



Does grazing change algal communities from grassland and pine afforested streams?: A laboratory approach



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ABSTRACT

Drastic changes in the composition and physiognomy of riparian vegetation, such as the conversion of grassland to forest, are expected to alter interactions among light availability, primary producers and herbivores. Our aim was to examine in laboratory the influence of a ubiquitous grazer on periphyton grown in a grassland unshaded stream (reference) vs. periphyton from a nearby pine afforested stream. Besides, we evaluated how the community responds to the removal of grazing. Given that grassland streams are exposed to higher light intensity and grazers are more abundant compared to afforested streams, we proposed that if biofilm grown in the afforested stream are dominated by grazing-vulnerable algal species, grazing pressure by *Helicopsyche* sp. should be stronger. In addition, if biofilm from the afforested stream has low quality or is less abundant as food for consumers, the effects of *Helicopsyche* sp. may be stronger or weaker depending on their feeding decisions. *Helicopsyche* sp. caused a decrease in richness and diversity in periphyton grown in the grassland stream and its net grazing effect on chlorophyll *a* (Chl *a*) was higher. Algal community composition from grassland stream was strongly changed after grazing, with a decrease in the proportion of overstory algae. In contrast, algal community structure of periphyton from the afforested stream was neither affected by grazing nor by grazing exclusion. *Helicopsyche* sp. produced significant changes in a short time in structural attributes of algal communities, mainly in periphyton from the grassland stream suggesting that herbivory, as a functional factor, is diminished following afforestation.

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Introduction

Land use changes at the catchment scale affect stream habitat condition, productivity and biotic interactions, and consequently alter stream functioning, water quality and ecosystem services (Allan and Castillo, 2007). In open-canopy streams, benthic algal community is the main source of organic carbon in the food web. Changes in the composition and physiognomy of riparian vegetation, such as the conversion of grassland to forest, may affect the availability of light and nutrients, discharge and temperature (Jobbágy et al., 2006, 2013), and hence profoundly alter algal

communities (Stevenson, 1996) and food webs (Thompson and Townsend, 2005).

Light in particular has been reported as a limiting factor in shaded streams, changing the physiognomic and taxonomic structure of algal communities (Steinman and McIntire, 1987; Lange et al., 2011). Light reduction leads to slower development, lower cell density and less vertical stratification of epilithic biofilms (Hudon and Bourget, 1983). Wellnitz et al. (1996) found that light level had a strong effect on the abundance of common algae. The green filamentous algae may outcompete diatoms under full light conditions, but in shaded conditions diatoms may be the dominant algal type (Steinman et al., 1989; Melody and Richardson, 2004; Villeneuve et al., 2010). In experimental channels, Bourassa and Cattaneo (2000) found higher proportion of cyanobacteria, single cells and colonial forms in shaded channels whereas filamentous chlorophytes and chain-forming diatoms were prevalent in open channels. Many studies have shown that irradiance can also influence chemical composition (Steinman et al., 1988), photosynthetic

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performance, primary production, and light utilisation efficiency (Boston and Hill, 1991; Hill et al., 1995; Hill and Dimick, 2002) resulting in higher chlorophyll *a* and higher periphyton biomass (Mallory and Richardson, 2005).

Periphyton is subject to complex interactions between top-down and bottom-up forces (Mallory and Richardson, 2005). Herbivore control could be of greater importance in some systems (Hillebrand, 2005; Schneck et al., 2013). Several studies have shown that grazing in lotic ecosystems can substantially influence algal growth form thus controlling assemblage physiognomy (Steinman et al., 1987; Wellnitz and Ward, 1998; Álvarez and Peckarsky, 2005), and reducing biomass significantly (Wallace and Webster, 1996; Álvarez and Peckarsky, 2005; Barbee, 2005). However, relatively few studies (Quinn et al., 1997a,b) have addressed the question of how the implantation of forests in grassland landscapes affects top-down control by grazing of stream algal communities.

