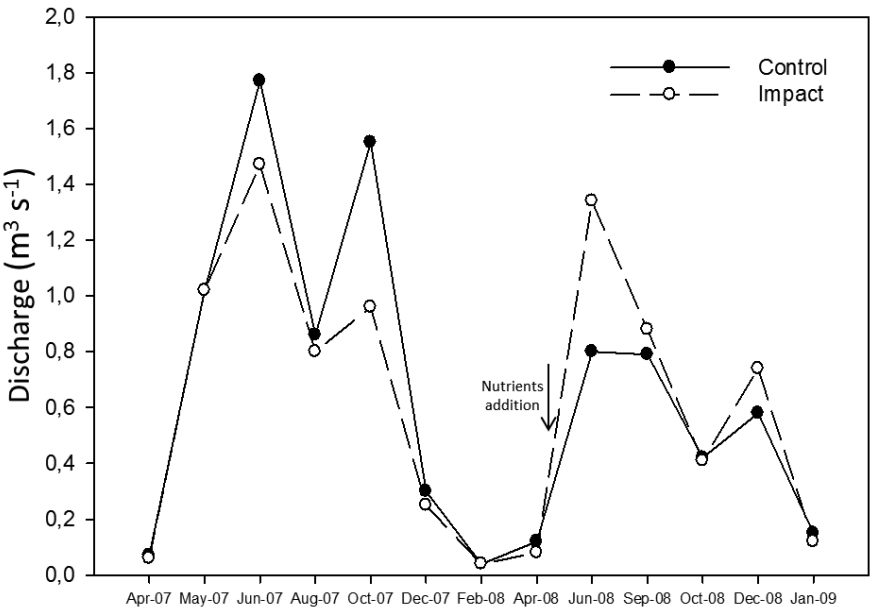

Figure 1. Discharge values of Tota stream for the Control (C) and Impact (I) reaches Before (B) and After (A) nutrient enrichment. Arrow indicates the time of nutrient addition.

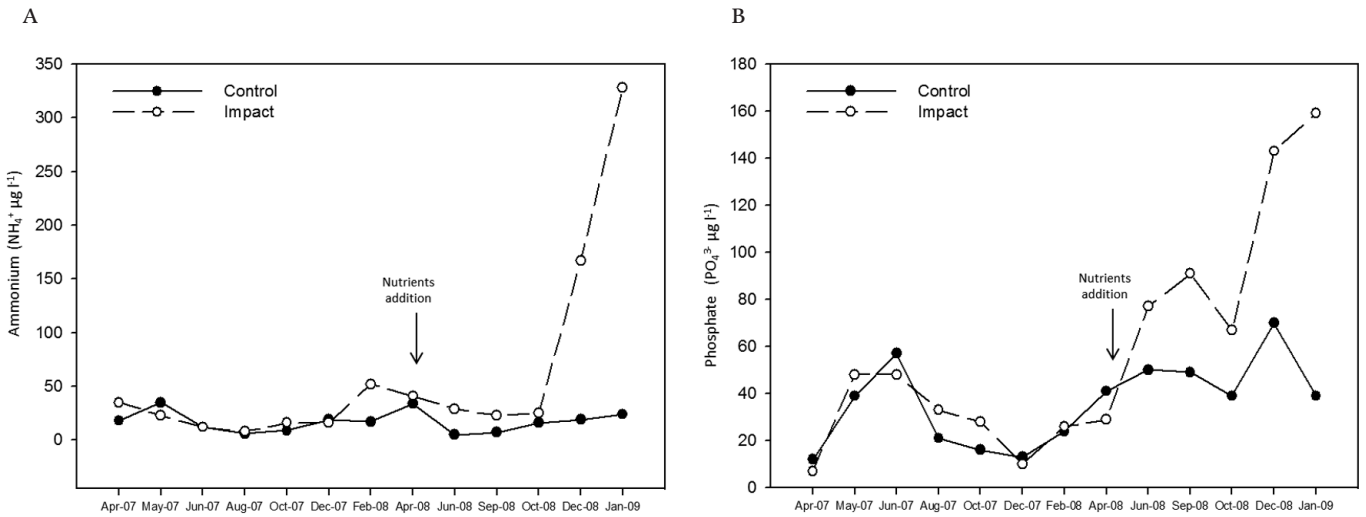

Figure 2. Ammonium (a) (NH_4^+) and Phosphate (b) (PO_4^{3-}) concentrations in the Control (C) and Impact (I) reaches during the experiment.

