

ORIGINAL ARTICLE

Disentangling grazing and light controls on algal communities in grassland and afforested streams

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Abstract

Our aim was to analyze the combined effect of grazing and light on composition, structure, functional diversity (FD) and succession of algal communities in two headwater streams (grassland and pine afforested). We hypothesized that algal communities in the grassland stream are top-down controlled, as a result of higher grazing; meanwhile, in the afforested stream, algal communities are bottom-up shaped, mainly due to sunlight reduction. In situ experiments, we used platforms to avoid grazing in both streams, and the substrates were extracted at 23, 45 and 73 days. Algal community composition was different between stream types. Seventy percent of structural and biomass variables responded to grazing. In the grassland stream, grazing reduced richness and ash-free dry mass (AFDM), especially after 45 days, while in the afforested stream, at the same time, grazed substrates showed higher richness and AFDM than ungrazed substrates. Net grazing effect was higher on algal biomass of grassland streams. Then, 30% of functional traits showed grazing effect. High profile algae predominated in the grassland stream, where ungrazed substrates showed higher proportions of species with pad mucilage and colonial life form. Algal communities in the afforested stream remained at an early stage of succession, with a predominance of small and low profile algae in both substrates, and FD and evenness indices were lower. Our results indicate that the relative strength of top-down versus bottom-up control is different among stream types and the primary regulatory factor of freshwater communities can be modified according to such forestry practice.

KEYWORDS

bottom-up, exotic pines, grassland, periphyton, top-down

1 | INTRODUCTION

The conversion of grasslands into forests for timber production has become a common phenomenon in the southern hemisphere over the past 50–70 years (Jobbágy et al., 2006; Raffaele, Núñez, & Relva, 2015; Simberloff et al., 2010). To maximize timber yields, grassland catchments are often planted with trees up to the stream margins. Grassland afforestation of stream margins has drastic consequences to stream ecosystems as they modify the sunlight input to

fluvial systems, especially when tree species are evergreen (Thompson & Townsend, 2005). A small, open-canopy stream with high levels of benthic algal production is likely to change if shaded. Algal growth and standing stock will be reduced and food webs may turn “brown” if allochthonous detritus becomes the primary energy source (Dodds, Gido, Whiles, Fritz, & Matthews, 2004; Thompson & Townsend, 2005; Whiting, Whiles, & Stone, 2011). In Argentina, semi-arid lands were afforested in the late 1970s as a result of a tax deferral plan implemented by the government.

Approximately 36,000 ha of the Comechingones mountains in Córdoba were afforested with exotic pines between 900 and 1,600 m above sea level (a.s.l., Plevich, Nuñez, Cantero, Demaestri, & Viale, 2002), which corresponds to 15% of upland grasslands. Farley, Piñeiro, Palmer, Jobbágy, and Jackson (2008) showed that pine afforestation in this area did not alter stream water acidity, although a 50% reduction in annual water yield was reported in afforested streams (Jobbágy, Acosta, & Nasetto, 2013). Previous studies in the area, comparing three afforested and three grassland streams, found that algal abundance, richness and indicator taxa were reduced in afforested streams compared to grassland ones (Cibils-Martina, Márquez, Principe, Gari, & Albariño, 2017). Furthermore, benthic invertebrates were heavily reduced under afforestation (mean density grassland streams: 23,180 ind/m², mean density afforested streams: 6,880 ind/m², Márquez, Cibils, Principe, & Albariño, 2015).

Under this new scenario following stream afforestation, algal-grazer interactions may be modified. Herbivory may have different, predictable effects on succession rates depending upon the growth forms available in the local species pool, and whether the more susceptible forms are early or late successional species (Díaz Villanueva & Modenutti, 2004; Tuchman & Stevenson, 1991). Hillebrand (2005) stated that grazer effects become stronger at high light supply, which indicated that high light favours algal growth types that are easily ingested and a community dominated by photosynthetic organisms. However, filamentous forms and high proportions of mucilage, which may be abundant in high light conditions, are difficult to graze (Wellnitz & Ward, 1998). Cibils-Martina, Principe, Márquez, Gari, and Albariño (2017) found that in an afforested stream, succession proceeded toward the dominance of prostrate, low-profile species, tolerant of low-light conditions. However, in grassland streams with higher sunlight availability, high-profile algae prevailed. Thus, algal community architecture can be different according to environmental conditions, with consequences through the food web. Stalked or high profile growth forms could outcompete other growth forms for light due to the erect structure, but in the presence of grazers, these same taxa may be disadvantaged due to higher consumption rates (Holomuzki, Feminella, & Power, 2010; Steinman, 1996).

The relative importance of top-down control by herbivores and bottom-up control by environmental conditions on primary production within ecosystems has been the subject of a long-standing debate in ecology (Elschot et al., 2017; Gruner et al., 2008; Hunter & Price, 1992). Environmental conditions may determine the direction and magnitude of top-down and bottom-up forces that control freshwater communities and influence species diversity (Thompson & Townsend, 2005; Werner & Matthiessen, 2013). In open mountain streams, benthic algae represent the primary

production supporting food webs (Roberts, Sabater, & Beardall, 2004), and their development is shaped by abiotic factors, such as resources (light, nutrients) and stressors (current shear stress, pH, salt, heavy metals), but also biotic factors, as positive (commensalism, mutualism) or negative (competition, grazing) interactions (Stevenson, 2010). Therefore, there are bottom-up and top-down forces that regulate the taxonomic and functional structure of algal communities, the rate and direction of succession (Larson & Passy, 2012) and their biomass (Hillebrand, 2002). The importance of species interactions in structuring ecological communities or regulating community processes within and among ecosystems has long been subject of interest to ecologists (Power et al., 1996). Many studies have shown the effect of grazers on algal biomass (Feminella & Hawkins, 1995; Lamberti, Ashkenas, Gregory, & Steinman, 1987; McNeely, Finlay, & Power, 2007; Rober, Stevenson, & Wyatt, 2015; Steinman, 1996; Taylor, McIntosh, & Peckarsky, 2002), composition (Abe, Uchida, Nagumo, & Tanaka, 2007; Díaz Villanueva & Modenutti, 2004), architecture (Wellnitz & Ward, 2000) and spatial heterogeneity (Álvarez & Peckarsky, 2005; Flecker & Taylor, 2004). Furthermore, there are studies of stream periphyton that emphasize the relationship between algal-grazer interactions and irradiance (Hill, Ryon, & Schilling, 1995; Lange, Liess, Piggott, Townsend, & Matthaei, 2011; Mallory & Richardson, 2005; Rosemond, Mulholland, & Brawley, 2000; Steinman, McIntire, Gregory, & Lamberti, 1989; Wellnitz & Rader, 2003; Wellnitz & Ward, 1998, 2000; Winkelmann et al., 2014). However, to our knowledge, few studies have analyzed the effects of riparian vegetation changes on algal-grazer interactions and the effects of grazing on the succession of algal communities (Díaz Villanueva & Modenutti, 2004; Tuchman & Stevenson, 1991).

