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Abstract: The theory of ecological stoichiometry holds that heterotrophs are mostly homeostatic and exhibit less variation in body stoichiometry than do autotrophs. Most studies of stream foodweb stoichiometry have been done in low-nutrient environments. Little is known about foodweb stoichiometry in nutrient-rich streams, in which a higher level of stoichiometric homeostasis should be expected, mainly because imbalances between resources and consumers are low and nutrient availability may meet biotic requirements. We analyzed elemental content (C, N, P) and stoichiometric ratios (C:N, C:P, N:P) of basal resources, macroinvertebrates, and fishes in a nutrient-rich Pampean stream and compared these values to those from other studies. We manipulated P and N in a 1-y fertilization experiment to analyze biotic stoichiometric responses to additional nutrient input to this naturally enriched system. Soluble reactive P concentration in the treatment reach was doubled relative to the background concentration. Consumers had lower C:P and N:P than those in other lotic systems, whereas P content and C:P and N:P of basal resources were within the ranges observed for other systems. Most components of the trophic web were not affected by fertilization, and only epiphyton, fine benthic organic matter, and 2 macroinvertebrate species (*Palaemonetes argentinus* and *Pomacea canaliculata*) changed their nutrient content or stoichiometric ratios. Imbalances in C:N and C:P occurred between primary consumers and their resources, particularly among macroinvertebrate collectors and detritivorous fishes feeding on FBOM. Most basal resources and consumers were strictly homeostatic for P content and the stoichiometric ratios, but a lower degree of homeostasis occurred in the epiphyton, *P. canaliculata*, and collectors feeding on epiphyton. A high degree of stoichiometric homeostasis exists across the various components of the food web in this nutrient-rich stream, regardless of their trophic position.

Key words: stoichiometry, basal resources, fertilization, macroinvertebrates, fishes

Ecological stoichiometry is a reliable framework for examining scaling of trophic dynamics across organization levels within an ecosystem (Sterner and Elser 2002, Persson et al. 2010). This theory predicts that the relative proportions of chemical elements, mostly C, N, and P, in the environment and in the biota affect the biological transformations of these elements through the food web.

Elemental imbalances can exist between resources and consumers because organisms meet their internal elemental demands by consuming food sources of varying stoichiometric ratios (C:N:P). These imbalances can constrain consumers' growth and reproduction (Frost et al. 2005) unless organisms can adjust their internal elemental composition following changes in resource stoichiometry. On short temporal scales, variations in elemental composition among organisms may be associated with changes in food quality. However, the evolution of different structures and tissues among taxa can lead to dissimilarities in

their internal elemental composition. Organisms' stoichiometry also can vary because of phylogenetic differences that operate over very long time scales (Lauridsen et al. 2012). Organisms that can maintain their internal stoichiometry regardless of changes in their food C:nutrient ratios are considered homeostatic (Sterner and Elser 2002, Persson et al. 2010). Biochemical and behavioral mechanisms to maintain stoichiometric homeostasis include food selection, increasing feeding activity to acquire the limiting element, regulating assimilation across the cell membrane or gut wall, post-assimilative metabolism, or any combination of the above (Hessen et al. 2013). Autotrophs are mostly nonhomeostatic and track the variations of dissolved nutrient concentrations, but consumers are mostly homeostatic and exhibit much less variation in their body stoichiometry (Sterner and Elser 2002, Cross et al. 2005, Persson et al. 2010).

Most studies of stream foodweb stoichiometry have been done in detritus-based streams with relatively low nutrient levels (soluble reactive P [SRP] concentration <0.1 mg/L), where nutrient limitation can be prominent (Cross et al. 2003, Sabater et al. 2011). Predictions based on stoichiometric theory for these systems have been tested by comparing the elemental composition of diverse components of the food web at different nutrient levels. Hence, most comparisons have been made based on pre-existing nutrient differences among stream reaches (Bowman et al. 2005, Ortiz et al. 2009) or among nearby streams (Small and Pringle 2010). Experimental nutrient additions also have been used to compare the response of control and enriched stream reaches (Cross et al. 2003, Sabater et al. 2011). Overall, these studies have confirmed that the elemental composition of autotrophs varies widely with changes in nutrient supply, whereas that of heterotrophs exhibits higher degrees of homeostasis.

The response of foodweb stoichiometry to changes in nutrient availability in nutrient-rich streams has received little attention (but see Lauridsen et al. 2012). In systems with low nutrient levels, autotrophs respond readily to the increment of limiting nutrients, and higher variability in their elemental nutrient contents should be expected (Tsoi et al. 2011). Imbalances between consumers and basal resources may be pronounced, and increases in water nutrient availability can lead to higher nutrient contents in basal resources that can be transferred later to other trophic levels. Hence, under low-nutrient conditions, the food web usually exhibits a low level of stoichiometric homeostasis. However, in eutrophic systems, imbalances between resources and consumers should be lower, and the availability of nutrients may suffice to meet requirements for growth and reproduction. In nutrient-rich systems, the challenge is for the organisms to regulate their internal elemental content (by retaining elements that are proportionally less available and eliminating those that are available in excess) so that optimal values in their stoichiometric ratios can be maintained. Over long time scales, this situation may favor genotypes with more efficient mechanisms for adjusting internal stoichiometry. Hence, a higher level of homeostasis can be expected in the biota of eutrophic than of oligotrophic streams (Lauridsen et al. 2012).

Most studies on foodweb stoichiometry have focused on heterotrophic streams, and little information is available on autotrophic lotic systems. In unshaded streams, autotrophic production is favored by high levels of irradiance, and available nutrients can be used rapidly and converted to autotrophic biomass, which becomes available to other trophic levels. Hence, when water nutrient availability increases, incorporation of nutrients into the food web will be faster in autotrophic than in detritus-based streams. In addition, in most autotrophic streams, basal resources consist mainly of algae and macrophytes, which have better nutritional quality than detritus. Thus, autotrophic streams may provide a good case for the study of the effects of enrichment of the stoichiometry of the food web.

