Potential responses of oligochaetes (Annelida, Clitellata) to global changes: Experimental fertilization in a lowland stream of Argentina (South America)

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ABSTRACT

One of the possible consequences of climatic change for streams and rivers in the pampean region of South America is an increment in nutrient loads. To analyze this possible perturbation on a biological scale, the response of oligochaetes to an experimental eutrophication of the La Choza Stream, Argentina was studied. We proposed that the addition of nutrients could increase the abundance, biomass, and species composition of the stream. Two stretches (Control and Treatment sites) were selected, with bimonthly samples being taken (March 2007 through February 2009) in two habitat types: the sediments and the aquatic vegetation. On each sampling occasion the environmental variables were measured. The nutrient addition consisted in the continuous dissolution of a commercial fertilizer. The oligochaete mean density and total biomass, the taxonomic richness, the Shannon diversity (H'), and the evenness (E) were calculated and the BACI ANOVA design used to compare the differences between the sites. Thirty-three species of the families Naididae (Naidinae, Pristininae, Tubificinae, and Rhyacodrilinae), Opistocystidae, Enchytraeidae plus Aphanoneura Aeolosomatidae were collected. The oligochaete abundance and biomass increased significantly in the sediments and on the aquatic vegetation, especially among the Naidinae and Pristininae during their asexual reproductive phase. The diversity and evenness varied significantly in the sediments with the nutrient addition. Significant differences in the species richness and diversity were found on the aquatic vegetation, with both increasing at the treatment site after the fertilization. A significant correlation (Spearman) was observed between the oligochaete density in the sediments and the NO_3-N and NH_4-N concentration in the water. The increment in the naidines resistant to the fertilizer throughout the experiment could be explained by the greater nutrient availability, their mode of reproduction, and their short life cycles. The results of our study suggested that the incorporation of nutrients modified the composition of the oligochaete assemblage in favor of herbivores and detrivores. The usefulness of these indicator organisms in monitoring freshwater systems is subsequently discussed.

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Introduction

Streams are habitats of worldwide ecological consequence, playing a significant role in global biogeochemical cycles through the processing, retention and/or transport downstream of solutes and nutrients, both autochthonous and terrestrial, to marine ecosystems (Martí et al., 2006). Streams are complex biotic networks occupied by organisms with diverse ecological functions. South America is one of the continents with the greatest proportion of freshwater systems and is accordingly recognized for the variety of its aquatic environments and the extent of its biodiversity (Rodrigues Capítulo et al., 2010). Nevertheless, the commodities and benefits the bodies of running water provide nowadays face an unprecedented array of environmental threats, many of which are the direct or indirect result of anthropogenic activities – e.g., eutrophication, toxic pollution, and climatic change (Perkins et al., 2010). Accordingly, streams and rivers have been used extensively to study the impacts of such perturbations on biological-community structures, but the ways in which those insults are manifested across different levels of biological organization (e.g., populations, communities, food webs, ecosystems) are still far from clear (Durance and Ormerod, 2007; Woodward, 2009).

Many studies (Hogg and Dudley Williams, 1996; Martí et al., 2006) have thus far attempted to understand and estimate the ecological consequences of climatic change on ecosystems and biodiversity, both of which biological commodities have currently become issues of central concern in environmental management.

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Recent investigations (Burgmer et al., 2007; Daufresne et al., 2007) have highlighted changes in the timing of life-cycle events, trophic interactions, and spatial shifts in response to temperature preferences, with a consequent modification of biodiversity and community composition. The alterations in the abundance, composition, and diversity of species have implications with respect to food-web stability and the fluxes of resources through the web. In this way, a temperature increase is generally considered to be detrimental to water quality, with aquatic invertebrates showing strong responses to climatic warming (Durance and Ormerod, 2007; Perkins et al., 2010).

River systems in temperate and tropical climates could experience a decrease in runoff and an increase in temperatures as a result of the present global change (IPCC, 2007), with a resulting habitat fragmentation and altered community dynamics. For the pampean region in particular, current models (Rodrigues Capítulo et al., 2010) predict temperature and rainfall increases along with changes in the runoff patterns that will lead to a greater input of nutrients and contaminants into streams and rivers. Since a particularly consequential ecological function of fluvial ecosystems is their capacity to process and retain nutrients (Martí et al., 2006), the predicted flow variations in river systems could lead to increased nutrient levels therein.

Oligochaeta are invertebrates frequently used as bioindicators of the health of freshwater ecosystems and, among other parameters, the trophic conditions in bodies of water (Särkkä, 1987; Lang, 1990; Lafont et al., 2001; Krodkiewska and Michalik-Kucharz, 2009). For example, the presence and abundance of oligochaetes can often be indicative of different levels of organic pollution and/or eutrophication (Verdonschot and Ter Braak, 1994; Lin and Yo, 2008; Armendáriz et al., 2011).