Cibils Martina, Márquez, Principe, Gari, and Albariño (2014) showed that larvae of the Trichoptera *Helicopsyche*, a common and abundant scraper, exerted greater control on algal communities developed in a grassland stream than when they were grown in an afforested one; they argued that stronger grazing was stimulated by higher periphyton quality (more autotrophic and with higher digestible organic matter) belonging to grassland streams. Principe, Márquez, Cibils Martina, Jobbágy, and Albariño (2015) which performed a 30-day field experiment in the same streams of this study with only grazing-exposed substrates, found that biofilm accrual (i.e., biomass per surface unit and time) was similar between three grassland and three afforested streams. Overall, those results suggest that opposing forces happening in grassland (high light levels plus strong grazing) and pine streams (low light and weak grazing) resulted in similar accrual rates. Long-term experiments, of more than 2 months of duration, provide a more realistic description of the herbivore role in streams, given that ungrazed treatments

capture better the temporal variability that periphyton would exhibit in absence of grazers (Feminella & Hawkins, 1995).

Our aim was to analyze the combined effect of grazing and light on composition, structure and succession of algal communities in two different headwater streams (grassland and afforested), with a long-term, field experiment. We manipulated grazing intensity by placing substrates on the bottom (ambient grazing) or elevated (reduced grazing) in grassland and afforested streams. We hypothesized that algal communities in the grassland stream are top-down controlled, by higher grazing; meanwhile, in the afforested stream, algal communities are bottom-up controlled, due to sunlight reduction (Figure 1). We expected that (a) greater sunlight exposure occurring in the grassland stream would favour the development of algal communities, especially grazing-vulnerable algal species. Therefore, the difference between grazed and ungrazed substrates would be higher than in the afforested stream. In this stream, (b) sunlight reduction would limit the development of algae in both substrates, so there would be no differences between treatments. In addition, we expected that (c) succession reaches an advanced senescent stage in ungrazed substrates in the grassland stream (i.e., higher proportion of high profile species)

and that algal communities remain in an earlier stage of development, dominated by low profile species, on both substrates in the afforested stream.

2 | MATERIALS AND METHODS

2.1 | Study area

The study was carried out in two streams of the Ctlamo-chita River upper basin, Córdoba, Argentina. This river is one of the main tributaries of the Carcarañá River and belongs to La Plata River basin. Headwaters of the Ctlamo-chita river are located in grasslands of the Comechingones mountains between 800 and 1,500 m a.s.l. The area of highland grassland and tabaquiño forests of the central Pampean Ranges of central Argentina have been classified as a biogeographic province belonging to the South American transition zone (Martínez, Arana, Oggero, & Natale, 2016). The lithology is dominated by granitic rocks with localized patches of metamorphic rocks (gneiss, schist, migmatite). Annual precipitation in the region reaches 725 mm, occurring mostly between spring and the end of summer

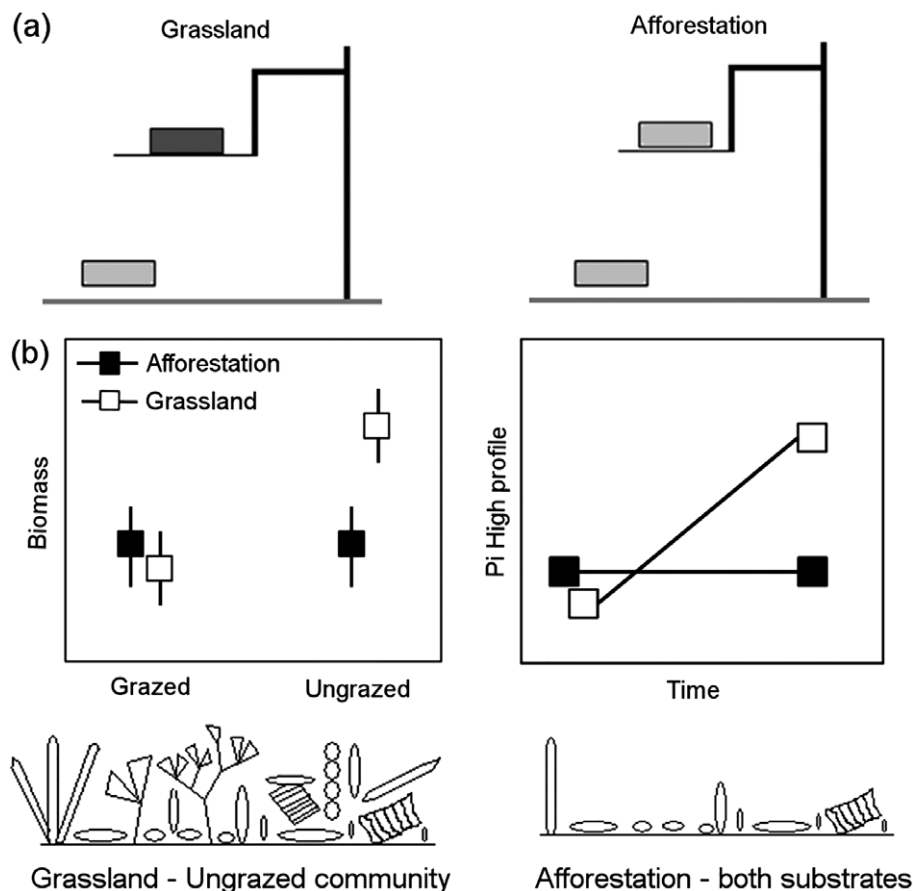


FIGURE 1 Conceptual graph of hypothesis and predictions: (a) algal communities in the grassland stream are top-down controlled, by higher grazing; meanwhile, in the afforested stream, algal communities are bottom-up controlled, due to sunlight reduction, so the difference in algal biomass between grazed (G) and ungrazed (UnG) substrates would be higher in the grassland stream than in the afforested stream, represented by different color of the substrates. (b) Succession reaches an advanced stage in ungrazed substrates in the grassland stream (i.e., higher biomass and proportion of high profile species) and algal communities remain at an earlier stage of development on both substrates in the afforested stream

(October–March) (Cabido et al., 2003). The maximum temperature reaches 34 °C in summer (December–March) and decreases to as low as −5 °C in winter (June–September). Vegetation varies according to altitude, with native grassland developing between 1,000 and 1,500 m a.s.l. (mainly *Festuca hieronymi* Hack., *Nasella* spp., *Schizachyrium condensatum* [Kunth] Nees and *Eragrostis airoides* Nees; Cabido et al., 2003; Oggero & Arana, 2012). The study area is affected by anthropogenic activities, mainly livestock and afforestation with exotic pines. *Pinus elliottii* Engelm. is the most abundant in the plantations.

2.2 | Experimental design and laboratory methods

We selected two first-order streams which were 1 km apart with similar altitude, slope, and drainage area. One stream drains grasslands (31°58'47"S, 64°48'41"W, 1175 m a.s.l., drainage area 87 ha), and the neighbouring stream drains a plantation of *P. elliottii* (31°59'22"S, 64°48'44"W, 1144 m a.s.l., drainage area 89 ha fully covered by the plantation). We performed a field grazing experiment by placing artificial substrates on the bottom (grazed) or elevated in platforms (ungrazed) in the grassland and the afforested streams, and extracting substrates at three different dates. We placed four platforms at different riffles of each stream. We conducted the experiment during the low flow season (July–September 2012) in order to avoid flooding effects on communities and experimental devices. At the beginning of the experiment we registered water depth, current velocity, dominant substrate type and channel wet width in four riffles, each corresponding to the location of tiles in each stream. Depth, width and current velocity were measured with a digital water velocity meter (Global Flow probe FP101), while substrate type was visually assessed (Gordon, McMahon, & Finlayson, 1994) and assigned to a category proposed by Thomson, Taylor, Fryirs, and Brierley (2001). Current velocity was measured for bottom and elevated substrates. Some physicochemical parameters were measured three times at each stream during the experiment, coinciding

with the extractions of tiles for algal community analysis: photosynthetically active radiation (PAR) intensity, pH, conductivity, temperature, total dissolved solids (TDS), carbonates, bicarbonates and nitrates. The PAR intensity was measured with a QSL–2100 Irradiance Sensor (Biospherical Instruments Inc., San Diego, CA) over the water surface midday along a 50 m reach in each stream. Water pH, conductivity and temperature were recorded with portable sensors. TDS, carbonates, bicarbonates and nitrates were analyzed by the area of Hydrology, Department of Geology, National University of Río Cuarto, according to standard methods (American Public Health Association [APHA], 1998). Carbonates and bicarbonates were measured by potentiometric titration with a Thermo Orion-selective electrode, while nitrates were determined by potentiometry using an ion selective electrode (Orion Model 9307), a reference electrode and an Orion potentiometer 710 A. To calibrate the potentiometer, six benchmarks (5, 10, 25, 50, 100 and 300 mg/L¹ NO₃[−]) were used. The detection limit for NO₃[−] was 0.2 mg of NO₃[−] L^{−1} and the analytical error of the measurements was 0.5%.