Streams in the Pampean region of central Argentina are eutrophic and exhibit predominantly autotrophic metabolisms fuelled by autochthonous organic matter production (Vilches and Giorgi 2010, Acuña et al. 2011). The occurrence of high nutrient concentrations is characteristic of Pampean streams and was documented even before the arrival of Spaniards to the region (Feijoó and Lombardo 2007). Thus, the potential response of biota to P availability is influenced by food elemental content, phylogenetic identity, and long-term coexistence of the organisms with enriched conditions. High nutrient levels, low discharge (<50 L/s), and lack of riparian forest vegetation, which allows high irradiance levels along the courses of these streams, promote development of dense macrophyte stands and elevated algal growth (Feijoó and Lombardo 2007). A warm climate favors high autotrophic production that is not limited by the temperature as it is in temperate systems. Hence, Pampean streams exhibit a wide range of basal food sources (seston, FBOM, epiphyton, and macrophytes) that supports a diverse consumer community with complex trophic interactions (Giorgi et al. 2005).

We investigated stoichiometric homeostasis in the food web of a chronically P-enriched Pampean stream (La Choza). Our study had 2 main objectives. The 1st objective was to examine whether the P and N contents of organisms of different trophic levels of the Pampean stream were consistently higher than those from low-nutrient environments. We studied the elemental stoichiometry of key foodweb components of La Choza intensively, and compared the values obtained to those from other river systems. We made this comparison among similar taxonomic and functional feeding groups to avoid phylogenetic differences in elemental composition that could hamper the

analysis. The 2nd objective was to assess whether the organisms in La Choza exhibit homeostatic responses to additional nutrient inputs. We enriched a stream reach with P and N, and assessed the effects on the biota's stoichiometric ratios by comparison to ratios in a control reach upstream. Considering the eutrophic status of La Choza, we did not expect the foodweb components to respond to nutrient increases, but rather to exhibit high stoichiometric homeostasis, regardless of their trophic position.

METHODS
Study site

We studied La Choza, a 2nd-order tributary of the Reconquista River (lat 34°44′S, long 59°06′W) with high nutrient levels (P-PO$_4$ = 0.2 mg/L; N-NO$_3$ = 0.9 mg/L). The stream is situated in the northeastern part of the Pampean region, a vast grassy plain that covers central Argentina. The climate is warm with annual precipitation between 600 and 1200 mm and a median annual temperature between 13 and 17°C. The mean stream discharge and current velocity are low, and the nutrient concentrations are high (Table 1). The stream bed consists of fine sediments (primarily silt and clay) without stones or pebbles. Hence, habitat heterogeneity is generated mainly by the abundance and diversity of the macrophytic community. The predominant land uses in the river basin are agriculture and cattle breeding.

Field survey and sample analysis

We selected 2 stream reaches, 100 m long and 5 km apart, for the experiment. The upstream reach (control) was untreated, whereas the downstream reach (treatment) was enriched with P and N. The pre-enrichment period started in March 2007 and ended in October 2007. Nutrient addition began on 20 November, and we considered

samples taken from mid-December 2007 to December 2008 as within the enrichment period. We calculated the proportion of added nutrients to maintain the N:P ratio in the water (Table 1). High P and N levels in the stream water would have hampered continuous addition of dissolved nutrients to the reach because of the very large solution volume required. Therefore, we used mesh bags with 750 g of commercial fertilizer (Nitrofoska, Basf, Amberes, Belgium; 12% P as PO$_4^{3-}$ and 10% N as NO$_3^-$) and 250 g of urea. We distributed 12 in-water bags along the treatment reach, and replaced the nutrient bags 2 or 3 times a week during the fertilization period to maintain a constant fertilization rate.

We sampled the 2 reaches bimonthly during the pre-enrichment and enrichment periods to assess their environmental characteristics, water chemistry, and biotic communities. Temperature, pH, dissolved O$_2$ concentration, and conductivity were measured with a Hach HQ40d18 portable meter (Hach, Loveland, Colorado). We estimated the water flow and velocity using the slug-injection method, with NaCl used as a conservative tracer (Gordon et al. 1992). We collected water samples in triplicate in both reaches on each sampling occasion for analysis of NO$_3^-$, NO$_2^-$, and NH$_4^+$ content. We also collected water samples in triplicate at both reaches during the pre-enrichment period for estimation of soluble reactive P (SRP) and every 5 to 10 d during the fertilization experiment to better capture changes in SRP concentration.

We sampled the various components of the trophic web bimonthly to analyze their C, N, and P content. We collected samples from each compartment in triplicate. We used a 100-cm^2 Eckman dredge for the benthos and a 625-cm^2 Plexiglas® square for aquatic vegetation. We harvested the dominant macrophyte species (*Ludwigia peploides* (Kunth) P. H. Raven) at 3 locations randomly distributed within each reach. We sampled epiphyton from

Table 1. Mean (±SE) values of physicochemical variables in the control and treatment reaches of La Choza before and after the onset of the fertilization experiment.