The present study focussed on the response of the assemblage of oligochaetes to the experimental eutrophication caused by a deliberate discharge of nutrients into a pampean stream (Argentina). The hypothesis proposed was that the effects of the nutrient loading could be reflected in a modification of the oligochaete assemblage composition as manifested in an increase in both their abundance and biomass. This work is part of a comprehensive study in which the effects on stream communities of experimental nutrient enrichment were assessed in three headwater streams within different biomes (in northeast Province of Buenos Aires, Argentina, a Mediterranean stream in northeast Spain, and an Andean stream in north Colombia).

Materials and methods

Study site

The study was carried out in a segment of the La Choza Stream – a tributary of the Reconquista River, La Plata Basin, Argentina (Fig. 1). The La Choza is a low discharge due to the mild downward slope in the surrounding terrain with high levels of suspended solids, abundant submerged and floating aquatic vegetation, and pastureland representing the dominant feature of the adjoining countryside. This stream constitutes a headwater system having areas of high biotic diversity with a low anthropic impact and diffuse pollution originating from the modest farming and cattle-raising activity of the surrounding area. The stream's width varies between 4 and 10 m with the narrow, winding, incised sectors of its course having greater current velocity and a hard and irregular bed of caliche. The wider segments are characterized by regular banks, shallower depths, lower discharges, and a uniform bottom composed of fine sediments (Colautti et al., 2009). For the present study two 100-m-long stretches of the stream were selected: the upstream Control Site at 34°44′23.8″S; 59°06′27.30″W and the

downstream Treatment Site at 34°42′7.74″S; 59°04′37.79″W, with a distance of 5 km intervening between the two.

Field sampling and treatments

Water-chemistry-analysis methods

Bimonthly samplings, from March 2007 through February 2009, were performed at each sites. On each sampling occasion physicochemical parameters were measured: temperature, pH, conductivity, dissolved-oxygen level, nitrogenous nutrients (*e.g.*, nitrates, nitrites, and ammonium ions), and soluble reactive phosphorus (SRP).

The process of experimental fertilization, begun at the end of October 2007, involved a continuous dissolution of nutrients at the treatment site of the stream. The low water velocity in the stream forced a distribution of fertilizer consisting in Compo Nitrofoska® (12% nitrogen, 12% phosphorus, 17% potassium), a mixture of frequent use in farming, along the reach. This addition was performed twice weekly in order to simulate the natural dynamic of nutrient input. At the same time, triplicate water samples were filtered through sintered-glass-fiber filters (Whatman GF/F, Whatman International Ltd., Maidstone, England) and then analyzed for ammonium, nitrite, nitrate, and SRP by standard methods (APHA, 1989; Artigas et al., submitted for publication: Ecological Applications).

Oligochaete sampling and identification

On each sampling opportunity (March 2007–2009), triplicate samples of the two habitat types, the sediments and the aquatic vegetation, at each site (Control and Treatment) were collected. The sediments were removed with an Eckman 100-cm^2 dredge and the vegetation with a 25-cm × 25-cm plexiglass square (area 625 cm^2); both samples were then fixed *in situ* in 5% (v/v) aqueous formaldehyde.

In the laboratory the samples were washed on a 500-μm – pore-size sieve and then stained with erythrosin B. The material was separated manually and examined under the stereoscopic and light microscopes. The annelids were classified down to the species level wherever possible, following Brinkhurst and Marchese (1992), Opinion 2167, ICZN (2007), Struck and Purschke (2005), and Glasby and Timm (2008) before a final preservation in 70% (v/v) aqueous alcohol.

After drying the total number of oligochaetes collected from a given sample to a constant value in a 60 °C oven, the total oligochaete biomass was estimated from the total weight of those organisms measured on an analytical balance (Ohaus Discovery, precision 0.01 mg).

Data analysis

To compare the physicochemical parameters measured between the control and treatment sites the Mann–Whitney Rank Sum Test was used.

The average density and biomass of oligochaetes at each site and habitat type were calculated and then the following indices assessed: taxonomic richness (R: total number of species or taxa present in each sample), the Shannon diversity index (H'), and evenness (E).