minimum higher than during the period before (Fig. 1). Significant differences were found in BACI analysis for nutrient concentrations, with Impact reach being higher after enrichment for NH_4^+ (n = 26, F = 4.685, p = 0.042) and PO_4^{3-} (n = 26. F = 6.638, p = 0.017) (Fig. 2).

The $\delta^{15}N$ signal clearly established three trophic levels: 1) basal level with CPOM and biofilm; 2) primary consumers (macroinvertebrates: collector-gatherers (*Heterelmis*, *Thraulodes* and *Trichorythodes*) and collector-filterers (*Simulium*); 3) predators-fish, (*Onchorhynchus mykiss* and *Trichomyterus bogotensis*, Fig. 3). The $\delta^{15}N$ of consumers was enriched compared to primary sources - mainly CPOM - in different proportions, depending on the feeding habits of each taxa (Table 2). The CPOM presented similar isotopic values in all phases of the experiment and constituted an indicator of the base of the food chain. However, the biofilm showed a higher $\delta^{15}N$ signal (between 3.5 and 4 ‰) than CPOM, except for the treatment reach after the nutrient addition where the $\delta^{15}N$ was 0.10 ‰. The average fractionation of nitrogen of the primary consumers with respect to CPOM was 4.7 ‰ (range, 3.8 - 5.5 ‰) and 1.7 ‰ with respect to biofilm (ranging between 0.3 and 4.0 ‰). Values for *Tricorythodes* $\delta^{15}N$ in impact reach after enrichment have not been taken into account in this range due to their unusual low values). Predators increased their