The grazers' ability to affect the structure of algal communities is probably related to differences in grazing vulnerability of algal species, which is associated to differences in algal physiognomy, microdistribution and palatability (Steinman, 1996). Grazers reduce the relative abundance of upright, overstory or loosely attached algal taxa, while algal species more closely associated with or tightly attached to the substratum usually increase their relative abundance (Holomuzki et al., 2010). Whereas prostrate growth forms are well adapted to high grazing pressure, upright or large forms are susceptible to it (Steinman, 1992). Large growth forms also may have a competitive advantage for light, as their higher profile enables them to intercept irradiance before the lower profile cells (Hudon and Bourget, 1983). Moreover, chemical composition of algal communities affects grazer–algal interactions since it modifies feeding activity of consumers. Hillebrand et al. (2009) found that individual grazers increase their intakes in response to low food quality but low-nutrient food often reduces herbivore growth efficiency and population growth rate. Therefore, grazing could shift species composition to grazer-tolerant organisms and/or non palatable species, which will be benefited by a higher access to light, nutrients and space (Holomuzki et al., 2010).

Some studies have demonstrated that streams in grassland landscapes converted to afforestation present lower algal productivity and higher standing crops of organic matter affecting food web structure (Thompson and Townsend, 2004, 2005). Surveys in our study area have revealed that the afforestation of grassland streams with pines changes macroinvertebrate community composition and abundance reducing grazer densities by half (J. Márquez, unpublished data). Interestingly, benthic biofilm accrual in a field experiment carried out in three catchments did not differ between both stream types, possibly due to higher grazing in grassland streams (R. Principe, unpublished data). However, biofilm standing stock was higher in grassland streams, so it is still unclear if grazers do control algal growth and how this interaction is affected by light regimes altered by pine afforestations.

In this study we aimed at answering how grazing controls periphyton from streams with contrasting light regimes by offering the ubiquitous grazer *Helicopsyche* sp. (Trichoptera, Insecta) biofilm grown both in a grassland (reference) and in a pine afforested stream. Given that grassland streams are exposed to higher light intensity, which favours the development of more productive algal communities (Thompson and Townsend, 2005), and grazers are more abundant compared to afforested streams (J. Márquez, unpublished data), we proposed two non-exclusive hypotheses. If biofilm assemblages grown in the afforested stream are dominated by grazing-vulnerable algal species, grazing pressure by *Helicopsyche* sp. (i.e. biofilm changes relative to the initial biofilm condition) is expected to be stronger than on assemblages grown in grassland streams (i). In addition, if biofilm from the afforested stream has less quality (as low light intensity yields less autotrophic biofilms)

or is less abundant as food for consumers, the effects of *Helicopsyche* sp. may be stronger or weaker depending on their feeding decisions (i.e. compensating or not the low food quality with high consumption) (ii).

Materials and methods

Study area

This study is included in a project that aims at assessing the potential changes in biodiversity and ecological processes of head-water streams draining natural grassland catchments converted to pine forests. The study area includes streams belonging to the headwaters of Ctalamochita river, which are situated between 800 and 1500 m a.s.l. on the east side of Córdoba hills, Argentina. Vegetation varies according to altitude, with grassland developing between 1000 and 1500 m a.s.l. (mainly *Festuca hieronymi* Hack. var. *hieronymii* and *Nasella* spp.; Cabido et al., 2003; Oggero and Arana, 2012). The study area is affected by anthropogenic activities, mainly livestock and afforestation with exotic pines. *Pinus ellioti* is the most abundant in plantations.

We selected two streams with different riparian cover, one flowing through grassland (31° 58' 52"S, 64° 46' 29"W, 1157 m a.s.l., drainage area 89 ha) and the other flowing through pine afforestations (31° 58' 24"S, 64° 45' 15"W, 1121 m a.s.l., drainage area 142 ha). PAR (photosynthetically active radiation) intensity was measured with a QSL–2100 irradiance sensor (Biospherical Instruments, Inc., San Diego, California) at midday along a 50 m reach at each site. PAR was reduced more than 60% in afforested stream (grassland: 2200 $\mu\text{mol m}^{-2} \text{s}^{-1}$; afforested: 900 $\mu\text{mol m}^{-2} \text{s}^{-1}$).

Experimental design and laboratory methods

During the low flow season (August–September 2011), twelve artificial substrata were placed in each stream (grassland and afforested) to allow algal community development under contrasting environmental conditions (i.e. lower light intensity, temperature and grazers abundance in afforested stream). Substrata consisted of sand-blasted glass slides, each measuring 9 cm². After 28 days, substrata were collected and transported to the laboratory in dark plastic containers filled with stream water. At the same time, *Helicopsyche* sp. larvae were collected by hand in the grassland stream and transported in similar conditions. This Trichoptera was selected for the experiment since it was reported as indicator (IndVal Method, Dufrêne and Legendre, 1997) of grassland streams in the study area (Márquez et al., 2010) and also is the only taxon of the community assigned as primarily scraper (Merritt and Cummins, 1996; Reynaga, 2009). *Helicopsyche* sp. was only collected in the grassland stream to test how grazers from a reference grassland stream face food resources from the same stream compared to food from an unshaded stream converted to afforestation and how algal assemblages respond to grazing.