Variable	Control reach		Treatment reach	
	Before	After	Before	After
Current velocity (m/s)	0.24 (0.38)	0.02 (0.03)	0.34 (0.48)	0.05 (0.05)
Discharge (L/s)	19.5 (15.9)	6.2 (4.6)	21.8 (12.4)	8.2 (6.8)
Conductivity (µS/cm)	867 (561)	1280 (99)	1160 (553)	1748 (101)
Dissolved O$_2$ (mg/L)	9.8 (5.5)	9.6 (3.3)	9.9 (4.3)	8.6 (2.4)
P-PO$_4^{3-}$ (mg/L)	0.21 (0.16)	0.09 (0.03)	0.25 (0.13)	0.44 (0.35)
N-NO$_3^-$ (mg/L)	0.58 (0.36)	0.86 (1.17)	1.30 (0.78)	0.81 (0.87)
N-NO$_2^-$ (mg/L)	0.05 (0.09)	0.05 (0.03)	0.05 (0.08)	0.05 (0.01)
N-NH$_4^+$ (mg/L)	0.02 (0.03)	0.02 (0.02)	0.02 (0.03)	0.02 (0.03)
N:P	9.6 (7.5)	26.0 (17.3)	17.2 (13.3)	21.2 (22.2)

apical shoots (10 cm long) of *L. peploides* kept in a glass bottle with filtered stream water (Whatman GF/F fiberglass filters; Whatman, Maidstone, UK). We collected fine benthic organic matter (FBOM) samples with a suctioning device (1 cm^2 diameter and 4 mL volume). We sampled macroinvertebrates in triplicate at the same places at which macrophytes and other food sources were sampled. We caught fishes by blocking the upstream and downstream ends of a subreach (25 m long) situated at the beginning of both reaches with trammel nets (5-mm mesh size) (Li and Li 2006). We identified macroinvertebrates and fishes at the species level, and assigned functional feeding groups according to Cummins et al. (2005) and Rosso (2006), respectively.

We filtered water samples in the laboratory through preweighed fiberglass filters (Whatman GF/F). We assayed SRP with the ascorbic acid method (APHA 1992), NO_3^- and NO_2^- by reaction with sulfanilamide, with a previous Cd reduction in the case of NO_3^- (APHA 1992), and NH_4^+ with the phenol hypochlorite method (Wetzel and Likens 1991).

We dried filters containing seston particles at 60°C and weighed them. We maintained macroinvertebrates in filtered stream water for 12 h to clean their guts (Hershey et al. 2006). We dried macrophytes and macroinvertebrates at 60°C to a constant mass and ground them in a mortar. We sonicated apical shoots for epiphytic elemental composition determination at low speed (3 + 3 + 3 min), filtered the final suspension through a preweighed Whatman GF/F fiberglass filter, and weighed the filters again. We sonicated FBOM samples 3 times at low speed for 3 min, filtered them through preweighed Whatman GF/F fiberglass filters, and dried the filters at 60°C to a constant mass. We dissected most fish and extracted their guts. We used their whole bodies later for measurement of elemental content. We used a subsample of muscle tissue from large detritivorous fish (Hershey et al. 2006). We dried the whole bodies of small fish and muscle tissues of large fish at 60°C to a constant mass and ground them in a mortar.

We analyzed the elemental content of all of the described compartments (filtered or dried samples). In the case of macrophytes, macroinvertebrates, and fishes, we measured elemental content at the species level. We measured C and N content with a Thermo EA 1108 elemental organic analyzer with vanadium pentoxide as an oxidation catalyst. We estimated P content after combusting the dried samples in a muffle furnace (500°C for 3 h) and digesting filtered and combusted samples for subsequent measurement of SRP (APHA 1992). We used various digestion agents based on recommendations in the literature for each compartment. We used a basic oxidant for seston, epiphyton, FBOM, and macroinvertebrates (Koroleff and Weinheimer 1983), HCl for macrophytes (AOAC 1984), and HNO_3 for fishes (APHA 1992).

We also collected samples of the different trophic compartments for stable-isotope analysis during the pre-enrichment period (November 2007). The sample preparation protocol for the different trophic compartments was the same as that for the measurement of elemental composition. We analyzed isotopic composition with a Flash EA1112 elemental organic analyzer (Thermo Electron, Milano, Italy) coupled to a Delta C isotope ratio mass spectrometer (Thermo Scientific, Bremen, Germany). We expressed the ratios of $^{13}C/^{12}C$ and $^{15}N/^{14}N$ as differences in parts per thousand ($\delta^{13}C$ or $\delta^{15}N$) between the sample ratio and a standard ratio (PDB carbonate or N_2 in air). We used this information to define the structure of the food web in the stream, given that trophic enrichment in ^{15}N is an indicator of the trophic level and that ^{13}C allows differentiation of sources of organic matter for consumers (Hershey et al. 2006). We also used analyses of gut contents of macroinvertebrates (Ocon et al. 2013) and field observations of the diets of consumers to investigate foodweb structure.

Data analysis

We tested differences in SRP concentration between the control and treatment reaches in the pre-enrichment and enrichment periods based on a before–after control–impact (BACI) design (Stewart-Oaten et al. 1986). In a BACI design, control and impact sites are sampled simultaneously before and after the impact. When changes in the impact site are large relative to natural variability (represented by the control site), changes at the impact site are assumed to be significant. We also analyzed the effects of nutrient enrichment on the elemental contents and stoichiometric ratios of the biota based on a BACI design. We tested the interaction between the factors, reach (control and treatment) and sampling time (months of sampling), with period (before and after enrichment) nested within sampling time (factor = reach × time[period]). In the case of macroinvertebrates and fish, we used the BACI design to analyze elemental content of the most common species. We adjusted all BACI probabilities for multiple tests with the Dunn–Šidák procedure (Šidák 1967). We arcsin(*x*)-transformed %C, N, and P to meet the assumption of normality.