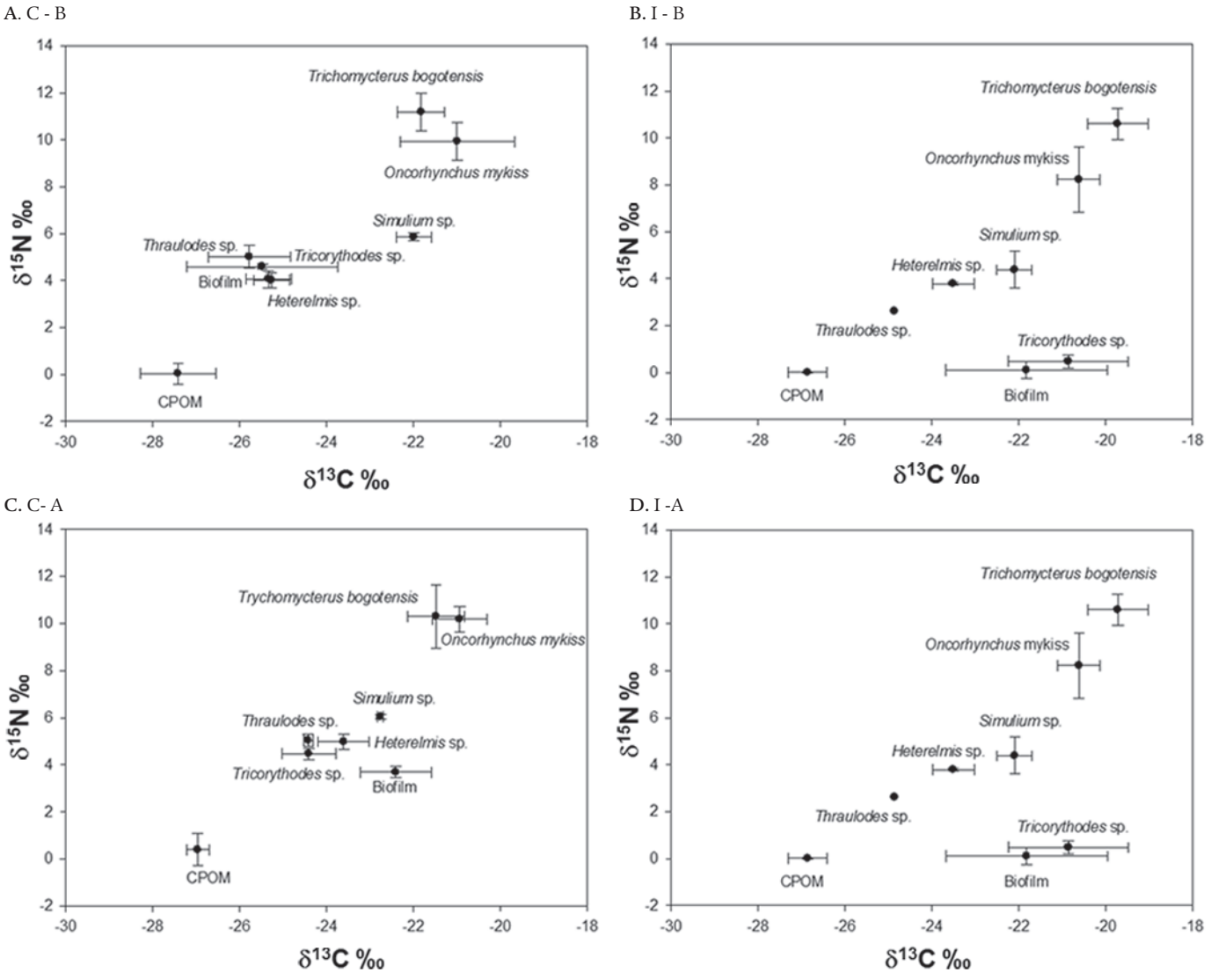

Figure 3. Carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}C$) signatures of basal resources (biofilm, CPOM), macroinvertebrates (*Heterelmis* sp., *Thraulodes* sp., *Tricorythodes* sp. and *Simulium* sp.) and fishes (O*ncorhynchus mykiss*, *Trichomycterus bogotensis*) in Control (C), Impact (I) reaches, Before (B) and After (A) the nutrient addition. Points are means ±1 SD. Thaulodes sp. in IA is a single sample.

$\delta^{15}N$ signal by 5.9 ‰ (from 4.3 to 10.17 ‰) with respect to primary consumers (Table 2).

In most cases, the $\delta^{13}C$ signal of biofilm overlapped with that of primary consumers, but a clear enrichment was observed with respect to CPOM. Following the $\delta^{13}C$ and the gut content results (see below), one would predict that almost all the invertebrates analyzed were actually feeding on CPOM and biofilm (Fig. 3). An increase in δ13C values of biofilm was observed in both reaches after the enrichment (n = 3, t = -5.259, p = 0.006 in control reach and t = -2.944, p = 0.05 in I reach), indicating an enrichment of 13C with respect to 12C, probably related to environmental changes (e.g. flow). *Oncorhychus mykiss* and *Trichomycterus bogotensis* fed on macro-invertebrates in both reaches, and in I reach *Oncorhychus* became a prey of *Trichomycterus* ($\delta^{15}N$ fractionation: 2.14 ‰ and 2.32 ‰, before and after respectively).

A depletion in $\delta^{15}N$ was observed in I with respect to C reach after fertilization in different compartments: biofilm (t = 13.453, p = 0.001), *Heterelmis* (t = 5.572, p = 0.01) *Simulium* (t = 4.019, p = 0.02) and *Tricorythodes* (t = 17.42, p < 0.001). Biofilm reflected the use of inorganic N from fertilizer ($\delta^{15}N$ = 0,1 ± 0 ‰) and invertebrate signals in its consumption. This depletion was not significant for top predators.