The BACI (Before-After-Control-Impact) or BACIPS (BACI-Paired Series) was considered the most appropriate analysis, as the addition of fertilizer to various stretches of the river was not feasible (García-Berthou et al., 2009). Through this analysis the differences in the temporal series of the treated (or impacted) zone and the control are compared, before and after the intervention or experimental treatment. In the present study, the effects of nutrient enrichment on oligochaete density and biomass and on the applied
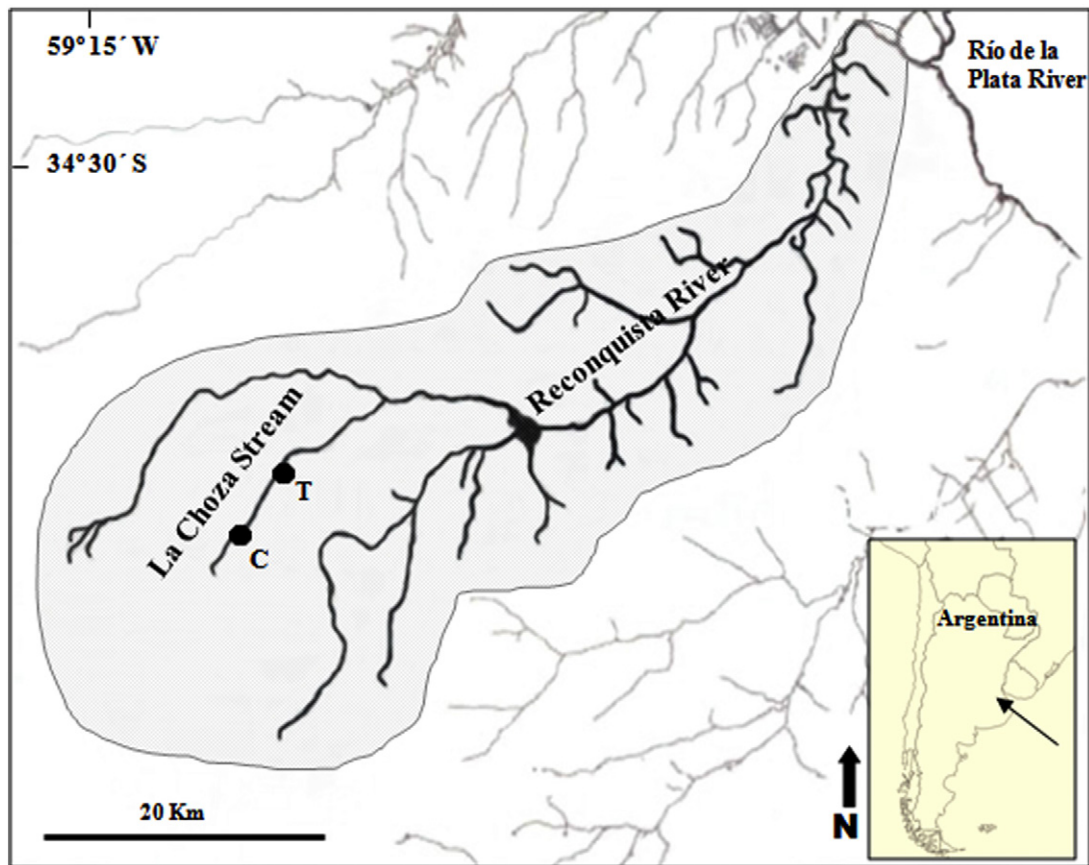
Fig. 1. Map of the Reconquista River Basin, Argentina (in gray). La Choza is a first-order stream and a tributary of the Reconquista River. Shown are the two sampling sites: control (C) and treatment (T).

indices were analyzed for each habitat individually according to a BACI-ANOVA design (Underwood, 1999), through the use of IBM SPSS Statistics 19.0. The effects of the treatment were considered statistically significant at *p* values <0.05.

The Spearman method was then used to analyze the relationship between every paired combination of the following variables: oligochaete densities *vs.* SRP or *vs.* nitrogen as nitrate, nitrite, or ammonium concentrations.

Results

The experimental fertilization of the La Choza Stream increased the average concentration of SRP in the water column above the natural value but barely altered the dissolved inorganic nitrogen levels (Fig. 2). We found statistically significant differences in SRP ($p < 0.001$) and conductivity ($p < 0.001$), between the control and the treatment sites.

The total of 22,900 annelids collected during the study pertained to 33 species within the oligochaete families Naididae (Naidinae, Pristininae, Tubificinae, and Rhyacodrilinae), Opistocystidae, and Enchytraeidae, together with the recently reclassified polychaete Aphanoneura Aeolosomatidae (Struck and Purschke, 2005) (Table 1).

The aquatic vegetation at the control site was represented by *Ludwigia peploides* (Kunth) Raven, and at the treatment site by *L. peploides*, *Bacopa monnieri* (L.) Penn and *Typha dominguensis* (Pers.). During August, October, and December the vegetation at the treatment site had decayed markedly, as had likewise that of the control site during the last of those months. For this reason, samples at both sites for those respective months could not be obtained. The sampling of the vegetation was thereafter resumed in February 2008

and continued for the remainder of the months except for July of that year.

The abundance of the oligochaetes after the fertilization treatment, showed statistically significant differences in the sediments (BACI-ANOVA: $F = 24.2$, $p < 0.001$) and on the aquatic vegetation ($F = 4.75$, $p = 0.003$). After the fertilization, the average abundance of the oligochaetes increased from 6700 (control) up to 16,000 ind/m^2 (treatment) in the sediments, and from 2500 (control) to 5700 ind/m^2 (treatment) on the vegetation (Fig. 3).

The sediments at the treatment site exhibited an increase in the abundance of Naidinae and Pristininae by December 2007, immediately after the artificial fertilization. In fact, the Naidinae maintained an increment in their density during all of the post-fertilization period, while in certain samplings their enhanced abundance was accompanied by high levels of members of the Tubificinae – *e.g.*, in February, May, and December 2008. After the fertilization the vegetation likewise evinced an elevation in the abundance of these two subfamilies in February 2008. At a much later time after the nutrient treatment, and continuing until the end of the study, the three groups that became registered at the highest densities were the Naidinae, the Pristininae, and the Aeolosomatidae (Fig. 4).