We estimated the degree of stoichiometric homeostasis of the foodweb components by regression analysis of the stoichiometric ratios of the organisms and their resources (log–log scale). The slope of this relationship is 1/H (eta), where H is the homeostatic coefficient (Persson et al. 2010). We paired species of consumers with their food sources by date and location and did regression analyses for %P, N:C, P:C, and N:P. We defined the degree of homeostasis as (Persson et al. 2010): 1) nonsignificant (*p* > 0.1) regression, 1/H set to 0, strictly homeostatic; 2) significant regression, 0 < 1/H < 0.25, homeostatic; 3) significant

regression, 0.25 < 1/H < 0.5, weakly homeostatic; 4) significant regression, 0.5 < 1/H < 0.75, weakly plastic; and 5) significant regression, 0.75 < 1/H ≤ 1, plastic. We pooled data from the control and treatment reaches to calculate the degree of homeostasis.

RESULTS
Environmental variables and biotic communities

Water velocity and flow were constantly low, and the water in the 2 reaches was well oxygenated with high conductivity (Table 1). Nutrient concentrations were high, and NO_3^- was the dominant fraction of the total dissolved inorganic N. Heavy rains in the first half of 2007 increased current velocity, flow, and SRP. The treatment reach was enriched with N and P, but the effect of the nutrient addition was more noticeable for P because mean SRP concentration in the treatment reach during the fertilization period was twice as high as the pre-enrichment background concentration (Table 1). SRP was significantly higher in the treatment than in the control reach ($F_{17,360}$ = 1.994, p = 0.011) during the experiment, but no other environmental variables differed between reaches. Mean N:P of the 2 reaches was similar during the fertilization experiment but displayed considerable temporal variation.

The food web of the La Choza stream consisted of several compartments and was characterized by high biological diversity. Autochthonous detritus and algal and vascular-plant communities provided the basal resources in the stream. $\delta^{13}C$ of FBOM was intermediate between the values for macrophytes and epiphyton, a result suggesting that FBOM was derived from both algae and vascular plants. Macrophyte patches were composed mostly of emergent plants, such as *L. peploides* and *Bacopa monnieri* (L.) Westtst. Macroalgal masses (mainly *Spirogyra* sp.) become dominant for a short period at the onset of the growing season (August–September). With the exception of macroinvertebrate predators, including water bugs (Hemiptera) and odonate nymphs, functional groups generally were represented by 1 taxonomic group. The gathering-collectors were mostly Crustacea (shrimps and river crabs), the filtering-collectors were bivalves, and the scrapers were gastropods (river snails). Fish diversity and abundance were very high and included carnivorous, omnivorous, herbivorous, and detritivorous species, primarily cichlids, characins, and catfish.

%P and stoichiometric ratios

We analyzed foodweb stoichiometry with data from the control reach because they represented the background situation in the stream (Fig. 1A–C, see Appendix S1 for mean stoichiometric ratios of the control and treatment reaches). Within the basal resources, mean C:N was higher in macrophytes and FBOM than in algae. FBOM had higher
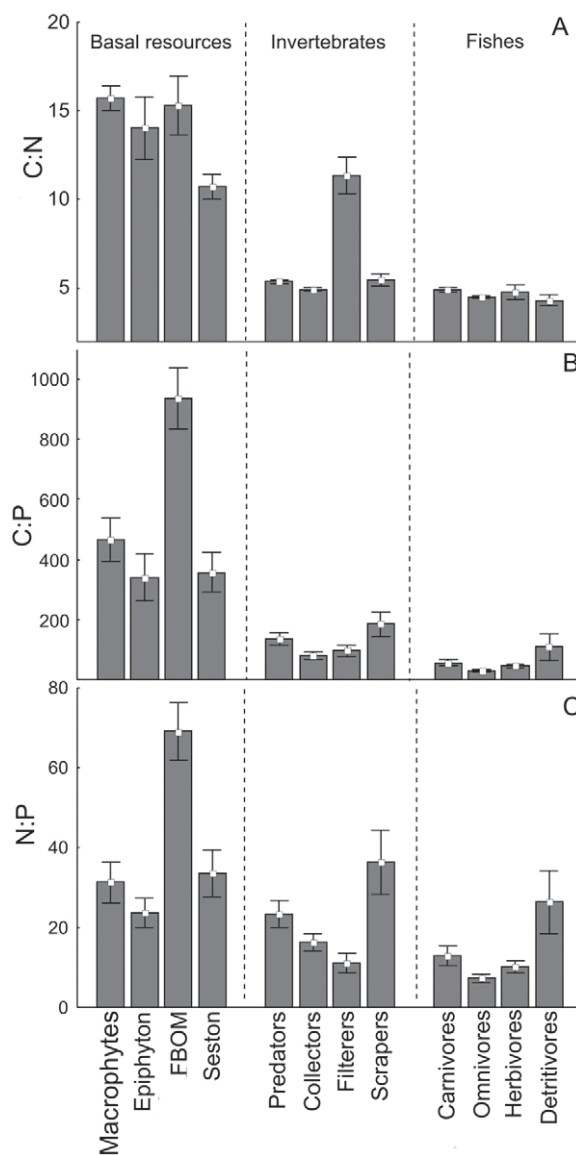

Figure 1. Mean (±1 SE) C:N (A), C:P (B), and N:P (C) for various foodweb components in the control reach throughout the sampling period. FBOM = fine benthic organic matter.

C:P and N:P than algae and macrophytes. Macroinvertebrate filterers had the highest C:N, whereas scrapers had the highest C:P and N:P (because of lower P content). Fish from various functional groups had the lowest C:P and N:P of all trophic compartments, reflecting the high P content of their bone tissues. Detritivorous fish had higher C:P and N:P than fish in other feeding groups, but this result might be related to our use of muscle tissue from detritivores to estimate their elemental contents and use of whole bodies for all other fish trophic groups.