We used 60 unglazed tiles of 7.5 × 7.5 cm as artificial substrates. To avoid grazing, we elevated half substrates 8–15 cm from the bottom and 2–4 cm under the water surface using a platform design with J inverted metal bars (Lamberti, Feminella, & Pringle, 2007). We placed the other half of the substrates in the bottom of each stream, exposed to grazers, next to J inverted metal bars (Figure 2). We placed metal bars in riffles of 1.4 m mean width, 10–60 cm from the nearest margin. This method excludes benthic grazers that do not swim or that exhibit low drift rates, but allows swimming or drifting nontarget species (e.g., mayflies, chironomids) to reach the platforms; thus, elevated tiles were controlled every 7–10 days, removing and collecting macroinvertebrates (Lamberti et al., 2007). Density of benthic invertebrates registered previously in grassland and afforested streams were 23,180 and 6,880 ind/m² (Márquez et al., 2015). We extracted five tiles from each grazing treatment for each stream at 23, 45 and 73 days of exposure. At

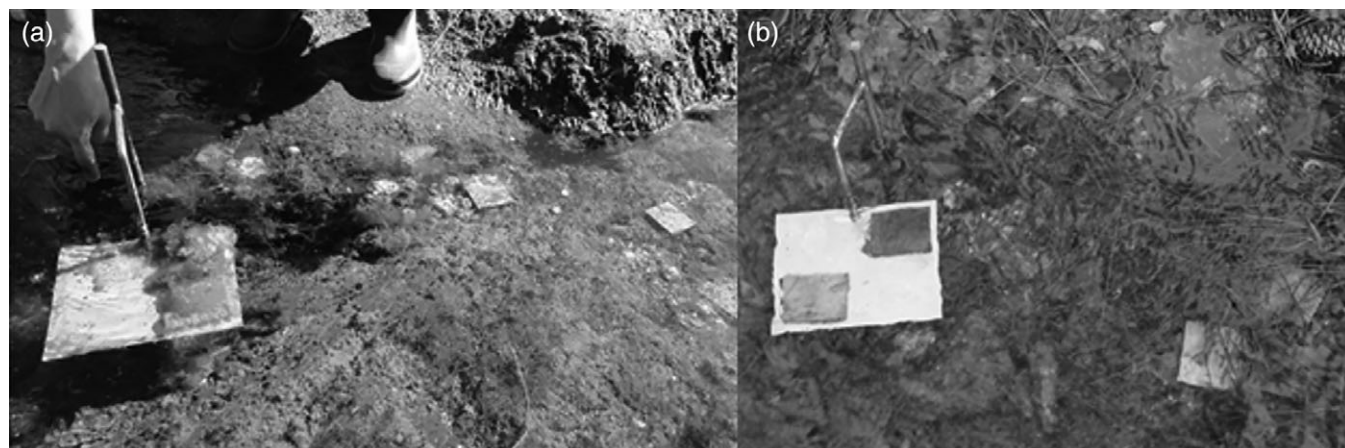


FIGURE 2 Grazed and ungrazed substrates at 54 days in the (a) grassland and (b) afforested streams

each extraction date, we took tiles to the laboratory in individual plastic containers refrigerated and in darkness. We scrubbed off algae from the upper surface of the substrates with a stiff nylon brush, after which, they were rinsed with clean tape water and all the dislodged material was collected. We homogenized and fractionated the periphyton suspension in three subsamples. We filtered one fraction through a pre-incinerated and preweighed glass-fibre filter MF/C (1.2 μm pore size, Munktell, Sweden), we dried it for 48 hr at 60°C, weighed it (to the nearest 0.01 mg), combusted at 500°C for 1 hr, and reweighed it, to determine ash-free dry mass (AFDM). We used another fraction to extract and quantify chlorophyll *a* (Chl *a*) concentration by 90% ethanol extraction in a hot bath and spectrophotometry (Nusch, 1980). We then calculated the autotrophic index from the Chl *a* and AFDM values ($\text{AI} = \text{AFDM in mg/m}^2 : \text{Chl } a \text{ in mg/m}^2$) for each substrate replicate (Weber, 1973); biofilm is considered to be more heterotrophic with higher values (Biggs & Kilroy, 2000). We fixed the third fraction with 4% formalin and used it for algal identification and density calculations. We analyzed algal communities quantitatively at $\times 400$ magnification with organisms grouped taxonomically by genus. For each sample, we counted three subsamples following transects along the coverslip to determine cell densities (cells/cm², based on Villafañe & Reid, 1995). The counting unit was an individual cell for unicellular and coenobial organisms, a 10 μm length for filaments and a 10 \times 10 μm area for colonies.

2.3 | Data analysis

We compared environmental variables between the grassland and the afforested stream using one-way ANOVA for those registered at the beginning of the experiment in four riffles at each stream, and repeated measures ANOVA for those measured three times at each stream.

We evaluated differences in benthic algal communities between riparian vegetation types, grazing treatments and time using nonmetric multidimensional scaling (NMDS) based on the Bray–Curtis dissimilarity matrix, and analysis of similarities (ANOSIM) to statistically test differences between groups. For these analyses, we used square root transformed abundance to decrease the influence of abundant species. Additionally, we performed similarity percentages (SIMPER) analysis to determine which species contributed most to the dissimilarity between sampling units (Clarke & Warwick, 2001; Quinn & Keough, 2002). We constructed rank-abundance curves with selected species (relative abundance >1%) of grazed and ungrazed substrates of the grassland and the afforested streams. We calculated relative abundance (Pi) and ordered species from the commonest to the less abundant in each stream and substrate type. Thus, these curves visually represent the changes in dominant species between streams.

We calculated structural attributes of algal communities: density, richness and Shannon diversity (H') and evenness (J') indices (calculated from algal densities and using \log_{10} in the formula). In addition, we assigned the genera to categories of some functional traits according to Cibils, Principe, Márquez, Gari, and Albariño (2015): size, morphological guild, attachment mechanism and life form (Table S1, Supporting Information). With these traits, we calculated two measures of functional diversity (FD) (Heino, 2005): FD, that is, Shannon–Wiener diversity index, describing both the number of functional groups and the division of individuals among the functional groups, and functional evenness (FE), based on Shannon–Wiener index and describing the division of individuals among the functional groups. We compared structural variables, Chl *a*, AFDM, AI, the proportion of algae corresponding to different categories of functional traits, FD and FE using repeated measures ANOVA, with three fixed factors: riparian vegetation (grassland-afforestation), grazing (grazed-ungrazed) and extraction time (23–45–73 days), and J inverted bars as a random factor. We calculated the NGE (modified from Buria, Albariño, Díaz Villanueva, Modenutti, & Balseiro, 2010) considering removal by feeding and movement activities as:

$$\text{NGE} = \text{UnG} - \text{G}$$

where UnG is the Chl *a* or AFDM in ungrazed substrates, G is the Chl *a* or AFDM in grazed substrates, showing the net biomass removed by the grazer. We compared NGE on the grassland and the afforested communities for each extraction time with two-way ANOVA.