Although the majority of the species were found at both sites, some were only represented exclusively at only one of the two sites: for example, *Paranais frici*, *Pristina breviseta*, *Slavina evelinae*, and the Aeolosomatidae at the treatment site and *A. leydigi*, *P. synclites*, *P. proboscidea*, *P. notopora*, and the opisthocistid *Trieminentia corderoi* at the control site (*cf.* Table 1). In contrast, *Limnodrilus hoffmeisteri* was registered at both sites, both in the sediments and on the vegetation, throughout the study without manifesting substantial changes in abundance after the fertiliza-

Table 1

Taxa recorded in La Choza Stream, Argentina. C: control site, T: treatment site, B: both control and treatment sites.

		03/07	06/07	08/07	10/07	12/07	02/08	05/08	07/08	09/08	12/08	02/09
Naididae Naidinae	*Dero (Dero) digitata* (Müller, 1773)[a,b]	B	B	C	B	B	B	B	B	B	B	T
	Dero (Dero) pectinata Aiyer, 1929[a,b]	B		C	B	B	T	B	C	B	B	B
	Dero (Aulophorus) furcatus (Müller, 1773)[a,b]			C	B			B				
	Dero (Aulophorus) costatus Marcus, 1944[a,b]			C							B	T
	Chaetogaster diaphanus (Gruithuisen, 1828)[a,b]		B	B					B	B	T	T
	Chaetogaster diastrophus (Gruithuisen, 1828)[a,b]							T	C			
	Bratislavia unidentata (Harman, 1973)[a,b]	C	C	C	T	T	T	T	T	T		T
	Nais variabilis Piguet, 1906[a,b]	B	T	B	T	T	T	C	B	B	B	
	Nais pardalis Piguet, 1906[a,b]	T	T	B	C	B	T	C	B	B		
	Nais communis Piguet, 1906[a,b]		T				T		B	C	T	T
	Stephensoniana trivandrana (Aiyer, 1926)[a,b]	T				T	B	B	B	C	C	
	Stylaria fossularis Leidy, 1852[a,b]	T	T	T					T	B	T	T
	Allonais paraguayensis (Michaelsen, 1905)[b]	T	C									
	Slavina isochaeta Cernosvitov, 1939[a,b]	T	B	B	B	B	B	T		B	B	B
	Slavina evelinae (Marcus, 1942)[a]	T		T	T							
	Paranais frici Hrabé, 1941[a,b]				T	T			T	T		
	Amphichaeta leydigi Tauber, 1879[b]								C			
Pristininae	*Pristina aequiseta* Bourne, 1891[a,b]	C	C				C	C	C		B	T
	Pristina leidyi Smith, 1896[a,b]	C			C		C	C	C	C		T
	Pristina americana Cernosvitov, 1937[a,b]	B	C		T	B	B	B			T	T
	Pristina jenkinae (Stephenson, 1931)[a,b]	B		C			B	B	C	C		B
	Pristina longidentata Harman, 1965[a,b]		T		B		T					T
	Pristina breviseta Bourne, 1891[a]		T									
	Pristina macrochaeta Stephenson, 1931[a,b]	C							C			T
	Pristina synclites Stephenson, 1925[a]			C								
	Pristina proboscidea Beddard, 1896[a,b]		C		C				C	C	C	
	Pristina notopora (Cernosvitov, 1937)[b]								C			
Tubificinae	*Limnodrilus hoffmeisteri* Claparede, 1862[a,b]	B	B	C	B	B	B	B	B	B	B	B
	Limnodrilus udekemianus Claparede, 1862[a,b]	T	T	C								
	Aulodrilus piguetti Kowalewski, 1914[a,b]	B	B	C	B	B	B	B	B		C	T
Rhyacodrilinae	*Bothrioneurum americanum* Beddard, 1894[a,b]	T	B	C		T	C	C				T
Enchytraeidae[a,b]		T	B	C	T		T	T	B		T	
Opistocystidae	*Trieminentia corderoi* (Harman, 1969)[b]	C					C					
Aphanoneura Aeolosomatidae	*Aeolosoma*[b]										T	T

[a] Taxa registered in sediments.