Mean %P and stoichiometric ratios in the basal resources in La Choza were within the range of values reported for streams elsewhere with lower P water concentra-

tions (Fig. 2A–C). However, macroinvertebrates generally had higher %P and lower C:P and N:P than similar taxonomic groups in oligotrophic streams (Fig. 3A, B). Cyprinodontiformes showed an analogous pattern (Table 2).
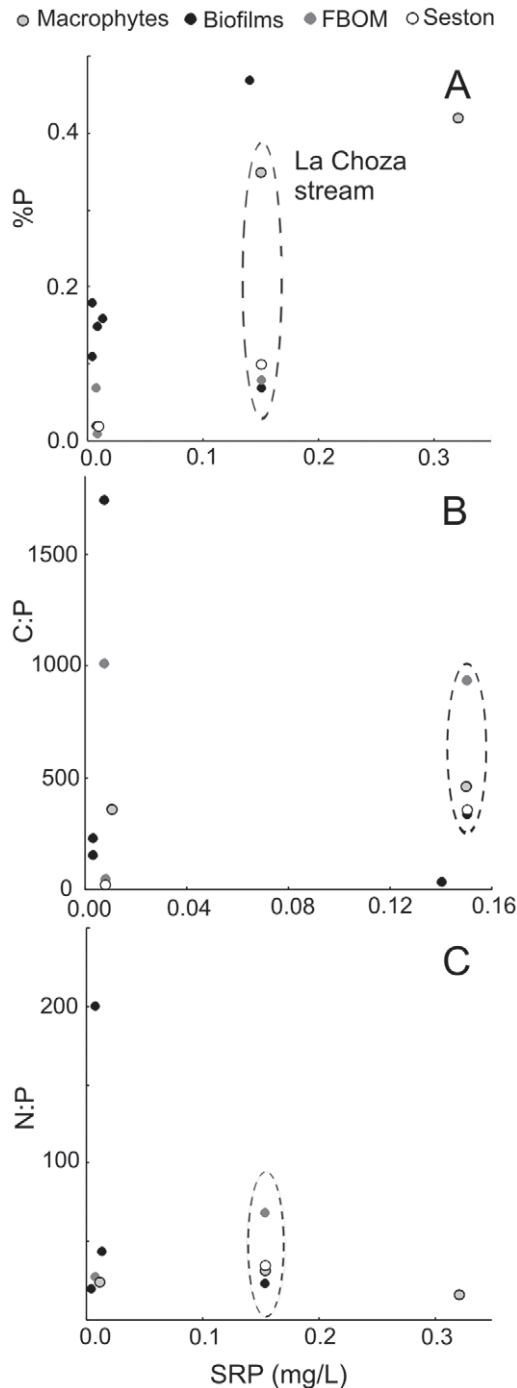

Figure 2. Comparisons of the mean %P (A), C:P (B), and N:P (C) in La Choza (control-reach data enclosed by broken line) and other streams (Feijoó et al. 1996, Cross et al. 2003, Slavik et al. 2004, Singer and Battin 2007, Small and Pringle 2010, Sabater et al. 2011, Tsoi et al. 2011). The data are arranged by increasing soluble reactive P (SRP) in water.

C:P and N:P of fish were lower and less variable in La Choza than in other streams, regardless of whether the estimates were made on whole bodies (Sterner and George 2000) or only muscle tissue (Tsoi et al. 2011) (Table 2).

Effect of nutrient enrichment

The same species of macroinvertebrates and fish were not found in all samples, so BACI analyses were applied only to the most abundant species of consumers, which were the gastropod *P. canaliculata* (a scraper), the crayfish *Palaemonetes argentinus* (a collector), and the fishes *Cyphocharax boga* (herbivorous) and *Oligosarcus jenynsii* (carnivorous). Percent C of the various foodweb components did not change as a result of the nutrient addition, except that %C of *P. canaliculata* increased slightly ($F_{4,27}$ = 3.74, p = 0.015). Percent N increased in *P. canaliculata* and *P. argentinus* in the treatment reach during the enrichment period ($F_{4,27}$ = 4.75, p = 0.005, and $F_{4,26}$ = 5.191, p = 0.003, respectively). Percent P increased in FBOM, *P. canaliculata*, and *P. argentinus* ($F_{7,34}$ = 3.813, p = 0.004; $F_{4,27}$ = 22.17, p < 0.001; $F_{4,26}$ = 16.427, p < 0.001, respectively). C:N and C:P decreased in epiphyton ($F_{8,16}$ = 3.913, p = 0.001; $F_{8,16}$ = 31.979, p < 0.0001, respectively) and FBOM ($F_{7,32}$ = 61.635, p < 0.0001; $F_{7,31}$ = 3.670, p = 0.005, respectively). N:P also decreased in epiphyton ($F_{8,16}$ = 22.179, p < 0.0001). C:N increased ($F_{4,26}$ = 206.7, p < 0.001), and C:P and N:P decreased in *P. argentinus* ($F_{4,26}$ = 3.086, p = 0.033; $F_{4,26}$ = 3.223, p = 0.028, respectively), but the elemental ratios of *P. canaliculata* were unchanged. None of the fishes tested responded to enrichment in terms of elemental content or stoichiometric ratios.