We performed multivariate analyses (NMDS, ANOSIM and SIMPER) in R version 3.3.2, using vegan library (Oksanen et al., 2013; R Core Team, 2013). We performed ANOVA using InfoStat, which implements an interface of platform R to estimate general and mixed linear models (Di Rienzo et al., 2012; Di Rienzo, Macchiavelli, & Casanoves, 2011). We performed validations of assumptions of the models reviewing standardized residuals versus predicted, the normal Q–Q plot of standardized residuals and the Shapiro–Wilks test. Variables that did not meet ANOVA assumptions were natural logarithm transformed. The Di Rienzo–Guzman–Casanoves test, which is a hierarchical method that controls type I errors while maintaining acceptable power, was used for multiple comparisons.

3 | RESULTS

Environmental parameters were similar between grassland and afforested streams (Table 1). Predominant substrates in the afforested stream were boulders and cobbles, and in the grassland stream, bedrock was dominant. Current velocities were similar between streams and were not affected by the elevation of substrates. The PAR intensity was 70% lower in the afforested stream, and given the transparency of water

TABLE 1 Environmental variables measured to characterize grassland and afforested streams

	Grassland		Afforestation		ANOVA results
Depth (cm)	15.56 (4.81)		7.95 (0.49)		$F_{1,7} = 9.95, p = 0.02$
Current velocity (m/s ¹)	0.06 (0.02)		0.09 (0.05)		$F_{1,7} = 1.08, p = 0.34$
	G 0.06 (0.02)	UnG 0.07 (0.01)	G 0.08 (0.05)	UnG 0.1 (0.06)	
Width (m)	1.60 (2.7)		1.22 (2.6)		$F_{1,7} = 3.92, p = 0.09$
Substrate type	Bedrock		Boulders and cobbles		
PAR ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	2,125 (169)		558 (353)		$F_{1,5} = 41.19, p = 0.007$
pH	8.95 (0.64)		8.70 (0.44)		$F_{1,5} = 0.23, p = 0.66$
Conductivity ($\mu\text{S/cm}$)	84.33 (16.20)		174.33 (72.7)		$F_{1,5} = 8.84, p = 0.06$
Temperature (°C)	15.17 (2.84)		10.33 (1.89)		$F_{1,5} = 4.55, p = 0.12$
TDS (mg/L ¹)	135.67 (103.65)		151.67 (54.6)		$F_{1,5} = 0.14, p = 0.73$
Carbonates (mg/L ¹)	2.83 (3.91)		0.00		$F_{1,5} = 1.69, p = 0.28$
Bicarbonates (mg/L ¹)	82.10 (69.65)		97.50 (38.49)		$F_{1,5} = 0.32, p = 0.61$
Nitrates (mg NO ₃ -N/L ¹)	0.33 (0.58)		0.00		$F_{1,5} = 1.20, p = 0.35$

Notes. Mean values, SD and results of ANOVAs (F , df and p values) are shown. Significant differences between grassland and afforested streams are in bold ($p < 0.05$). One-way ANOVAs were performed for depth, current velocity, width and repeated measures ANOVAs were performed for PAR, pH, conductivity, temperature, TDS, carbonate, bicarbonate and nitrate. PAR = photosynthetically active radiation; TDS = total dissolved solids. G = grazed, UnG = ungrazed substrates. $N = 8$ for depth, current velocity and width, and $n = 6$ for PAR intensity, pH, conductivity, temperature, TDS, carbonates, bicarbonates and nitrates.

there were no differences between substrates. In addition, the afforested stream showed higher conductivity than grassland streams, although ANOVA was marginally significant (Table 1).

Metal bars effectively prevented grazer access and feeding, even when we found and removed occasional invertebrates in weekly inspections of ungrazed substrates (mean value along the experiment: one individual/substrate/stream). Densities in elevated substrates were 49 ind/m² in the grassland stream and 167 ind/m² in the afforested one, representing more than 90% of reduction in both cases. In substrates of the afforested stream, we found and collected mainly simuliids and chironomids, while ephemeropterans were present in ungrazed substrates in the grassland stream. Each week we photographed substrates, and gradually larger differences between grazed and ungrazed substrates in the grassland stream were apparent, compared to the afforested

stream (Figure 2a,b). In grazed substrates of the grassland stream, *Helicopsyche* larvae were common.

Furthermore, in the grassland stream, there was a large growth of the algae *Tetraspora lubrica* (Roth) C. Agardh (Figure 2a) covering ungrazed substrates completely, mainly at 23 days. After 38 days of experiment, we observed a high volume of mucilage in substrates of the grassland stream, but they were absent 10 days before the final substrate removal. Biomass measured as Chl *a* increased with time, mainly in the grassland stream where it reached higher values in grazed substrates (Table 2, Figure 3a). AFDM also showed an increase with time, more evident in the grassland stream, where higher values occurred in ungrazed substrates contrasting with Chl *a*. Autotrophic index depended on the interaction of the three factors, with higher values (mean value 1,400) in grazed tiles from the grassland stream in the second extraction date, and the lower values (mean value

TABLE 2 Repeated measures ANOVAs results for biomass and structural variables of algal communities developed in afforested (Af) and grassland (Gs) streams (factor vegetation), in grazed (G) and ungrazed substrates (UnG) (factor grazing), extracted at three dates (factor time, 1, 2 and 3)

Variable	Factor	df	F	p	A posteriori
Ln Chl <i>a</i>	Veg × time	2	5.69	0.006	Af1 = Gs1 < Af3 = Gs2 = Af2 < Gs3
	Veg × grazing	1	6.22	0.02	AfUnG<GsG = AfG = GsUnG
AFDM	Grazing × time	2	4.49	0.02	UnG1 = G1 = G3 < UnG2 = UnG3 = G2
	Veg × grazing	1	9.71	0.003	AfUnG<AfG = GsG < GsUnG
Ln AI	Veg × grazing × time	2	3.78	0.03	GsG3 < AfUnG2 = AfG2 = AfG3 = AfG1 = GsUnG2 = AfUnG1 = AfUnG3 = GsUnG3 = GsUnG1 = GsG1 < GsG2
Ln density	Veg × grazing	1	4.75	0.04	AfUnG<AfG = GsG = GsUnG
	Time	2	18.21	<0.001	1 < 2 = 3
Richness	Veg × grazing	1	7.87	0.02	AfUnG<AfG = GsG < GsUnG
	Time	2	11.18	0.002	1 < 2 = 3
Diversity	Veg × time	2	6.93	0.003	Af3 = Af2 < Gs1 = Af1 = Gs2 = Gs3
Evenness	Veg × time	2	6.91	0.003	Af3 = Af2 < Gs1 = Gs2 = Gs3 = Af1

Note. For each variable, factors that showed a significant effect are shown, with F value, degrees of freedom, p value and a posteriori results.