Fine detritus (FD) was the most abundant food in the four invertebrate species whose guts were analyzed. Proportions of algae (diatom and filamentous algae) were always 10 % lower in gut contents. *Heterelmis* showed significant differences in FD (n = 5 t = -5.159, p < 0,01) and PM (n = 5, t = 3.533,

Table 2. $\delta^{13}C$ and $\delta^{15}N$ values of the different compartments for the Control (C) and Impact (I) reaches, Before (B) and After (A) nutrient enrichment in Tota stream.

		B				A			
		C		I		C		I	
Compartment	Isotope	Mean	SD	Mean	SD	Mean	SD	Mean	SD
CPOM	$\delta^{13}C$	-27.41	0.87	-27.01	0.57	-26.96	0.26	-26.85	0.44
	$\delta^{15}N$	0.04	0.44	-0.09	0.57	0.39	0.69	0.01	0.01
Biofilm	$\delta^{13}C$	-25.33	0.52	-24.86	0.53	-22.41	0.82	-21.82	1.85
	$\delta^{15}N$	4.07	0.36	3.43	1.01	3.68	0.24	0.10	0.37
Heterelmis sp.	$\delta^{13}C$	-25.27	0.41	-24.22	1.26	-23.60	0.59	-23.51	0.47
	$\delta^{15}N$	4.02	0.32	4.04	0.57	4.98	0.31	3.79	0.07
Thraulodes sp.	$\delta^{13}C$	-25.78	0.94	-25.53	0.90	-24.42	0.11	-24.86	
	$\delta^{15}N$	5.02	0.49	3.54	1.18	5.04	0.27	2.61	
Tricorythodes sp.	$\delta^{13}C$	-25.48	1.74	-25.45	1.59	-24.40	0.63	-20.86	1.38
	$\delta^{15}N$	4.60	0.12	4.14	0.37	4.47	0.24	0.48	0.29
Simulium sp.	$\delta^{13}C$	-21.99	0.41	-24.44	0.39	-22.76	0.07	-22.09	0.40
	$\delta^{15}N$	5.86	0.17	5.55	0.13	6.04	0.10	4.38	0.79
Oncorhynchus mykiss	$\delta^{13}C$	-20.99	1.31	-22.56	0.87	-20.94	0.64	-20.62	0.49
	$\delta^{15}N$	9.93	0.82	9.39	0.93	10.19	0.54	8.22	1.38
Trichomycterus bogotensis	$\delta^{13}C$	-21.82	0.55	-20.46	0.76	-21.48	0.64	-19.72	0.70
	$\delta^{15}N$	11.19	0.81	11.53	0.41	10.31	1.36	10.61	0.67

p = 0.001) between C and I reaches after nutrient addition. *Simulium* in PM (n = 5, t = -2.496, p = 0.017), *Thraulodes* in CD (n = 5, t = 6.760, $p < 0.001$) and FD (n = 40, t = 7.027, $p < 0.001$). *Tricorythodes* did not show significant differences in any of the food categories (Fig. 4).

DISCUSSION

In spite of progress in the study of isotopes in food webs, there is little information available regarding tropical high mountain systems. In our study, the Tota stream food web shows about three to four trophic levels according to the spatial work scale of the study (Post *et al.*, 2000). This high mountain creek is hydrologically very dynamic, and shows nutrient limitations (Rivera and Donato, 2008) that lead to low productivity (Abuhatab, 2011). All these could be important factors for limiting connectivity between species (Schmid-Araya *et al.*, 2002; Jardine *et al.*, 2012).

$\delta^{13}C$ values obtained for biofilm (-25.33 to -21.82) and CPOM (-27.41 to -26.85) in the Tota stream are similar to those of other tropical streams, although slightly higher than those reported for leaves in other small tropical streams (March and Pringle, 2003; Dudgeon *et al.*, 2010). In addition, our values correspond to those given by Peterson and Fry (1987) for C_3 plants. Several factors can affect algal fractionation of C (Finlay *et al.*, 2002), and result in a broad range of values. Fine detritus is derived from both algal and detrital components and has intermediate $\delta^{13}C$ values (Hershey *et al.*, 2007). It is an important source for consumer diet, it was common in the guts of Tota invertebrates, but, unfortunately, we did not analyze this compartment for stable isotopes.