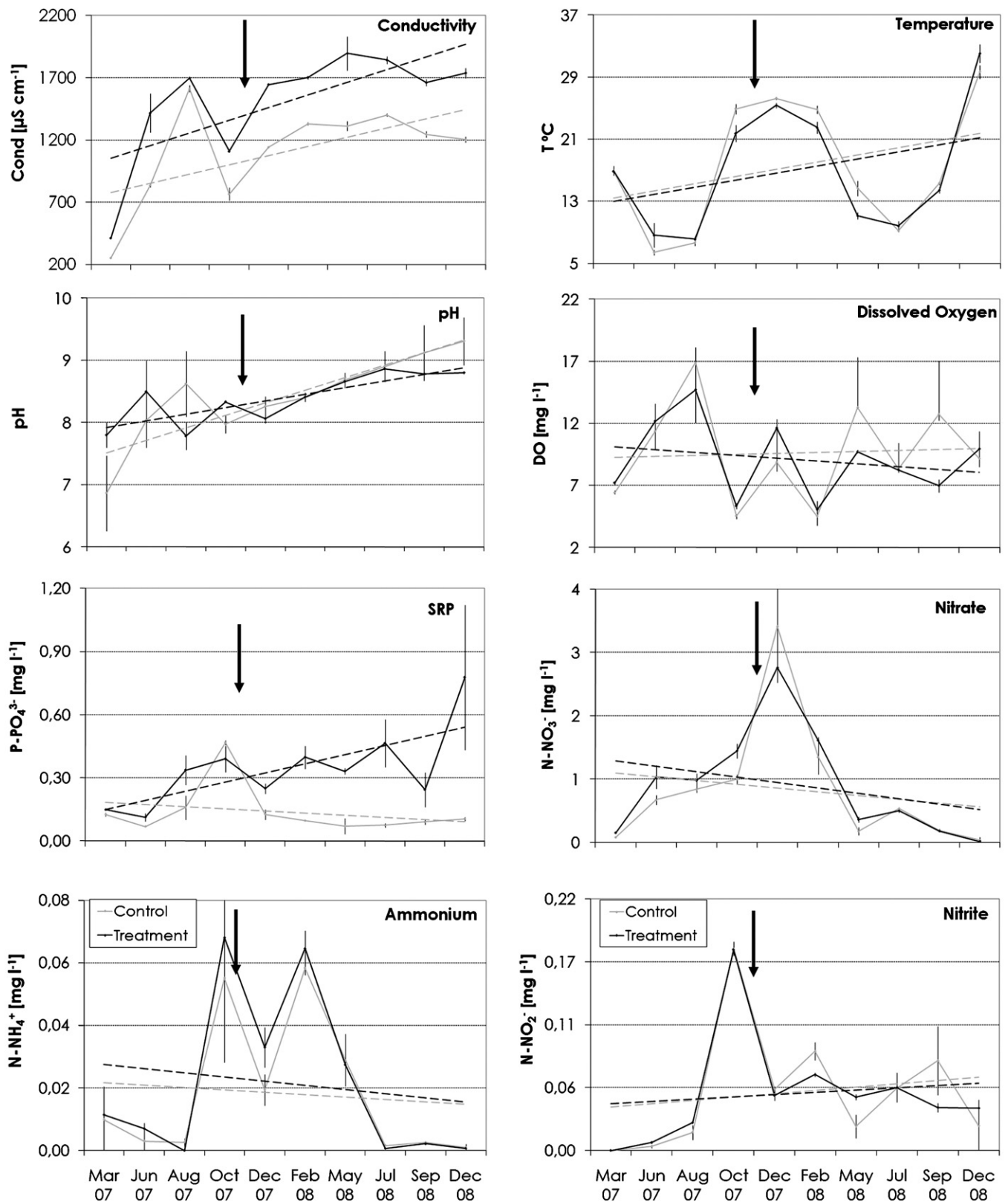[b] Taxa associated to vegetation.

Fig. 2. Physicochemical variables and nutrient contents at the control (in gray) and treatment (in black) sites in the La Choza Stream, Argentina. The error bars bracket the standard deviation. The arrow indicates the beginning of the experimental fertilization. The dashes represent the trendlines.

tion. *Stylaria fossularis*, however, was recorded almost exclusively at the treatment site, likewise in the sediments as well as on the vegetation; but was also present at the control site, though in scant numbers (33 ind/m^2), in only September 2008. In the

treatment-site sediment the density of this species rose from 800 to 2500 ind/m^2 between June 2007 and July 2008, whereas on the vegetation the abundance increased from about 140 to almost 460 ind/m^2, but between March 2007 and February 2009. One
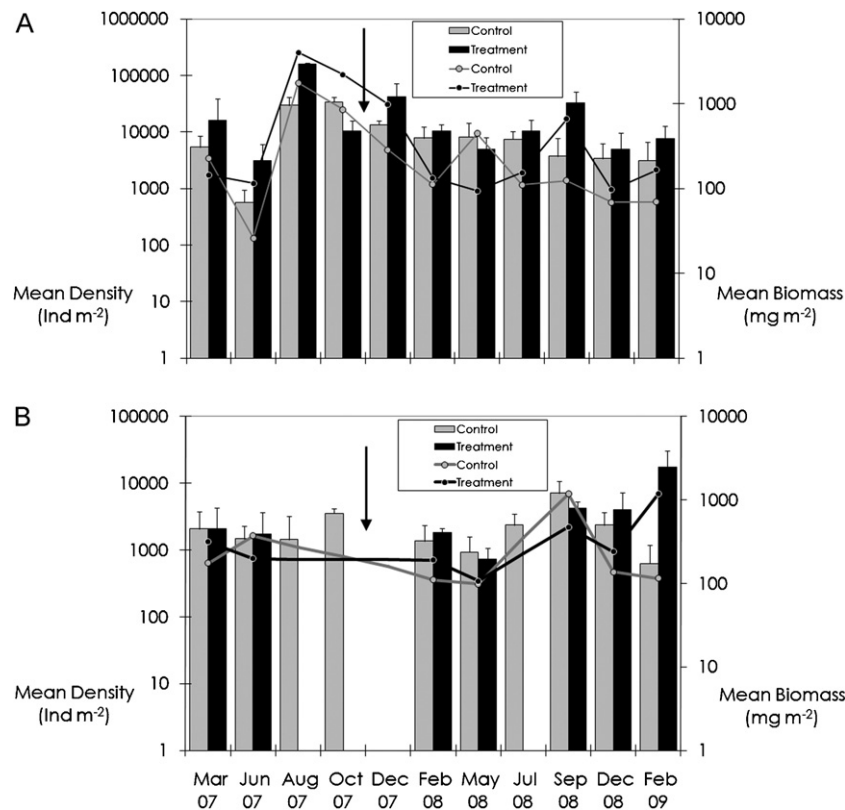
Fig. 3. The average density (bars) and biomass (lines) of the oligochaetes at the control (in gray) and treatment sites (in black), in the La Choza Stream, Argentina. (A) Oligochaetes in sediments. (B) Oligochaetes on aquatic vegetation. The error bars bracket the standard deviation. The arrow indicates the beginning of the experimental fertilization.