In the laboratory, two plastic white trays (5L volume) were used as microcosms. Each one contained a water pump to create a constant flow (200 Lh⁻¹), and plastic beakers to contain glass substrata and grazers acting as experimental units. A 4 × 4 cm opening was cut in two opposite sides of the beakers and screened with 1 mm² mesh, which allowed water circulation preventing *Helicopsyche* sp. larvae from escaping (Zanotto Arpellino et al., 2011). Each microcosm was filled with net-filtered water (25 μm mesh) either from the grassland or the afforested stream and was replaced twice daily. Water chemistry was similar between streams (see Farley et al., 2008) and water temperature was kept at 24 °C (± 2 °C). Although temperature is lower under afforestations, the experiments were run in late winter when stream water

temperature differences between streams are minor. It is likely that different thermal regimes occurring in both streams, particularly in spring and summer months would increase the differences found in our experiments, as biofilm development and invertebrate feeding activity is expected to be lower under afforested streams. To simulate the difference in light intensity between grassland and afforested streams, one microcosm was exposed to PAR intensity of $170 \mu\text{mol m}^{-2} \text{s}^{-1}$ and the other to $36 \mu\text{mol m}^{-2} \text{s}^{-1}$ in a light:dark photoperiod 12:12 to mimic contrasting light intensities between grassland and afforested stream in the field. Despite PAR intensities in the laboratory were much lower than in natural conditions, the short experimental period (2 days, see below) and the different experimental light intensities should guarantee confident results.

Four glass substrata from each stream were used to estimate initial periphyton biomass (as Chl *a*) and to determine the composition and structure of algal community offered to grazers (initial condition). At t_0 , glass substrata from each stream were placed inside the beakers (one glass per beaker) under the conditions of water and light intensity corresponding to the simulated grassland or afforested stream. In both light conditions, each of the four beakers was supplied with two individuals of *Helicopsyche* sp. (i.e. 22 individuals m^{-2} , grazed treatment), and four beakers were used as controls (ungrazed treatment). Grazer density was set according to that registered in grassland streams in the study area (20 individuals m^{-2} ; J. Márquez, unpublished data).

All glass substrata were removed from beakers on 23 September, two days after placement in microcosms, due to the great depletion of biofilm in grazed treatments. Algae from the upper surface of glass substrata (initial, grazed and ungrazed conditions) were scrubbed with a stiff nylon brush, rinsed with clean water and all the material dislodged was collected. The periphyton suspension was homogenized and fractionated in two subsamples. One fraction was filtered through a glass-fiber filter GF/C to extract and quantify Chl *a* by spectrophotometry (Nusch, 1980). After that, each filter was dried at 60°C for 48 h, weighed (at the nearest 0.01 mg), combusted at 500°C for 1 h, and re-weighed, to determine ash free dry mass (AFDM). AFDM was used to calculate the autotrophic index (the ratio of AFDM:Chl *a* in mg m^{-2}) for each substrate replicate and values were corrected by surface of substrate; biofilm is considered to be more heterotrophic for higher values (Biggs and Kilroy, 2000). Another aliquot was fixed with 4% formalin and was used for algal identification and density calculations. Algal communities were analyzed quantitatively at $400\times$ magnification with organisms grouped taxonomically by genus. For each sample three subsamples were counted following transects along the coverslip to determine cell densities (cells cm^{-2} , based on Villafañe and Reid, 1995) and taxa richness. The counting unit was an individual cell for unicellular and coenobial organisms, a $10 \mu\text{m}$ length for filaments and a $10 \times 10 \mu\text{m}$ area for colonies.

Data analysis

Structural attributes of the community were calculated (density, richness, Shannon's diversity index (H'), evenness (J'), Chl *a*) and compared with linear models with two factors: riparian vegetation type (grassland and forest) and grazing (initial community, grazed and ungrazed). Validations of simple assumptions of the models were performed reviewing standardized residuals vs. predicted and the normal Q-Q plot of standardized residuals. Heterogeneity of variances between treatments was observed for density and diversity, and the model had the best fit (using the AIC and BIC criterion) using a variance function (varIdent) with 'grazing' as grouping criteria. DGC test (Di Rienzo-Guzman-Casanoves), a hierarchical method which controls type I error while maintaining acceptable power, was used for multiple comparisons. Analyses were performed using InfoStat that implements an interface of platform R

for estimating general and mixed linear models (Di Rienzo et al., 2011, 2012).