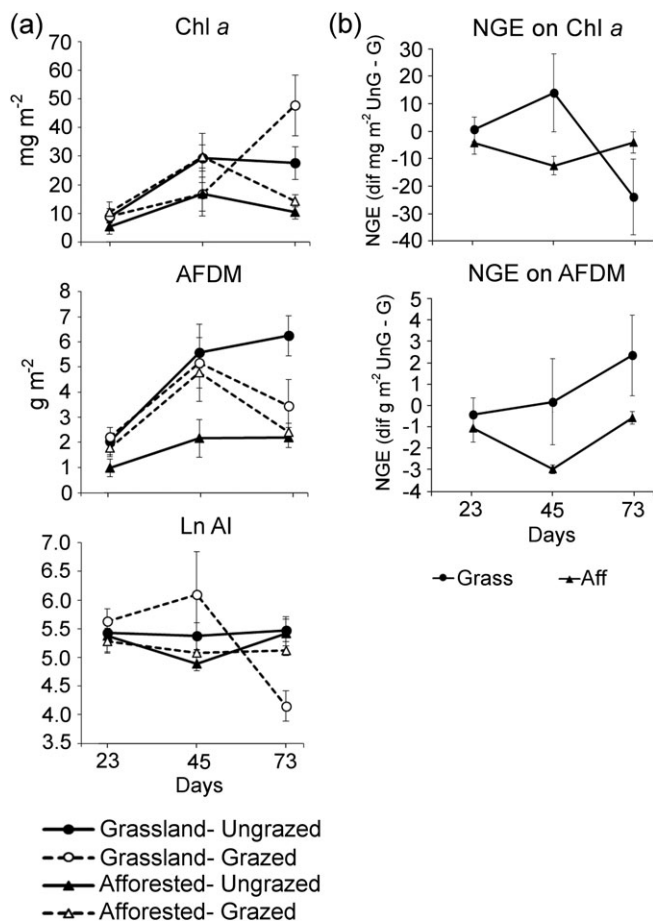


FIGURE 3 (a) Biomass variables of algal communities developed in the afforested and the grassland streams, in grazed and ungrazed substrates, extracted at 23, 45 and 73 days. (b) Net grazing effect (NGE) on Chl *a* and on AFDM registered in the afforested and the grassland streams at 23, 45 and 73 days. For each variable, mean values and SD are shown

70) in the same substrates but at 73 days. Respecting NGE, many negative values were obtained (Figure 3b). NGE on Chl *a* showed differences between vegetation types but depending on extraction time ($F_{2,26} = 4.74$, $p = 0.02$). At 23 days, there were no differences between the grassland and the afforested stream. At 45 days, NGE was higher in the grassland stream but at 73 days grazing effect was reduced. In the afforested stream, NGE was around zero. NGE on AFDM was higher in the grassland stream ($F_{1,26} = 6.7$, $p = 0.02$).

Algal assemblages developed in both types of substrates along the experiment recruited 95 genera, 45% corresponding to diatoms, 20% to Cyanobacteria, 20% Chlorophyta, 12% Charophyta and two genera of Euglenozoa (Table S1). Algal assemblages differed significantly between the grassland and the afforested streams (ANOSIM, $R = 0.40$, $p = 0.001$) as seen in the NMDS ordination (Figure 4, stress = 0.19). Also, there were differences among extraction dates (ANOSIM, $R = 0.20$, $p = 0.001$), but grazing effect was not observed (ANOSIM, $R = 0.03$, $p = 0.11$). SIMPER analysis revealed some genera contributing most to differences between riparian vegetation types: *Fragilaria*, *Achnanthes*, *Encyonema* and *Gomphonema*. These same genera contributed to the difference between extraction dates, adding *Phormidium* which contributed to the separation of the first extraction time. In the afforested stream, a rise in the proportion of *Achnanthes* was observed (Figure 5), mainly in grazed substrates where *Phormidium* predominated at 23 days (first extraction date). Thus, there were more differences between grazed and ungrazed substrates at 23 than 73 days. In contrast, grassland stream

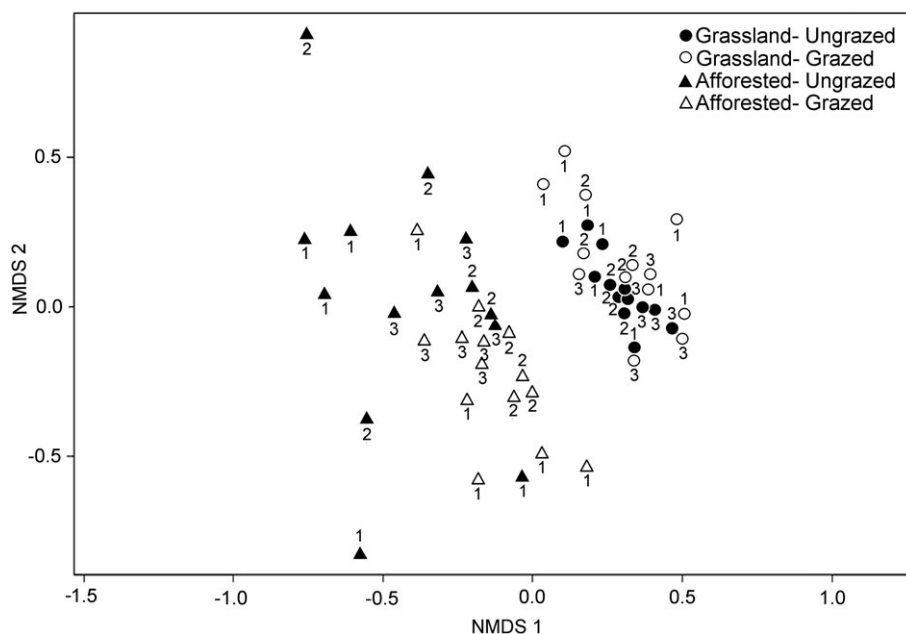


FIGURE 4 NMDS of algal communities in ungrazed and grazed substrates extracted at three dates (1:23 days, 2:45 days and 3:73 days), from the grassland and the afforested stream