species, *S. evelinae*, exhibited an extraordinary pattern, it being registered at the treatment site before the fertilization but then was never collected again at that site.

The differences registered in the total abundance became reflected in the oligochaete total biomass. Statistically significant differences in this parameter were found in the sediments ($F = 4.336$, $p = 0.005$) and on the vegetation ($F = 4.636$, $p = 0.006$). After the experimental fertilization the average oligochaete biomass increased from about 160 to some 340 mg/m^2 in the sediments and from around 340 to 460 mg/m^2 on the vegetation (Fig. 3).

Statistically significant correlations were registered between the oligochaete density in the sediments *vs.* the NO$_3$-N concentration in water ($r = 0.366$, $p = 0.046$) and *vs.* the NH$_4$-N concentration in water ($r = 0.587$, $p = 0.005$). No significant correlations were found, however, between the oligochaete abundance in sediments and the concentrations of SRP and NO$_2$-N in the water ($p > 0.05$), nor between the oligochaete density on the vegetation *vs.* the nutrients in the water ($p > 0.05$).

We found significant variations in the species diversity and evenness in the sediments (BACI-ANOVA: $F = 3.294$, $p = 0.004$ and $F = 4.292$, $p < 0.001$, respectively). On the aquatic vegetation, significant differences in the species richness ($F = 3.962$, $p = 0.008$) and diversity ($F = 3.986$, $p = 0.007$) were also found, with these parameters increasing at the treatment site after the fertilization (Fig. 5).

Discussion

Modern agriculture has greatly influenced freshwater ecosystems worldwide, especially through the undesirable side effects of the use of fertilizers and pesticides on the land that later may enter adjacent freshwater ecosystems or the subterranean water (Brock et al., 1995; Cuppen et al., 1995). In aquatic environments

from urbanized, industrialized and/or agricultural areas, eutrophication in this manner is usually accompanied by chemical pollution that results in the presence of toxicants or pesticides in the effluent waterways. Furthermore, the global climate change is predicted to affect the physical, chemical and hydrologic properties of pampean lotic systems and consequently, the structure and function of their biological communities (Rodrigues Capítulo et al., 2010).

Because of the differing chemical properties of nitrogen and SRP, the amount of nutrients reaching fluvial ecosystems not only becomes severely increased, but the relative proportions of those two classes of inorganic compounds are also altered (Martí et al., 2006). Multiple influences have been identified that change nutrient dynamics in streams and rivers, and the efficiency of nutrient removal is reduced in polluted streams. Moreover, stressors are themselves by their nature often multivariant, and synergistic interactions among them can amplify their individual effects (Strayer and Malcom, 2007; Woodward, 2009).