Foodweb structure, imbalances, and stoichiometric homeostasis

Stoichiometric imbalances (especially for C:N and C:P) between basal resources and consumers were large, except for macroinvertebrate filterers, which had C:N similar to seston, and scrapers, which had N:P similar to macrophytes and epiphyton (Fig. 1A–C). Consumer–resource pairs were established on the basis of the results of the isotopic analyses, the analyses of the gut contents of the macroinvertebrates, and field observations. The pairs were used to analyze the potential imbalances and degrees of stoichiometric homeostasis of the different foodweb components. Detritus was the most important food source for consumers, regardless of the functional group to which they were assigned. Gut-content analyses were consistent with information reported in the literature (Rosso 2006) on the lack of a strict dietary specialization in macroinvertebrates and fish. Isotopic signatures indicated that macrophytes were not an important food source for consumers, except for *C. boga* (herbivorous fish). However, gut-content analyses indicated that large scrapers, such as *P. canaliculata*, consume macrophytes and epi-
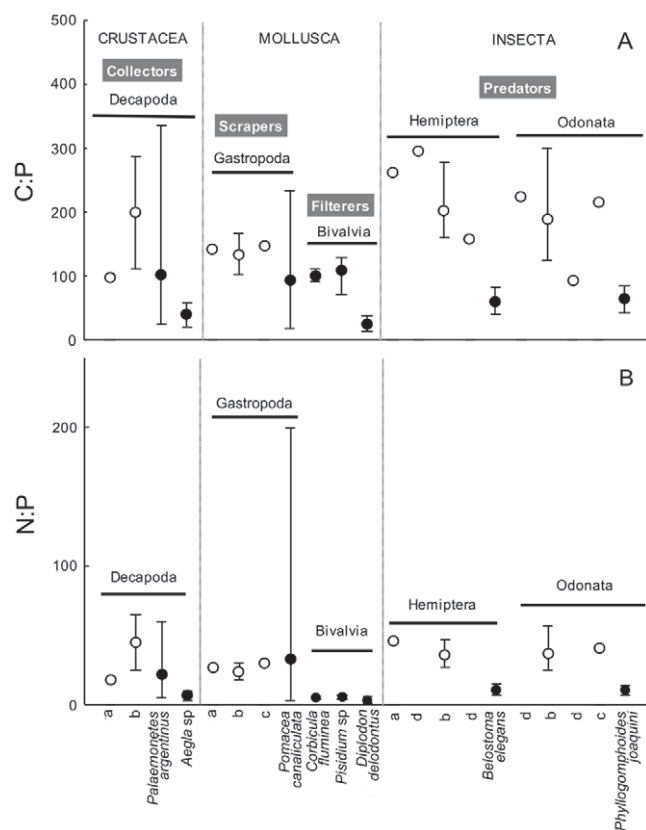
Figure 3. Mean (range) values for C:P (A) and N:P (B) of macroinvertebrates in La Choza (control-reach data indicated by black points) and those reported by Evans-White et al. (2005) (a), Tsoi et al. (2011) (b), Lauridsen et al. (2012) (c), and Small and Pringle (2010) (d). Corresponding functional groups are indicated in the gray boxes.

phyton. FBOM and epiphyton may be food sources for macroinvertebrate collectors, whereas filterers were associated with seston, and detritivorous fish were associated with FBOM. Isotopic signatures also indicated that om-

nivorous fish may consume epiphyton. Isotopic composition analysis indicated similar trophic structures in the 2 reaches. Based on all of this information, we established the following consumer pairs: *P. argentinus* and *Aegla* sp. vs epiphyton and FBOM, *Corbicula fluminea* vs seston, *P. canaliculata* vs macrophytes and epiphyton, *Jenynsia multidentata* vs epiphyton, *C. voga* vs macrophytes, and *Hypostomus commersoni* vs FBOM (Table 3). Basal resources (macrophytes, epiphyton, and seston) were paired with the nutrient concentration in the water.

Epiphyton was weakly homeostatic for %P, whereas the remaining basal resources were strictly homeostatic for %P and N:P ratio (Table 3). All macroinvertebrates were strictly homeostatic for %P content and weakly to strictly homeostatic for C:N, C:P and N:P. The exception was *Aegla* sp. when feeding on epiphyton, which exhibited weak plasticity for N:P. Collectors had a lower degree of homeostasis when feeding on epiphyton than when feeding on FBOM. Fishes showed strict homeostasis for all stoichiometric ratios.

DISCUSSION

Consumers in La Choza had lower C:P and N:P than consumers in oligotrophic streams, and their basal resources had %P, C:P, and N:P within the ranges reported by other authors. We did not detect general effects of experimental fertilization on components of the food web. Only epiphyton, FBOM, and 2 macroinvertebrate species (*P. argentinus* and *P. canaliculata*) had changes in nutrient content or stoichiometric ratios. We observed high degrees of stoichiometric homeostasis at almost all trophic levels.

The nutrient concentrations in La Choza exceeded the values in naturally low-nutrient streams by at least an order of magnitude (Slavik et al. 2004, Bowman et al. 2005, Sabater et al. 2011) but were similar to those in im-

Table 2. Comparison of the stoichiometric ratios in Cyprinodontiformes from La Choza (control reach) and other aquatic systems. Means and ranges (in brackets) are reported, unless otherwise indicated.

Species	Group	C:N	C:P	N:P	Reference
Xiphophorus maculatus	Family Poeciliidae	4.3 (4.0–4.5)	218 (95–366)	51 (22–84)	Tsoi et al. 2011
Xiphophorus helleri	Order Cyprinodontiformes	4.3 (4.1–4.5)	318 (130–601)	74 (31–138)	
Gambusia holbrooki		4.3 (4.0–4.4)	458 (186–963)	108 (47–235)	
Phoxinus eos	Family Cyprinidae	5.55 (0.76)[a]	88.5 (35.2)[a]	15.7 (5.23)[a]	Sterner and George 2000
Phoxinus neogaeus	Order Cyprinodontiformes				
Margariscus margarita					
Pimphales promelas					
Cnesterodon decemmaculatus	Family Poeciliidae	4.7 (4.3–4.9)	22.7 (3–52)	4.7 (0.7–10.7)	This study
	Order Cyprinodontiformes				
Jenynsia multidentata	Family Anablepidae	4.4 (3.9–5.29)	29 (14–65)	8.7 (3.2–28.5)	
	Order Cyprinodontiformes				

[a] Standard deviation

Table 3. Values of 1/H (H = homeostasis coefficient) and significance of relationships between consumer–resource pairs for %P and the stoichiometric ratios. When $p > 0.1$ or 1/H < 0, the organism was strictly homeostatic. When $p < 0.1$ and 0 < 1/H < 1, the organisms were classified as: 0 < 1/H < 0.25 = homeostatic; 0.25 < 1/H < 0.5 = weakly homeostatic; 0.5 < 1/H < 0.75 = weakly plastic; and 0.75 < 1/H < 1 = plastic. SRP = soluble reactive P, FBOM = fine benthic organic matter, ns = not significant.