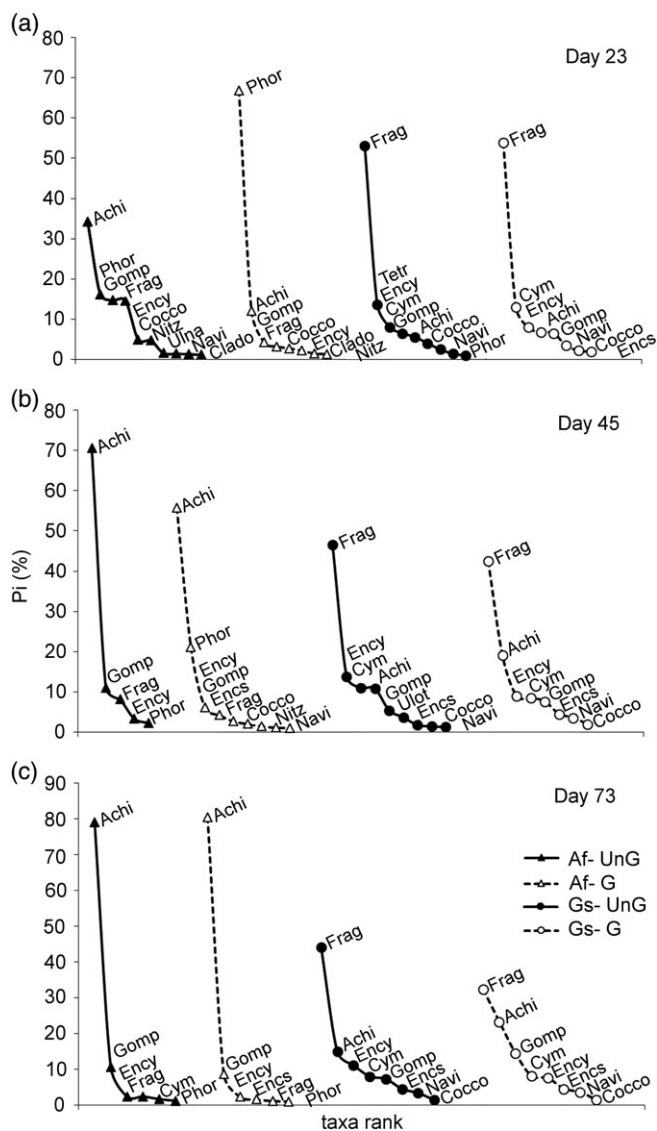


FIGURE 5 Rank-abundance curves for abundant genera (relative abundance $P_i > 1\%$) in ungrazed (UnG) and grazed (G) substrates extracted from the afforested (Af) and the grassland (Gs) stream at three dates (a) 23, (b) 45 and (c) 73 days. Achi: *Achnanthes*, Clado: *Cladophora*, Cocco: *Cocconeis*, Cym: *Cymbella*, Ency: *Encyonema*, Encs: *Encyonopsis*, Gomp: *Gomphonema*, Frag: *Fragilaria*, Navi: *Navicula*, Nitz: *Nitzschia*, Phor: *Phormidium*, Tetr: *Tetraspora*, Ulna: *Ulnaria*, Ulot: *Ulothrix*

communities were similar during the whole experiment. Some differences were the higher proportion of *Tetraspora* in ungrazed substrates at 23 days and the reduction of *Fragilaria* proportion in grazed substrates (Figure 5).

When we analyzed independently samples from streams with different riparian vegetation, we found that in the grassland stream, samples corresponding to different dates were separated (stress = 0.17, ANOSIM for time, $R = 0.32$, $p = 0.002$), but there were no differences due to grazing (ANOSIM for grazing, $R = 0.002$, $p = 0.33$). Instead, in the afforested stream, we observed differences among assemblages from each extraction date (stress = 0.18, ANOSIM for time, $R = 0.27$; $p = 0.003$) and also between grazed and ungrazed tiles (ANOSIM for grazing, $R = 0.19$, $p = 0.01$),

due to differences in the first extraction date. Genera that contributed to differences in the afforested stream were *Achnanthes* and *Phormidium*.

Regarding structural variables, most were affected by the three factors, with exception of diversity and evenness that did not show grazing effects (Table 2). Algal density and richness were affected by time, with an increase in the number of organisms and taxa (Figure 6). There was a grazing effect but this varied with riparian vegetation. Communities from the afforested stream showed lower density and richness in ungrazed substrates, while in the grassland stream, ungrazed substrates showed the highest number of taxa. Diversity and evenness of assemblages from the afforested stream decreased with time, while assemblages from the grassland stream showed an increase. Communities from the grassland stream were more diverse and even than communities from the afforested stream, which showed lower values at the end of the experiment.

Regarding the functional traits, few categories were affected by grazing (Table 3). The proportion of algae of different sizes changed with time and between streams. Small algae (size class 1) predominated in the afforested stream, while larger algae decreased with time. In the grassland stream, algae of intermediate size predominated (size classes 2 and 3), with a higher proportion of algae of size class 3 in grazed substrates. Morphological guilds varied with time depending on riparian vegetation. In the grassland stream, high profile algae predominated and motile were abundant at 73 days. In the afforested stream, there were a higher proportion of low profile algae, which increased with time, while high profile algae decreased with succession. Motile algae showed higher proportion in grazed substrates. With regard to attachment mechanisms, only algae with mucilage pads and stalks were affected by grazing. Algae with pads showed a higher proportion in the grassland stream, and the difference between grazed and ungrazed substrates increased with time, with a lower proportion in grazed substrates. Stalked algae increased with time in both streams, predominating in the afforested stream (mainly represented by *Achnanthes*). Life forms analysis showed that colonial algae were more abundant in the grassland stream, and decreased with time. In addition, the proportion of colonial algae was affected by grazing, with higher abundance in ungrazed substrates. Filamentous forms were more abundant in the afforested stream but also decreased with time. Unicellular algae increased with time in both streams and were more abundant in the afforested streams. FD and FE of functional traits (Table 3, Figure 7) increased with time in the grassland stream and decreased in the afforested stream.

4 | DISCUSSION

We expected algal communities to be controlled by grazing in grassland streams while to be limited by light in afforested

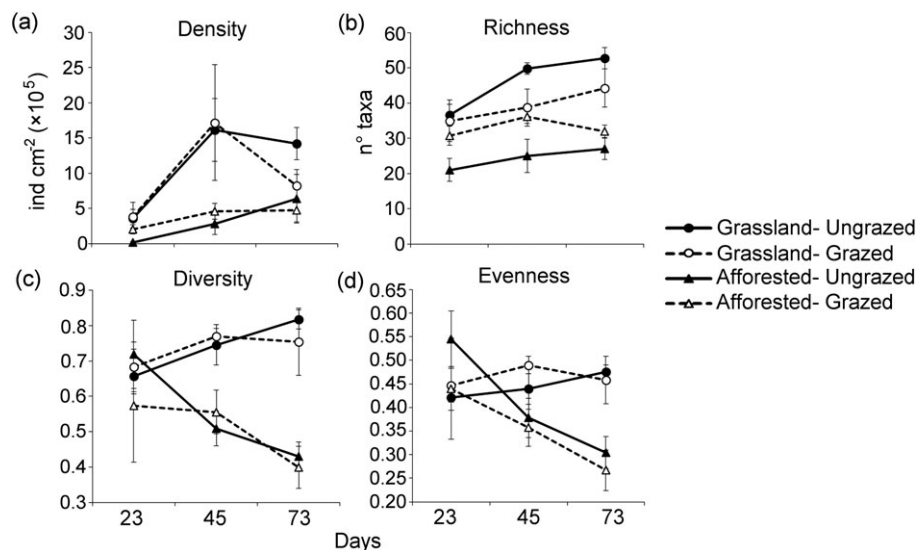


FIGURE 6 Structural variables of algal communities developed in the afforested and the grassland streams, in grazed and ungrazed substrates, extracted at 23, 45 and 73 days. (a) Density, (b) richness, (c) diversity and (d) evenness. For each variable, mean values and *SD* are shown

streams (Figure 1). The afforested stream showed the expected results but there are many factors involved in the more complex communities of grasslands streams, given that

grazing affected some of the variables that we measured. Then, 70% of structural and biomass variables responded to grazing. In the grassland stream, grazing reduced richness

TABLE 3 Repeated measures ANOVAs results for functional traits and functional diversity (FD) and evenness (FE) of algal communities developed in afforested (Af) and grassland (Gs) streams (factor vegetation, veg), in grazed (G) and ungrazed substrates (UnG) (factor grazing, Graz), extracted at three dates (factor time, 1, 2 and 3)