The net grazing effect (NGE%, modified from Buria et al., 2010) considering removal by feeding and movement activities was calculated as:

$$\text{NGE\%} = \frac{(((\text{UnG} - \text{G}) / \text{UnG}) \times 100)}{\text{initial}}$$

where UnG is the Chl *a* in ungrazed substrata, G is the Chl *a* in grazed substrata, and Initial is Chl *a* in the initial community, showing the net percentage of Chl *a* removed by the grazer per unit of periphyton offered. NGE on grassland and afforested communities was compared with one-way ANOVA.

The proportion of abundant genera (with a relative abundance greater than 1%) on substrata from grassland and afforested streams was analyzed in initial, grazed and ungrazed communities. To evaluate changes in algal community in the grassland and afforested streams due to the grazer, a correspondence analysis (CA) was performed using CANOCO 4.5 (ter Braak and Šmilauer, 1997–2002, 1998).

Results

Algal communities developed under contrasting environmental conditions due to afforestation of grasslands showed similar cell density (i.e. t_0). Algal density of grazed substrata was remarkably lower compared with that of their initial community and of ungrazed controls in both riparian stream conditions ($F_{2, 18(\text{grazing})} = 10.57$, $P = 0.0009$), but cell density of ungrazed assemblages was similar to that of their respective initial communities (Fig. 1). In contrast, the grazer effect on richness and diversity depended on the origin of algal community ($F_{2, 18(\text{vegetation} \times \text{grazing})} = 7.51$, $P = 0.0042$ and $F_{2, 18(\text{vegetation} \times \text{grazing})} = 3.72$, $P = 0.0446$, respectively, Fig. 1). Initial assemblages from grassland stream showed higher richness than assemblages from the afforested stream, and the same occurred when grazing was avoided. In the grassland condition, algal richness was reduced under grazing but increased in the ungrazed treatment (DGC test, $P < 0.05$). In substrates colonized in the afforested stream, algal richness was not affected by grazing (DGC test, NS) but it also increased in the ungrazed treatment. Shannon diversity was similar in substrata colonized in both streams (i.e. initial condition) but the trajectory of diversity depended on stream type. In substrata from grassland condition, diversity showed a similar pattern as for richness; it was heavily reduced by grazing and increased when grazing was absent. In substrata from the afforested stream, diversity did not change either with or without grazing. Evenness was very variable within treatments and resulted in no significant differences among them ($F_{1, 18(\text{vegetation})} = 1.46$, $P = 0.2422$; $F_{2, 18(\text{grazing})} = 0.28$, $P = 0.7582$; $F_{2, 18(\text{vegetation} \times \text{grazing})} = 0.57$, $P = 0.5761$).

Chl *a* was similar in initial assemblages of both streams and remained with no significant changes under grazing. In ungrazed treatments of both grassland and afforested conditions Chl *a* increased ($F_{2, 18(\text{grazing})} = 6.51$, $P = 0.0092$), though in biofilm from grassland stream it was 6x higher compared with the initial assemblage (Fig. 1). In consequence, net grazing effect of *Helicopsyche* sp. on Chl *a* was two-fold higher on substrata from the grassland stream (Fig. 2), although marginally significant ($F_{1, 6(\text{vegetation})} = 3.55$, $P = 0.11$).

Contrasting algal communities were developed in substrates colonized in afforested and grassland streams (CA Eigenvalues: axis 1 = 0.298, axis 2 = 0.043, axis 3 = 0.012, axis 4 = 0.007, and 94.1% of accumulated variance, Fig. 3). A higher proportion of chlorophytes and cyanobacteria were observed in assemblages from the grassland stream as opposed to the dominance of diatoms (90%)