In general it is very difficult to obtain clean samples of periphyton from the field since algal cells grow into the biofilm matrix together with bacteria, microfauna and detritus, thus resulting in higher values of $\delta^{15}N$ than other basal resources. This makes it more difficult to calculate nitrogen fractionation for primary consumers with respect to resources. Values in our system are near those predicted in the literature (an average of 3.4 ‰, Post, 2002) although with high variability (ranging between 0.3 and 4.0 ‰) when biofilm is used in calculations. Fish nitrogen fractionation values were an average of 5.9 ‰ and clear $\delta^{13}C$ enrichment was observed with respect to the invertebrates, showing their feeding dependence.

We found a strong relationship between collector-gatherers and biofilm in the two reaches (C, I) and periods (B, A), indicating strong reliance on algal carbon in this feeding group. *Tricorythodes* was the collector-gatherer that had the closest connection with the biofilm. A previous study (Donato-Rondon *et al.*, 2010) has already shown that this species is strongly associated with periphyton resources, while Tomanova *et al.* (2006) indicated that most invertebrate collectors in tropical rivers are not food specialized and their dietary changes are related to the availability of resources.

Finlay *et al.* (2002) argued that trout isotopic ratios vary seasonally, depending on food resource availability. In our case, *Onchorhynchus mykiss* ratios were clearly related to primary consumers. In the case of our other top predator, *Trichomycterus bogotensis*, there are no published reports on its diet. However, records of diets presented by Habit *et al.* (2005) and Roman-Valencia (2001) for *T. areolatus* and *T.caliensis*, respectively, show an insectivore behavior. In our study it is evident that