Trait	Variable	Factor	df	F	p	A posteriori
Size	Ln c1	Veg	1	55.59	<0.0001	Gs < Af
		Time	2	35.36	<0.0001	1 < 2 = 3
	c2	Veg	1	181.56	<0.0001	Af < Gs
		Time	2	4.15	0.03	3 = 2 < 1
	Ln c3	Veg × time	2	7.70	0.002	Af3 = <Af2 = Gs1 = Af1 < Gs2 = Gs3
		Grazing	1	5.10	0.04	UnG < G
Morphological	Ln c5	Veg × time	2	14.97	<0.0001	Af3 < Af2 = Gs3 = Gs2 = Gs1 < Af1
		Veg × time	2	8.41	0.001	Af3 < Af2 < Af1 = Gs3 = Gs2 < Gs1
	High profile	Veg × time	2	11.08	0.0002	Gs1 < Gs2 = Gs3 = Af1 < Af2 < Af3
		Veg × time	2	5.18	0.01	Af3 = Af2 < Gs1 = Af1 = Gs2 = Gs3
	Low profile	Grazing	1	4.99	0.04	UnG < G
		Veg	1	34.09	0.0001	Af < Gs
Attachment	Ln Adnate	Veg × Graz × time	2	3.59	0.04	AfUnG3 = AfG3 = AfG2 = AfG1 = AfUnG2 = AfUnG1 < GsG3 = GsG2 < GsUnG3 = GsUnG2 = GsG1 = GsUnG1
		Veg × time	2	8.43	0.001	Gs1 < Af1 = Gs2 = Gs3 < Af2 < Af3
	Stalked	Veg × grazing	1	7.05	0.02	GsUnG = GsG < AfG = AfUnG
		Veg × time	2	10.03	0.001	Af3 = Af2 = Gs1 = Gs3 = Gs2 < Af1
	Ln holdfast	Veg × time	2	20.84	<0.0001	Af3 = Gs2 = Gs1 = Gs3 = Af2 < Af1
		Veg × time	2	5.27	0.04	GsUnG = GsG < AfG = AfUnG
Life forms	Ln Unicellular	Time	2	28.18	<0.0001	1 < 2 < 3
		Veg × time	2	4.66	0.02	Af3 < Af2 = Af1 < Gs3 < Gs2 < Gs1
	Colonial	Grazing	1	9.73	0.009	G < UnG
		Veg × time	2	7.84	0.002	Af3 = Af2 = Gs1 = Gs2 = Af1 < Gs3
	Ln Filamentous	Veg × time	2	5.93	0.007	Gs2 = Gs3 = Af3 = Gs1 < Af2 < Af1
		Veg × time	1	25.55	<0.0001	Af3 = Gs1 < Af2 = Gs2 = Gs3 = Af1
FD	FE	Veg × time	1	27.28	<0.0001	Af3 = Gs1 < Gs2 = Af2 = Gs3 < Af1

Notes. For each variable, factors that showed a significant effect are shown, with *F* value, degrees of freedom (*df*), *p* value and a posteriori results. References: c1 < 99 μm³, c2 100–299 μm³, c3 300–599 μm³, c4 600–1,499 μm³, c5 > 1,500 μm³, Ln natural logarithm.

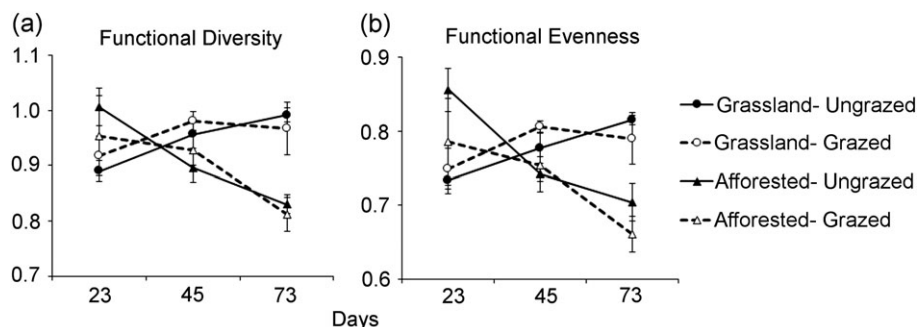


FIGURE 7 (a) Functional diversity and (b) functional evenness of algal communities developed in the afforested and the grassland streams, in grazed and ungrazed substrates, extracted at 23, 45 and 73 days. For each variable, mean values and *SD* are shown

and AFDM, especially after 45 days, while in the afforested stream, at the same time, grazed substrates showed higher richness and AFDM than ungrazed substrates. NGE showed a higher effect of grazing on algal biomass of grassland streams. In addition, 30% of functional traits showed grazing effect, but in both stream types. High profile algae predominated in grassland streams, but with a similar proportion in grazed and ungrazed substrates. Nevertheless, ungrazed substrates showed higher proportions of species with pad mucilage and colonial life forms, similar to the expected results. Afforested communities were dominated by low profile species in both substrates, suggesting a higher importance of light in the regulation of algal communities in this stream, in agreement with other studies (Cibils et al., 2015; Cibils-Martina, Principe, et al., 2017; Majdi, Boiché, Traunspurger, & Lecerf, 2015). In addition, in a previous study which included three grassland and three afforested streams, lower algal abundance and richness, fewer indicator taxa and changes in composition in communities of afforested streams were found; this was mainly attributed to light intensity reduction (Cibils-Martina, Márquez, et al., 2017). Even when the replication of the afforested situation was not possible, we considered that the results of this study contribute to the understanding of how this land use change could affect factors that regulate algal communities.

Community composition showed differences with time in both streams, similar to the reported by Cibils-Martina, Principe, et al. (2017), but multivariate analysis allowed detecting differences between grazed and ungrazed substrates only in the afforested stream. This result is contrary to our prediction and to the findings of other authors (Cibils-Martina et al., 2014; Steinman et al., 1989; Wellnitz & Rader, 2003; Wellnitz & Ward, 1998) that reported higher grazing effects on substrates exposed to higher light intensity, which favours the development of more productive algal communities. However, as Wellnitz and Rader (2003) stated, periphyton is shaped by multiple factors, and scouring may influence algal composition and response to grazers and light. In our in situ experiment, the differences in the afforested stream were evident with the change in dominance of *Phormidium* on grazed substrates at the first

extraction to the dominance of *Achnanthes*. It is likely that *Phormidium*, which forms a mat over substrates, was easily removed by the growth of algae in the understory, by water flow or by the movement of macroinvertebrates (Holomuzki et al., 2010; Saravia, Giorgi, & Momo, 2012; Tuchman & Stevenson, 1991). It would be less likely to attribute the decrease of *Phormidium* to consumption given its growth form and size since it is a filamentous cyanobacteria that forms densely layered mats which are difficult to consume (Holomuzki et al., 2010). According to Biggs (2000), some invertebrate species seem to avoid large sized algal species as well as filamentous algae since they are difficult to manipulate, and can be of low nutritional value or have chemical compounds that make them unpalatable. The small size and shade tolerance of *Achnanthes minutissimum* explains its predominance in the afforested stream (Díaz Villanueva & Modenutti, 2004; Johnson, Tuchman, & Peterson, 1997).