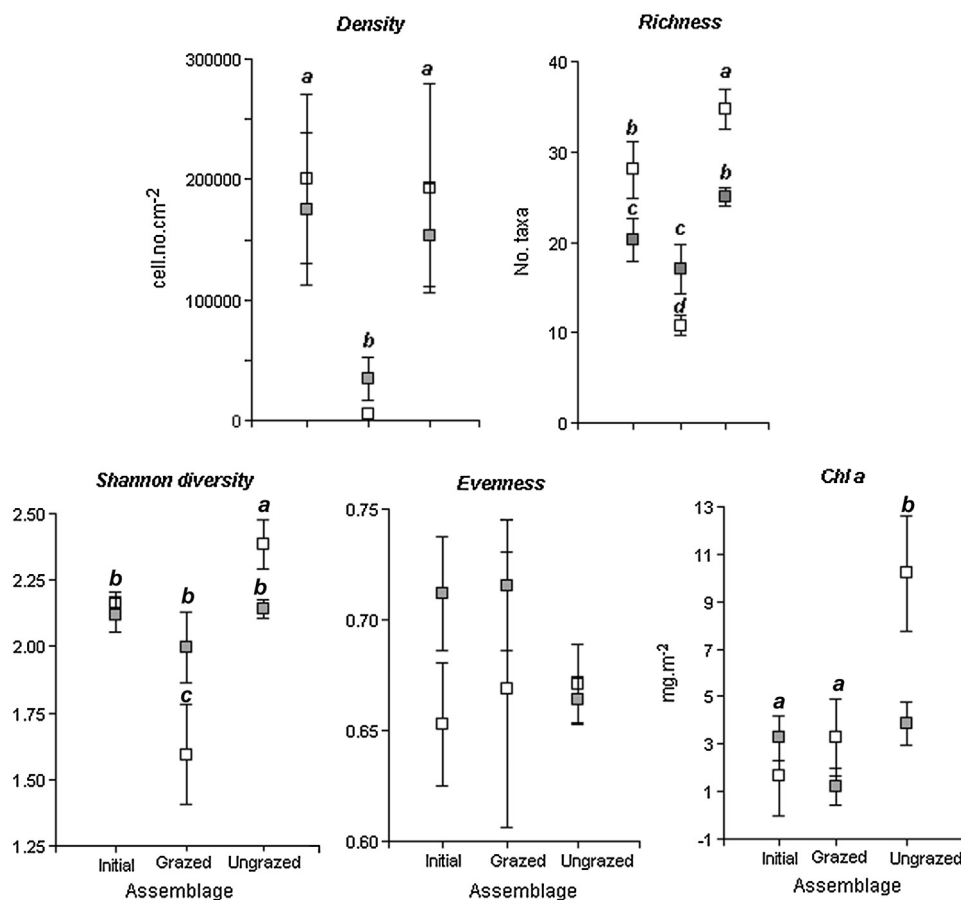


Fig. 1. Structural attributes of algal community developed on glass substrata from grassland stream (open symbols) and afforested stream (closed symbols), at the beginning of the experience (initial community) and for grazed and ungrazed treatments. Media and standard error are represented. Different letters indicate significant differences (post hoc DGC test, $P < 0.05$).

observed in the afforested stream, mainly *Achnanthydium* sp. and *Cocconeis* sp. (Fig. 4). Grazing in substrata from the afforested stream did not change algal community compared to initial or ungrazed conditions (Fig. 3). Instead, grassland substrata exposed

to the grazer showed a decrease in abundance of *Oedogonium* sp., *Spirogyra* sp. and *Ulothrix* sp., which are located in the upper layer of the algal mat, and an increase in the proportion of *Achnanthydium* sp. It is worth noting that substrata from the pine forest had higher amounts of mucilage, fungi and bacteria as opposed to biofilm developed on grassland stream substrata. In fact, the autotrophic index (AFDM:Chl *a*) in substrata colonized in the afforested stream was ~1300 against ~400 in substrata grown in grassland conditions.

Discussion

The scraper *Helicopsyche* sp. produced changes in structural attributes of algal communities, mostly in periphyton developed in the grassland stream. Moreover, the positive effect of light, when the negative effect of grazing was experimentally eliminated, was much more important in grassland substrata. This suggests that herbivory is a relevant functional factor determining algal structure in grassland streams, and is remarkably significant compared to afforested streams.