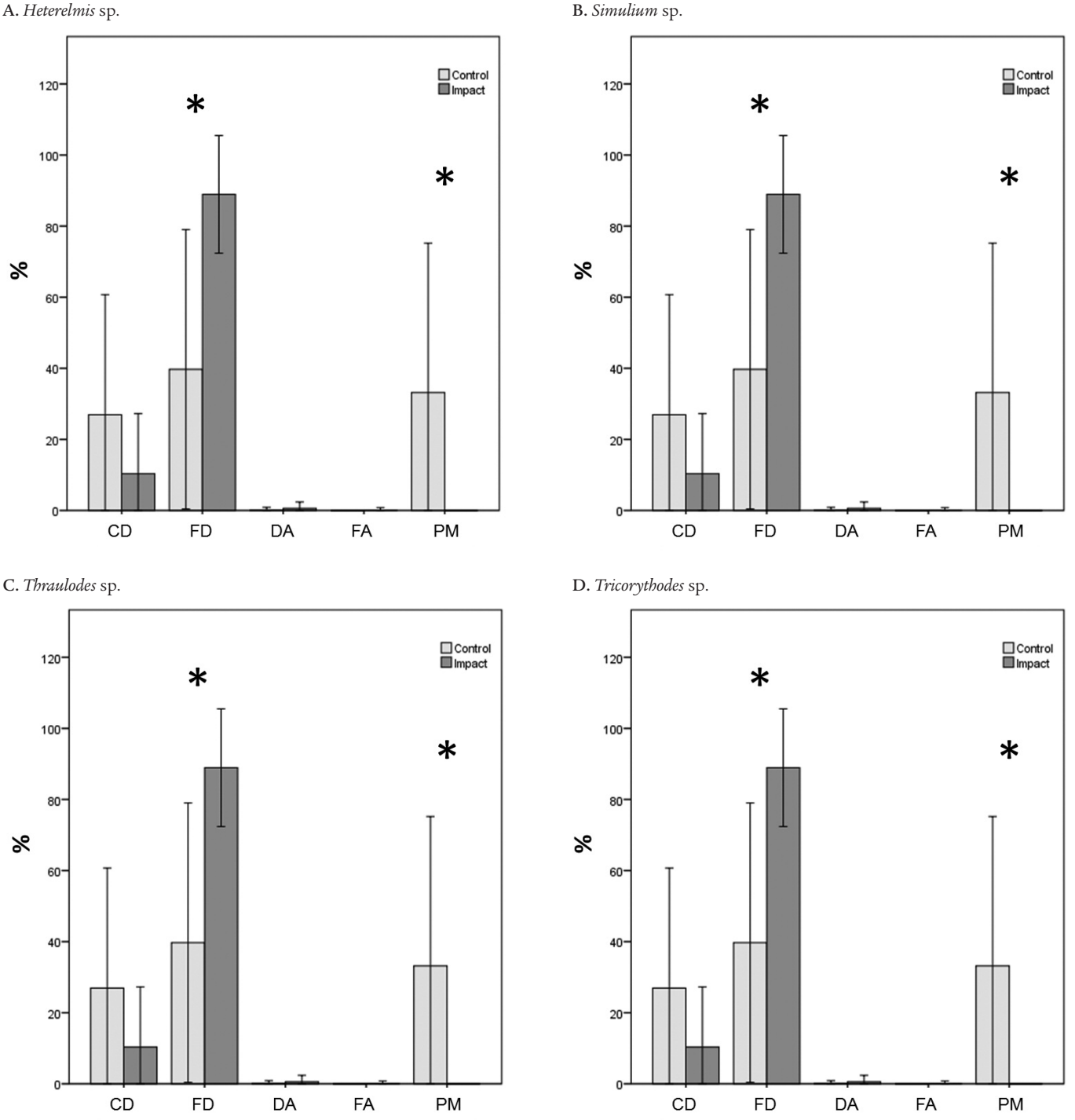

Figure 4. Gut content percentage found in macroinvertebrates during the enrichment period. * Corresponds to significant differences between the control and Impact reaches ($p < 0.05$) for the Student's t-test. CD (coarse detritus), FD (fine detritus), DA (diatom algae), FA (filamentous algae), PM (plant matter).

diet was composed by insects and in the impact reach also by *Oncorynchus mykiss*. In the same way, Chará *et al.* (2006) showed that *Trichomycterus* is insectivore and partially piscivore. It is hard to explain the differences found in diet between our two study reaches for this species. Perhaps the dietary differences were derived from differences in habitat, as in the Impact reach this species was more abundant in the deep pools, where food items might differ from those in other habitats.

An increase in $\delta^{13}C$ values of biofilm was observed in both reaches after nutrient addition. This increase could be a product of discharge temporality (before samples were taken

at the beginning of the high discharge period and the after ones were taken in the low discharge period). In this period a thicker periphyton, active photosynthesis, combined with diffusion-limited movement from the water to cells, is likely to cause the depletion of inorganic carbon within the periphyton matrix and higher values of $\delta^{13}C$ (Hill y Middleton, 2006). On the contrary, Hladyz *et al.* (2011) raise the point that maximum enrichment in cobble biofilm $\delta^{13}C$ signature occurred following periods of high discharge, while maximum depletion occurred during the low discharge period. These findings highlight the fact that trophic links between basal resources and primary consumers can be altered profoundly and that changes in hydrology can alter food chains and energy fluxes to the higher trophic levels (Perkins *et al.*, 2010). As we hypothesized, nutrient addition, partly in nitrogen form, reduced the $\delta^{15}N$ signature of biofilm and of most of the primary consumers, although no evidence of higher consumption was observed in gut contents. Conversely, detritus entering from riparian forest was not affected. Moreover, the proportion of fine detritus in gut contents significantly increased in *Heterelmis* and *Thraulodes* after addition. Both results may indicate a higher consumption of fine detritus and biofilm in the impacted reach, even though no clear significant differences were found in their quality as a consequence of fertilization. However, this depletion was not reflected in fish. Their high mobility along the river (Jardine *et al.*, 2012) and long life cycle would lead to different patterns for these top predators.

The natural distribution of isotopic abundance in Tota stream showed the links between a resource or prey and predator in the two reaches and periods studied, and the nitrogen addition works as a tracer approach confirming those links. Complementary to this, the gut-contents data help to decide which link is the most correct and to discern dietary changes due to nutrient addition.

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