In the grassland stream, the same genera predominated on both grazed and ungrazed substrates, similar to the reported by Peterson, Vormittag, and Valett (1998), which offered biofilm developed in 7 days under different grazing conditions (elevated and nonelevated platforms) to two common insect grazers. The lack of differences between substrates may be a result of the predominant genus, *Fragilaria*, which is tolerant to grazing and compensates loss in abundance with higher reproduction and recolonization rates (Stevenson, Peterson, Kirschtel, King, & Tuchman, 1991; Sumner & McIntire, 1982; Wellnitz & Poff, 2006). Peterson et al. (1998) proposed that there is a trade-off between ingestion resistance and digestion by herbivorous invertebrates, where natural selection would favour digestion resistance in algae easily consumed. This would be the case of *Fragilaria*, given that these authors found it was more susceptible to be ingested by macroinvertebrates but it was less digestible, allowing it to promptly recolonize substrates. In agreement with our results, Lamberti, Gregory, Ashkenas, Steinman, and McIntire (1989) found a higher grazing effect on the taxonomic structure at lower light incidences, and null grazing effects at higher light intensities, suggesting that algal

communities reach a similar successional state at high irradiances.

Regarding structural variables, the results obtained for richness are consistent with what was predicted, and are in agreement with the findings of Liess and Hillebrand (2004), given that in the grassland stream a higher number of genera compared to the afforested stream were found in ungrazed substrates. This was also accompanied by higher algal density and biomass. However, higher values of Chl *a* and lower AFDM were registered in grazed substrates, indicating that grassland stream communities subject to heavy grazing displayed fast recovery. In support of this, Abe et al. (2007) proposed that a higher productivity in assemblages with lower biomass can mitigate grazing effect; as grazers reduce periphyton biomass the ability to generate more biomass increases. NGE results on Chl *a* and AFDM supported the higher grazing effect on grassland substrates compared to the afforestation. In a previous study in the area, Principe et al. (2015) proposed that higher grazing in grassland streams and heavy shading by afforestation contributed to the lack of differences in biofilm accrual between both stream types, which is confirmed by our results.

Grazing can have a positive effect and stimulate primary production by removing dying and dead cells, changing the assemblage to species more active photosynthetically (e.g., diatoms), facilitating light and nutrients access to lower layers of biofilm, and renewing nutrients by grazer excretions (Holomuzki et al., 2010; Lamberti et al., 1989; Lamberti & Resh, 1983; Liess & Haglund, 2007; Rober, Wyatt, & Stevenson, 2011; Wallace & Webster, 1996). This phenomenon could explain the higher values of Chl *a* in grazed substrates of the grassland stream, suggesting a higher efficiency and metabolic activity of the community exposed to grazers (Huchette, Beveridge, Baird, & Ireland, 2000; Liess & Hillebrand, 2004). Furthermore, grazed substrates showed higher values of the autotrophic index at 45 days. At that time, we observed a high vertical development of the community, which a few days later was sloughed, probably because of bubble formation and senescence of inner layers of biofilm, similar to the findings of other researchers (Boulêtreau, Garabétian, Sauvage, & Sánchez-Pérez, 2006; DeNicola, McIntire, Lamberti, Gregory, & Ashkenas, 1990; Lamberti et al., 1989; Saravia et al., 2012). In this way, the community was reset leading to a more autotrophic community at 73 days. Interestingly, communities in the afforested stream never reached that state of development and “sloughing off” mechanism. This could explain the reduction of diversity with time in both substrates of the afforested stream, given that a higher frequency of disturbances (due to grazing or current) or self-generated detachment occurring in the grassland stream may increase diversity according to intermediate disturbance hypothesis (Connell, 1978; Peterson, 1996). Ungrazed substrates of the grassland stream were completely covered by masses of

T. lubrica during the experiment, but they were absent 10 days before the final substrate removal, probably as a consequence of periphytic sloughing.

In the afforested stream, algal biomass (Chl *a* and AFDM) did not increase with time, suggesting growth limitation by light (Cibils-Martina, Principe, et al., 2017; Stevenson, 1996). Cibils-Martina, Principe, et al. (2017) also found that succession trajectories were different between grassland and afforested streams, with a longer accrual phase occurring in grassland streams and lower algal biomass in afforested streams. As we expected, in this stream, there were no important differences between grazed and ungrazed substrates in structural or functional attributes. The analysis on functional traits revealed that communities in the afforested stream remained at an early stage of succession, with a predominance of small and low profile algae, consistent with what we expected. Several researchers agree that grazers selectively remove algae more exposed and loosely attached, while prostrate and firmly attached forms resist grazing (Feminella & Hawkins, 1995; Holomuzki et al., 2010; Liess & Hillebrand, 2004; McCormick, 1994; McCormick & Stevenson, 1989). A low proportion of trait categories (33%) revealed grazing effects, similar to the findings of Wellnitz and Ward (1998), who stated that the substrate which algae are attached to (e.g., detritus or other algae) and their position in the matrix can be better indicators of grazing susceptibility than physiognomy. However, grazed substrates in the grassland stream showed a higher proportion of motile algae and a reduction of algae with pad mucilage and colonial forms. Motile algae have been reported as good competitors since they can avoid unfavourable conditions (Johnson et al., 1997; Lange et al., 2011; Passy, 2007). The reduction of algae with pad mucilage and colonial growth could reflect the consumption of erect algae, which are more exposed in the biofilm, consistent with other studies (Cibils Martina et al., 2014; Holomuzki et al., 2010; Kawamura & Hirano, 1992; Lange et al., 2011). FD and FE indices were lower in the afforested stream at the end of the experiment, which is in agreement with the results of Cibils et al. (2015). They used other FD indices, one similar to Simpson's index of taxonomic diversity and an index of variance. However, similar to our study, they found that grassland streams had a higher FD than afforested streams. They stated that afforestation reduces FD given that lower light availability lead to periphyton architecture simplification. Instead, higher light levels in grassland streams result in complex algal communities. FD is an important ecological issue because it is linked to the way species share the niche space available in a community (Mason, Mouillot, Lee, & Wilson, 2005) and has important consequences for the functioning of ecosystems (Díaz et al., 2007).

This study showed that grazers can affect several variables of the algal community's structure and dynamics of succession, and that afforestation has altered the

development of algal communities and their interaction with grazers. Many researchers have reported marked effects of grazing on stream algal communities, though usually there are interactions with other factors such as light, nutrients, seasonality or higher trophic levels (Buria et al., 2010; Mal-lory & Richardson, 2005; Moulton, Lourenço-Amorim, Sasada-Sato, Neres-Lima, & Zandonà, 2015; Opsahl, Wellnitz, & Poff, 2003; Rober et al., 2011; Rosemond et al., 2000; Wellnitz & Ward, 1998). These factors affect abundance and behaviour of grazers (Wellnitz, 2015). Some studies did not find significant effects of grazing because of low grazer densities or high levels of physical disturbances (Hillebrand & Kahlert, 2002; Lange et al., 2011; Merten, Hintz, Lightbody, & Wellnitz, 2010), and it is important to consider life history, adaptations and physiological limits of species that dominate grazer assemblage (Wellnitz, 2015). In addition, as pine afforestation not only change light availability but also the input of organic matter, it would be interesting to determine the relative importance of algae in relation to other food resources, as biofilm developed in needle accumulations; likewise, to assess the inhibitory effect of substances released by needles (Bärlocher & Oertli, 1978; Thompson & Townsend, 2004).

5 | CONCLUSION

In conclusion, this experiment showed that top-down and bottom-up forces interact in different ways in grassland and afforested streams. Some structural attributes and functional traits revealed more grazing effects in the grassland stream, and a higher importance of light in the regulation of algal communities in the afforested stream. However, further research can help to understand these complex interactions. This type of study allows assessing changes in time, given that grazing effects tend not to be evident at the beginning of experiments. While algal communities are under dual control of resources and consumers (Rober et al., 2015), the relative strength of bottom-up versus top-down control is different among stream types and the primary regulatory factor can be modified according to land use change.

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CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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