The fertilization of the La Choza Stream resulted in higher SRP levels in the water column. The other nutrients added were most probably incorporated into organic material and sediment. This finding suggests that the amount of nutrients entering through the experimental fertilization could have been incorporated into the ecosystem by algae, macrophytes, and/or bacteria as had been noted previously by other authors (Verdonschot and Ter Braak, 1994; Brock et al., 1995; Artigas et al., submitted for publication: Ecological Applications). This differential stimulation produced a characteristic bottom-up regulation within the stream communities along with an overall shift towards autotrophy. Significant correlations were found between the oligochaete density and the concentration of NH$_4$ and NO$_3$ in the water. Nevertheless, the lack of correlation between the oligochaete density and SRP concentrations could be explained by delayed shifts in the response of the
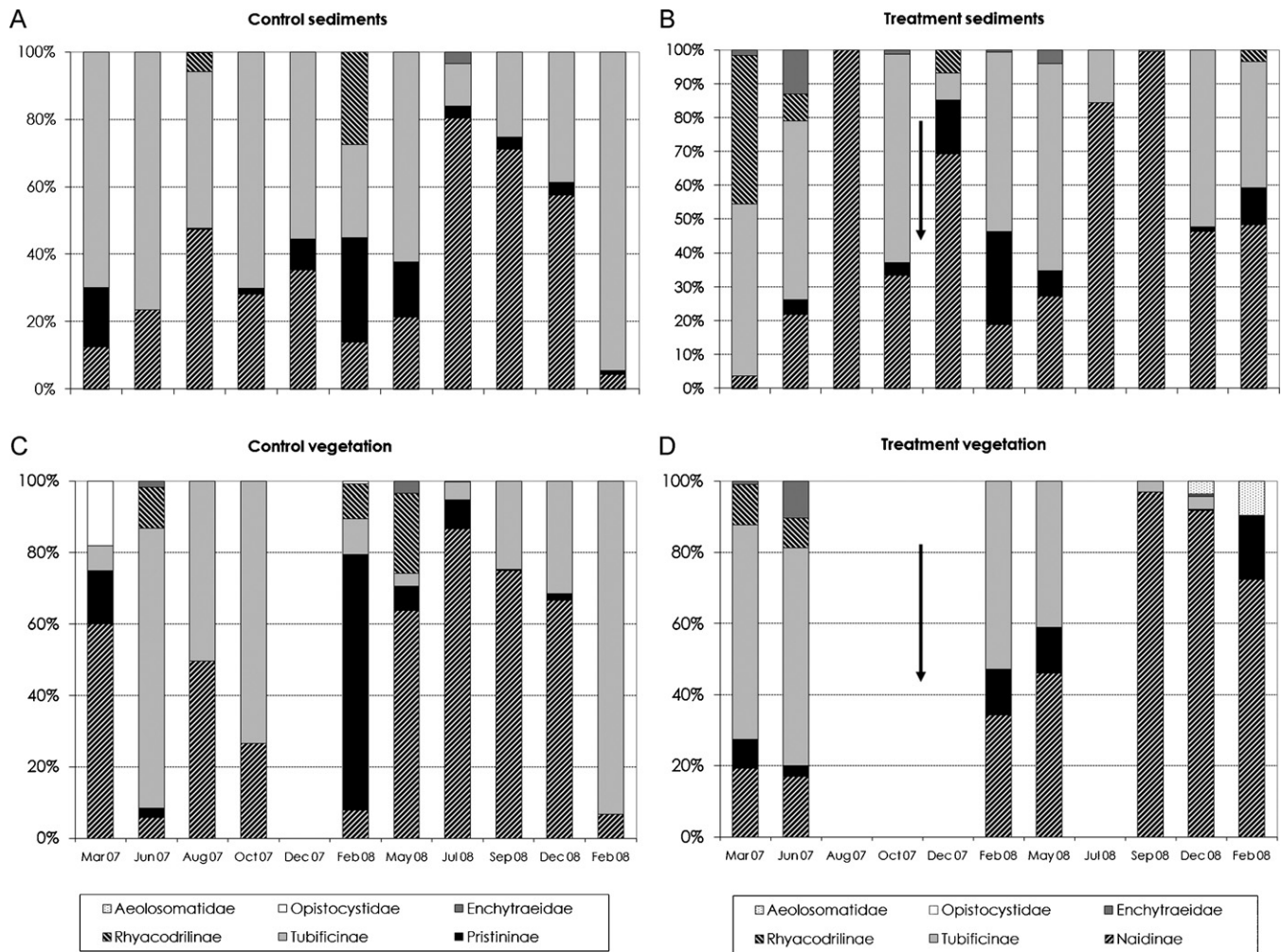
Fig. 4. Relative abundance of the main oligochaete families and subfamilies registered at the control and treatment sites in La Choza Stream. (A) Oligochaetes in sediments from control site. (B) Oligochaetes in sediments from treatment site. (C) Oligochaetes on aquatic vegetation from control site. (D) Oligochaetes on aquatic vegetation from treatment site. The arrow indicates the beginning of the experimental fertilization.

worms to an abrupt elevation in the levels of this ion within the ambience. In this regard, annelid populations have been found to react slowly to increases in SRP concentrations (Lang, 1990).

Notwithstanding, the assemblage of oligochaetes, by the end of the study, began to register changes with respect to certain variables (*e.g.*, abundance, total biomass, taxonomic richness, diversity, and evenness) in response to the added nutrients. The observed increase in biomass was related to an increment in the abundance of those specific individuals (mainly of Naidinae and Pristininae) with an active phase of asexual reproduction (personal observation), and not to an increase in the body size of each individual collected.

On the aquatic vegetation of the La Choza Stream the fertilization produced an increase in the oligochaete taxonomic richness and diversity, mediated through changes in the quantity and/or the quality of food available to those populations and the complex short-term composition of the microhabitat. Members of the Naidinae prefer plants and plant detritus as substrate, while those of the Tubificinae seek a mineral substrate (Barbour et al., 1999; Krodkiewska and Michalik-Kucharz, 2009). Notably, in our study, the abundance of *Stylaria fossularis* increased after the artificial enrichment, immediately following the periphyton increase reported by Rodrigues Capítulo et al. (2010) and Artigas et al. (submitted for publication). These authors found that the bacteria and algae associated with the sediment responded significantly

to the addition of the nutrients by increasing their biomass (by 20–30% and 49%, respectively), while changes in the structure of the diatom taxocenosis were also noted. The naidine oligochaete *Stylaria fossularis* was observed to crawl on the filamentous algae instead of the substratum or bore into decayed stems of submerged plants (Lin and Yo, 2008). The pronounced increase in the numbers of naidines at the end of the experimental period, although annelids are not affected by the fertilizer used, might be explained by increased amounts of available food, their asexual modes of reproduction, and their relatively short life cycles (Cheng and Chang, 1999). The feeding habits of a closely related species, *Stylaria lacustris* – and of other naidines such as *Dero digitata*, *Bratislavia unidentata*, and *Slavina* spp. is herbivorous–detrivorous. These annelids consume periphytic algae, particularly diatoms, and senescent macrophyte material, so that the constituent members of this subfamily are thus generally classified as gatherers, collectors, and/or grazers (Brock et al., 1995; Cuppen et al., 1995).