Our first hypothesis could not be tested as the initial assemblages developed in substrata exposed to two contrasting stream conditions were opposed to our expectations, which had been: (i) periphyton from grassland stream should be rich in tolerant species, as heavy grazing would deplete upright, overstory and loosely attached algal taxa shifting benthic assemblages toward prostrate, understory forms (Rosemond et al., 2000; Holomuzki et al., 2010), and (ii) periphyton from the pine afforested stream should be dominated, contrastingly, by grazing vulnerable algal

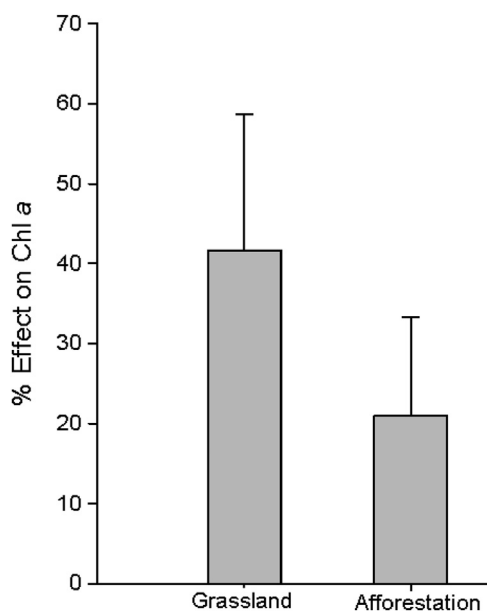


Fig. 2. Net grazing effect on periphyton biomass (Chl *a*) of glass substrata from grassland and afforested streams. Media and standard error are represented.

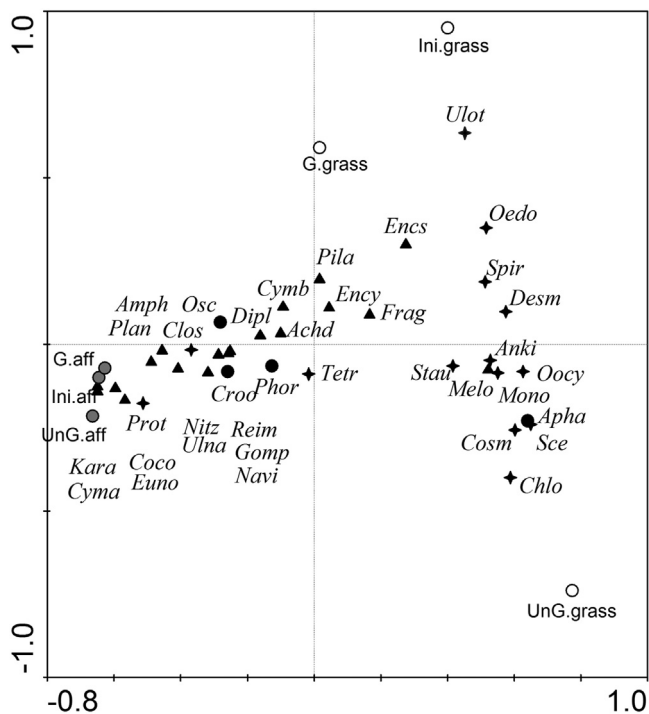


Fig. 3. CA of periphyton samples and 36 genera taken at grassland (white circles) and afforested stream (grey circles). Ini: Initial community, G: grazed, UnG: ungrazed, grass: grassland condition, aff: afforested condition; Symbols, triangles: diatoms, stars: chlorophytes and charophytes, circles: cyanobacteria; Achd = *Achnanthydium*, Amph = *Amphora*, Coco = *Cocconeis*, Cyma = *Cymatopleura*, Cymb = *Cymbella*, Dipl = *Diploneis*, Ency = *Encyonema*, Encs = *Encyonopsis*, Euno = *Eunotia*, Frag = *Fragilaria*, Gomp = *Gomphonema*, Kara = *Karayevia*, Melo = *Melosira*, Navi = *Navicula*, Nitz = *Nitzschia*, Pila = *Pinnularia*, Plan = *Planothidium*, Reim = *Reimeria*, Ulna = *Ulnaria*, Anki = *Ankistrodesmus*, Chlo = *Chlorococcal*, Clos = *Closterium*, Cosm = *Cosmarium*, Desm = *Desmodesmus*, Mono = *Monoraphidium*, Oedo = *Oedogonium*, Ooco = *Oocystis*, Sce = *Scenedesmus*, Spir = *Spirogyra*, Stau = *Staurastrum*, Ulot = *Ulothrix*, Tetr = *Tetraspora*, Apha = *Aphanocapsa*, Croo = *Chroococcus*, Osc = *Oscillatoria*, Phor = *Phormidium*.

taxa. As Passy (2008) stated, high resource levels allow the coexistence of tolerant species, which present a short habit and an understory location, with sensitive species, which have high resource demands and a beneficial spatial position in the overstory. However, the benefits of this spatial arrangement are reversed under grazing pressure (Steinman, 1996).