Group	Functional group	Species	Resource	%P			N:C			P:C			N:P		
				1/H	p	n	1/H	p	n	1/H	p	n	1/H	p	n
Producers	Macrophytes	Ludwigia peploides	SRP	0.21	ns	48							0.14	ns	44
	Epiphyton		SRP	0.27	0.011	56							0.19	ns	34
	Seston		SRP	-0.01	ns	59							0.07	ns	53
Invertebrates	Collectors	Palaemonetes argentinus	Epiphyton	-0.17	ns	33	0.40	<0.0001	33	0.24	0.067	33	0.37	0.038	33
			FBOM	0.39	ns	27	0.49	ns	24	0.45	0.110	24	0.34	ns	27
		Aegla sp.	Epiphyton	-1.14	ns	30	0.16	0.012	30	0.31	ns	29	0.64	0.015	29
			FBOM	-0.25	ns	24	0.08	ns	24	0.06	ns	23	-0.1	ns	23
	Filterers	Corbicula fluminea	Seston	0.13	ns	30	0.29	ns	30	0.29	0.084	28	0.26	ns	28
	Scrapers	Pomacea canaliculata	Macrophytes	-0.31	ns	34	0.36	0.034	34	0.28	ns	32	0.42	0.037	33
			Epiphyton	-0.23	ns	40	0.07	ns	40	0.35	0.016	27	0.46	0.031	27
Fishes	Omnivores	Jenynsia multidentata	Epiphyton	-0.13	ns	28	0.07	ns	28	0.10	ns	12	0.06	ns	12
	Herbivores	Cyphocharax voga	Macrophytes	0.00	ns	13	0.28	ns	13	0.14	ns	10	0.15	ns	11
	Detritivores	Hypostomus commersoni	FBOM	0.54	ns	8	0.05	ns	8	0.09	ns	8	0.14	ns	8

paired streams (Lauridsen et al. 2012). The P contents of the basal resources in La Choza were not higher than those of basal resources elsewhere (Cross et al. 2003, Bowman et al. 2005, Demars and Edwards 2007, Singer and Battin 2007, Sabater et al. 2011), and C:N and C:P were within the ranges reported in the literature. This absence of effects of the P enrichment could be related to a dilution effect in the autotrophs. That is, an increase in the P concentration of water would accelerate P uptake by autotrophs that would be used to stimulate new biomass production and not as a storage element in their biomass. Hence, even though the %P of algal and macrophyte biomass will not change, the total quantity of P in the basal resources at the reach scale will increase. The observation that the experimental nutrient addition to La Choza produced a moderate but significant increase in the algal and macrophyte biomass (Artigas et al. 2013) provides evidence to support this hypothesis.

C:P and N:P of consumers were lower in macroinvertebrates and Cyprinodontiformes than in analogous consumers in other systems, indicating high %P. Percent P in basal resources and across invertebrate assemblages usually is high in streams with chronically high P concentrations (Small and Pringle 2010). Lauridsen et al. (2012) found low C:P and N:P ratios in basal resources but not in consumers in a nutrient-rich stream. However, in La Choza, we observed P enrichment and reduced C:P and N:P only at the consumer level. This lack of a general pattern could be an effect of variability in climatic conditions, different levels of autotrophy, or the specific organisms in the systems, but in any case, the lack of a general pattern shows that relationships are more complex than they might appear.

Basal resources of La Choza differed in their stoichiometric ratios. Macrophytes and FBOM had high C:nutrient ratios, but epiphytic algae had the highest %N and %P. Thus, nutritional quality of detritus and macrophytes is poor and of algae is high. Among the macroinvertebrates, filterers (bivalves) had higher C:N and scrapers (gastropods) had lower %P than other invertebrate taxa. Therefore, mollusks in La Choza had the lowest body nutrient contents. In other studies, C:N, C:P, and N:P were higher in aquatic insects than in mollusks (Evans-White et al. 2005, Lauridsen et al. 2012). This difference might be related to the presence of detritus in the diets of bivalves and gastropods in La Choza and to the intake of macrophyte material by gastropods (in addition to epiphyton).

We sought to test whether the effects of experimental nutrient enrichment are transferred to the elemental composition of the multiple components of a stream's food web. We focused on P because it is generally the limiting nutrient in Pampean streams. However, we observed a proportionally lower increase in dissolved inorganic N concentration in water compared to the increase in P during the nutrient addition. This observation, together with the results concerning the changes in N content and C:N of some basal resources and macroinvertebrate species during the fertilization, suggests higher N uptake in the fertilized than in the control reach. Moreover, the addition of P might stimulate N uptake by the biota to maintain relative constancy in their internal N:P (Small et al. 2009). This hypothesis will require further testing and additional data.