In contrast, the major food sources for deposit feeders are the sedimentary organic matter along with the associated bacteria and microalgae. The variation in the time of bioavailability and in the quantity of the detritus influences the growth and survival of these benthic animals, the occupation depth of which is limited by depth of the aerobic layer of the sediment (Cheng, 1995; Cheng and Chang, 1999). In a study involving an insecticide-treated stream, the oligochaetes were found to be the dominant noninsect
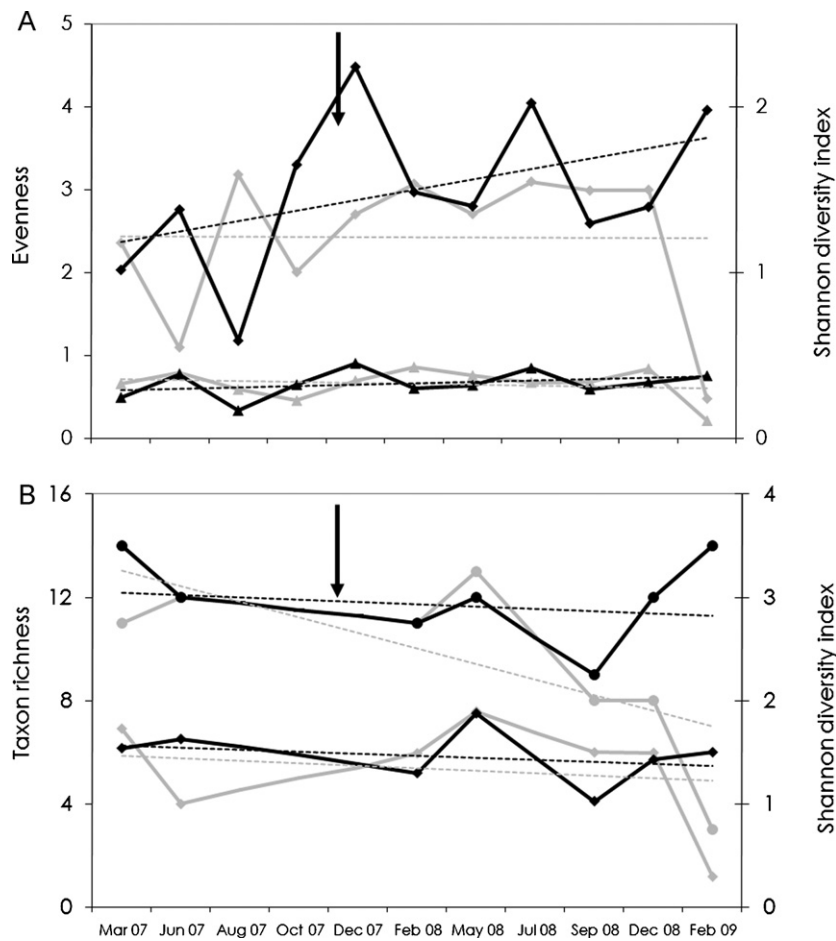
Fig. 5. Taxon richness (number of taxa/sample, R, ●), Shannon diversity index (*H*, ♦) and evenness (*E*, ▲) for control (in gray) and treatment (in black) sites at the La Choza Stream, Argentina. (A) Sediment samples. (B) Aquatic-vegetation samples. The arrows indicate the beginning of the experimental fertilization. The dashed lines represent the trendlines.

contributors to the total production, with the gatherers (the Oligochaeta along with the Chironomidae and the Copepoda) being the principal components of the macrofaunal community (Lugthart and Wallace, 1992).

Our results suggest that in nutrient-enriched systems, an increase in the abundance, biomass, diversity and evenness of the oligochaetes was accompanied by an uptake and storage of nutrients by the sediments – the main source of food for these annelids. These results indicate the potential for using oligochaetes at the species level as indicators of the environmental conditions, and particularly the trophic state, of a lotic system. For example, with respect to functional responses in this experiment, the fertilization resulted in an increase in diversity along with a shift in composition in favor of herbivores and detrivores. The replacement of sensitive species in a community often results in a greater tolerance of the overall population to environmental stress, although this transition brings about a decrease in the efficiency of resource use. Future research should examine whether the environmental influences within a stream's ecological networks are altered by the addition of nutrients to the surroundings, taking into account the interactions (*i.e.*, the food webs) between the oligochaetes as the consumers and the algal and/or the bacterial communities as the producers.

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