On the other hand, grazing on periphyton from the afforested stream was diminished possibly due to its low quality (more heterotrophic and with less digestible organic matter), supporting our second hypothesis. As periphyton abundance was similar at t_0 , our results suggest that the feeding strategy of this ubiquitous and abundant grazer gives importance to resource quality. They fed actively on biofilm from grassland stream but not on biofilm from afforestation. In support of this, Steinman (1996) stated that algal biomass may not be affected by grazing if (1) consumption rates or grazer density are low, (2) grazer's feeding morphology is not well matched with the dominant algal growth form, or (3) biomass accrual is constrained by limited resources (bottom-up control).

The higher grazing effect under high light levels in our experiment is in line with previous findings on grazer–periphyton interactions in other studies (Wellnitz and Ward, 1998; Hillebrand, 2005; Lange et al., 2011). Regarding diversity, we found stronger grazer effects on the grassland substrata; both richness and H diversity decreased significantly by grazing, maybe due to the presence of chlorophytes that were easily consumed by the grazer. In contrast, grazing did not affect diversity on substrata from the afforested stream. Interestingly, this pattern was shared for richness and H diversity independently of the initial condition: algal

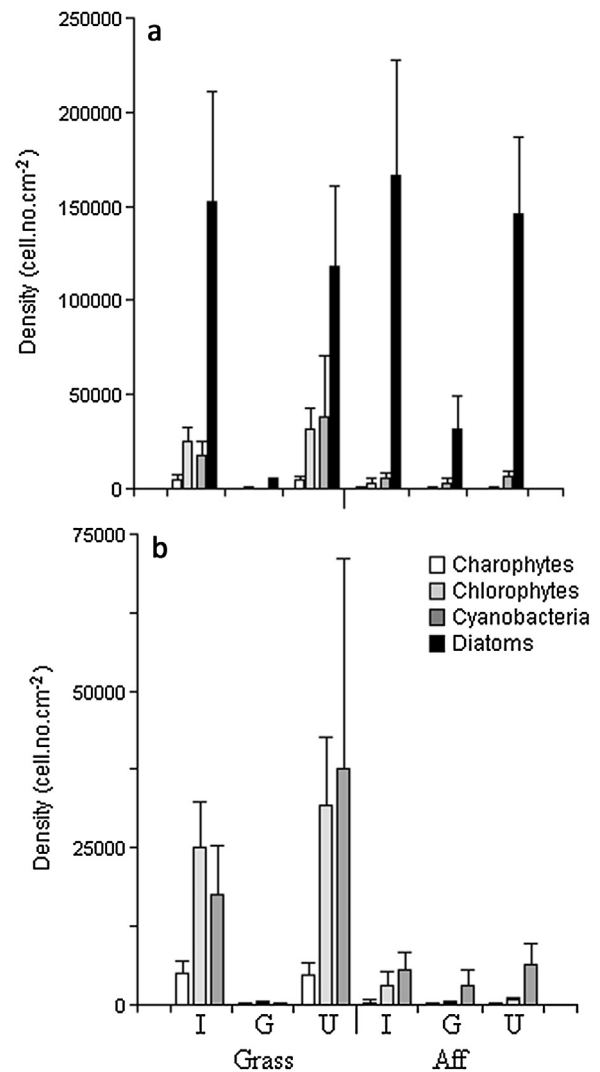


Fig. 4. Density of all algal divisions (a) and excluding diatoms (b) represented in substrata from grassland (Grass) and afforested (Aff) streams, at initial (I), grazed (G) and ungrazed (U) communities. Mean values and standard error are plotted.

richness in grassland substrata was higher than the observed in substrata from afforested stream but diversity was similar. Moreover, grazing exclusion treatments yielded higher diversity values in grassland substrata emphasizing the strong control of grazing on diversity under field and laboratory conditions.