Nutrient enrichment did cause an increase in P in FBOM and decreases in C:N and C:P in FBOM and epiphyton. Similar responses to enrichment have been reported in streams with lower nutrient levels (Cross et al. 2003, Slavik et al. 2004, Bowman et al. 2005, Sabater et al. 2011). Increases in %N and %P were detected in *P. canaliculata* and *P. argentinus*, with concomitant changes in C:N, C:P, and N:P in *P. argentinus*. Nutrient contents and stoichiometric ratios in fish did not change significantly. Low or undetectable responses of macroinvertebrate stoichiometry to experimental nutrient addition also have been reported in previous studies. Cross et al. (2003) observed an increase in P content in some macroinvertebrate taxa in response to nutrient input, but other authors have reported no response to nutrient addition (Bowman et al. 2005, Ortiz et al. 2009) or delayed response after 2 y of continuous fertilization (Sabater et al. 2011).

It is generally accepted that the elemental composition of basal resources tracks changes in dissolved nutrient concentrations, whereas animals maintain their elemental composition within a relatively small range (strict homeostasis), regardless of the elemental composition of their food (Sterner and Elser 2002, Elser and Hessen 2005). The results of several field studies have shown that consumer homeostasis is generally much stronger than basal resource homeostasis (Cross et al. 2003, 2005, Evans-White et al. 2005, Ortiz et al. 2009, Persson et al. 2010, Tsoi et al. 2011). We assessed the level of homeostasis of various foodweb components by relating the stoichiometry of the organisms to the stoichiometry of their resources. Strictly speaking, homeostasis should be evaluated in experiments in which the diet (and its elemental content) are controlled, but in the field, uncertainty in the diet can be decreased by establishing reliable consumer–resource associations using various approaches (stable-isotope analysis, review of bibliographic and field information, and gut-content analyses). Uncertainty can be further reduced by considering a large number of replicates to increase the reliability of estimates of the level of homeostasis at the species level. We established that, in La Choza, macrophytes and seston are homeostatic or strictly homeostatic for %P and N:P but epiphyton is weakly homeostatic for %P. Lower regulation of elemental content in epiphyton was also demonstrated by the changes in C:N and C:P in response to experimental nutrient addition. These results agree with those of other authors who reported greater stoi-

chiometric flexibility in algal communities and biofilms (Cross et al. 2005, Persson et al. 2010, Tsoi et al. 2011) and strict homeostasis for C:N:P ratios in macrophytes (Demars and Edwards 2007). The stoichiometric homeostasis of seston may be explained by the short time that this basal resource spent in the experimental reach (~20 min), which clearly is not sufficient to change their stoichiometric ratios. In the case of macrophytes, lower stoichiometric flexibility could be related to the lower turnover rate of macrophytic biomass in comparison to algal communities such as epiphyton.

Consistent with the findings of other investigators (Cross et al. 2003, Ortíz et al. 2009, Persson et al. 2010), the primary consumers analyzed in our study were strictly homeostatic for %P. Most species also were strictly to weakly homeostatic for the stoichiometric ratios, in spite of substantial imbalances in C:N and C:P in their basal resources. The consistent stoichiometric ratios in macroinvertebrates and fishes during the fertilization experiment are additional evidence of the homeostatic response of consumers in La Choza. Sabater et al. (2011) suggested that the delayed response of elemental content of basal resources and macroinvertebrates to experimental fertilization of a forested stream was associated with light limitation. Unlimited light in Pampean streams guarantees their autotrophic character and use of available resources, conditions sufficient to cause a rapid response of community metabolism to nutrient increases (Vilches and Giorgi 2010, Acuña et al. 2011) and strong effects on the stoichiometry of the food web.

Primary consumers that can exploit various food sources may reduce stoichiometric imbalances by increasing the proportion of high-quality resources in the diet (Lauridsen et al. 2012). Macroinvertebrate collectors in La Choza feed on both epiphyton and FBOM. However, imbalances in C:P and N:P were higher for FBOM than for epiphyton, indicating that collectors might prefer epiphyton as a main food source. This assumption is supported by the lower degree of homeostasis of *P. argentinus* and *Aegla* sp. for C:N, C:P, and N:P when they consume epiphyton and the strict homeostasis for FBOM. Considering that epiphyton was weakly homeostatic for P and that *P. argentinus* responded to the fertilization by increasing its P content and reducing C:P and N:P, our results suggest the potential for bottom-up response of collectors to dissolved P in water via its incorporation in the epiphyton. At the species level, a possible mechanism to explain this weak homeostasis would be a higher storage of nonRNA P when more P is available through food intake. For instance, Small et al. (2011) observed that chironomids from high-P streams allocated comparatively less P in nucleic acids than chironomids from low-P streams. They suggested that other forms of P storage (e.g., in polyphosphate granules) could occur in organisms from high-P

streams, increasing their fitness in that environment. It is possible that P storage in non-nucleic acid compounds could be a usual mechanism in consumers of nutrient-rich systems, such as La Choza stream.

In summary, our results support the hypothesis of a high degree of stoichiometric homeostasis in almost all components of the food web (regardless of their trophic position) in La Choza. Further evidence to support this hypothesis is provided by the lack of a strong response to the experimental P addition for most basal resources and consumers. Evidence exists to indicate that aquatic communities with higher numbers of species take better advantage of niche opportunities than those with fewer species because coexistence of different species that are best adapted for different habitats allows diverse systems to capture greater proportions of nutrients (Cardinale 2011). Hence, in ecosystems like Pampean streams, where rich communities develop in high-nutrient environments, biodiversity may help buffer ecosystems against the ecological impacts of nutrient pollution. In addition, in these streams, biota are not exposed to nutrient shortages that may alter their stoichiometric composition and should, therefore, maintain relatively homeostatic elemental contents. However, as Lauridsen et al. (2012) have suggested, a certain degree of plasticity in primary consumer elemental composition may be expected because of temporal variations in the quality and availability of basal resources. The high stoichiometric homeostasis observed across the trophic web of the La Choza stream probably can be explained by the long coexistence of the biota in an enriched environment.

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