Grazers decreased the abundance of genera which exploit the upper layers of biofilm only in substrata from grassland stream. Grazer identity is important in controlling benthic algal composition, and caddisflies and snails have been reported to strongly reduce overstory algal forms when compared to other benthic grazers (Steinman, 1996). It is likely that grazing activity of *Helicopsyche* larvae had removed upright chlorophytes, abundant in grassland substrata, consistent with findings of previous studies (DeNicola et al., 1990; Holomuzki et al., 2010). Vaughn (1986) found that *Helicopsyche* larvae did not actively select any of the algal types offered in preference to others, but adult emergence was higher on the green filamentous algal diet. The dominance of prostrate diatoms in the afforested stream and the heterotrophic character of this biofilm contributed to reduce *Helicopsyche* feeding activity and its effect on biofilm composition.

In our study, biomass (as Chl *a*) was higher in control ungrazed substrata, consistent with other studies (Giorgi and Tiraboschi, 1999; Álvarez and Peckarsky, 2005; Schneck et al., 2013). The initial

Chl *a* concentration was similar between periphyton from different sources and changed drastically under higher light conditions when grazing was avoided (ungrazed treatment), thus suggesting a strong grazer's control of periphyton in grassland streams, in concordance with previous findings (Feminella and Hawkins, 1995; Hillebrand, 2005). In the same way, richness and density were higher in ungrazed substrata, mainly in communities grown in the grassland stream. This shows that starting from a grassland stream biofilm developed under natural grazing conditions, the experimental removal of grazing pressure led to a greater community development (higher richness and structural complexity) and to increased differences with substrates from the afforested stream. In support of this, Hudon and Bourget (1983) proposed a model of diatom community development where favourable conditions, as low grazing pressure and high light levels, lead to a stratified community, with greater structural complexity. In addition, Rosemond et al. (2000) found greater differences in biomass between grazed and ungrazed treatments under elevated resources (light and nutrients). Their results were associated with the capacity of herbivores to maintain low algal biomass even under high resource conditions, and the increase in algal productivity in response to increased resource levels when herbivores were removed.

Several researchers have reported a decrease in biomass due to different grazers, being caddisflies the ones which caused the most important effect, possibly because of their feeding mode, motility and high consumptive rate (Lamberti et al., 1987; DeNicola et al., 1990; Holomuzki et al., 2010). In particular, *Helicopsyche* sp. is one of the grazers that affect periphyton abundance the most (Feminella and Hawkins, 1995; Reynaga, 2009). We observed significant effects by exposing algal communities from different environmental conditions to only one grazer species during a short time, similar to other studies (DeNicola et al., 1990; Holomuzki et al., 2006). It is worth noting that the differential effect of grazing previous to the start of the experiment (i.e. due to the higher density of grazers reported in our grassland streams, J. Márquez, unpublished data) reflected the real situation of algal communities in each stream, and allowed us to evaluate how algal community from grassland responded to the elimination of grazing pressure. Besides, as *Helicopsyche* sp. individuals were collected in the grassland stream, our experiment showed how they responded to the new conditions imposed by afforestation, which altered food resources and light intensity.

Grazing appears as a crucial factor determining the structure and abundance of algal communities in the study area and it seems particularly more important in grassland streams. Conversion of grassland to pine forest changes energy sources of aquatic systems, potentially affecting consumers that rely on specific resources. In addition, pines are widely dispersed and can be effective invaders of grasslands, thus increasing the potential impacts on natural ecosystems (Sarasola et al., 2006; Pollice et al., 2013). Forest implantation of rapid-growth species in grassland landscapes is growing due to an increasing demand for timber. In our study region in particular, extensive areas have already been afforested with introduced pine species and there is little knowledge about the effects they exert on the environment. This is why larger spatial and temporal scale studies are needed to better understanding how top-down (grazing control) and bottom-up (mediated by riparian vegetation) forces interact in reference streams (i.e. grassland) and to what extent those patterns are changed by afforestation.

Conclusions

Helicopsyche sp. produced significant changes in structural attributes of algal communities from a grassland stream, suggesting that herbivory is a relevant functional factor determining algal

structure in open streams and is diminished following afforestation. This was supported by the grazing exclusion treatment in substrates from the grassland stream that showed strong structural changes in periphyton. Biofilm grown in both streams was different in assemblage composition but tolerant algae did not dominate the assemblages developed in grassland stream, and periphyton from the pine afforested stream was not dominated, contrastingly, by grazing vulnerable algal taxa. However, given that biofilm offered to *Helicopsyche* was similar in abundance (same density and Chl *a*), our results suggest that its feeding strategy gives importance to resource quality. This study contributes to understanding how drastic changes in riparian vegetation altering sunlight regime affect the interaction of algal communities and herbivores in headwater streams.

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