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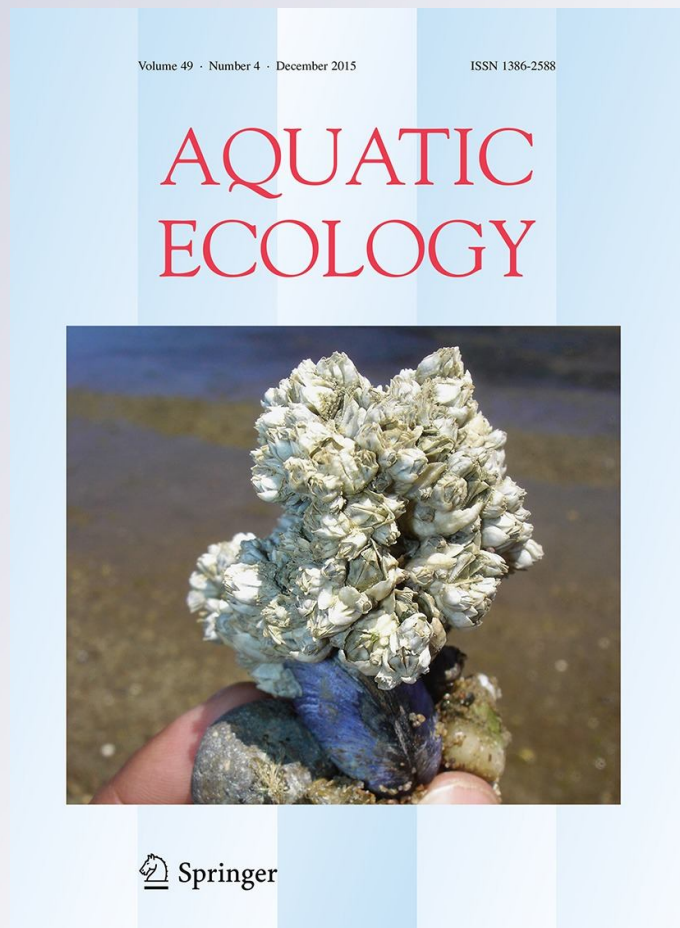
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Functional diversity of algal communities from headwater grassland streams: How does it change following afforestation?

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Abstract Pine afforestation of grassland streams may lead to changes in species traits and therefore functional diversity of epilithic algal community. Here, we studied trait-based responses in three grassland and three afforested streams in a mountain watershed of Córdoba, Argentina. We hypothesized that afforestation would reduce functional diversity through a simplification of periphyton architecture resulting from reduction in light availability, and that changes in hydrological periods would influence community responses. Algal samples were collected at each stream during two different hydrological periods (high flow and low flow), and physicochemical variables were recorded. Selected traits included strategies and morphological characters related to resource access and disturbance resistance (size, morphological guild, resource requirement, attachment mechanism and life-form). We calculated two

indices of functional diversity: Rao's quadratic entropy (FD_Q) and functional variance. Most trait categories showed a significant effect of one or both factors; 26 % discriminated between vegetation types, 26 % reflect the changes between hydrological periods, and 47 % were sensitive to both of them. Our results revealed some categories of traits that can be used to distinguish changes in riparian vegetation, such as unicellular life-form and high-profile guild. Functional diversity of single traits was affected differently by pine afforestation. However, the most integrative index, the FD_Q mean, partially supported our hypotheses. Afforestation reduced FD_Q mean by 50 %, but only during low-flow period. FD_Q mean was high and similar between streams at high flow, when environmental factors, such as discharge and temperature, could prevail on differences in riparian vegetation.

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Introduction

Functional classification of freshwater communities has been implemented in the monitoring of human impacts on aquatic ecosystems (e.g., Cummins et al. 2005; Welcomme et al. 2006; Kruk et al. 2010). There is a growing number of studies of physiognomic changes in stream periphytic communities in response

to disturbances (e.g., Passy 2007; Lange et al. 2011; Rimet and Bouchez 2011; Stenger-Kovács et al. 2013; Wagenhoff et al. 2013; Hlúbíková et al. 2014), but the use of functional traits and functional diversity indices of periphyton communities has been less explored. Functional diversity is an important ecological issue because it is linked to the way species share the niche space available in a community (Mason et al. 2005) and has important consequences for the functioning of ecosystems (Díaz et al. 2007).

Functional traits are morphological, physiological or phenological features measurable at the individual level, that impact fitness of the organism through their effects on growth, reproduction and survival (Violle et al. 2007). It is widely considered that species differ from each other in terms of some traits (Díaz and Cabido 2001), and thus that functional diversity is based on the “extent of trait dissimilarity among species in a community” (Tilman 2001; Petchey and Gaston 2002). The function of single species is determined mainly by their ability to capture, allocate and conserve resources, or to withstand the pressure of competitors, consumers and environmental stress (Grime 2001). However, direct measurements of these “hard traits” are usually not available for many species. Instead, more easily measured, often morphological, “soft traits” can be used as surrogates (Lepš et al. 2006). Every environmental change that affects the growth, survival or fecundity of individuals will affect population sizes, community structure and ultimately ecosystem functioning (Doléec and Bonada 2013).

Stream ecosystems around the world receive a diverse range of human impacts (both in extension and intensity). One major driver of stream impairment comes from timber extraction which results in catchment deforestation and conversion to other land uses, and in the replacement of native forests by monocultures of rapidly growing trees. A less studied impairment is the creation of tree plantations in what are otherwise grassland ecosystems, which happens in semiarid areas of the world, including South America (Simberloff et al. 2010). When grassland streams are afforested with exotic pines, the ecological processes in which benthic algae play a key role (e.g., stream nutrient cycling, productivity of basal resources which support food webs) may become altered. Reduced light availability that results from evergreen trees being planted in riparian areas reduces the physiologic options of members of periphytic algal

communities, since only under ample light conditions can structural complexity develop (Lowe et al. 1986). Afforestation can also have a significant impact on the catchments that drain plantations (Farley et al. 2005; Jackson et al. 2005). In mountain streams of Córdoba, Argentina, 50 % reduction in annual water yield has been reported for afforested catchments compared to grasslands (Jobbágy et al. 2013), likely the result of increased water interception by needles, absorption by deep tree roots and evapotranspiration compared to grasslands. Differences in stream flow and in the hydrological regime can be an important factor controlling periphyton development that can affect its structure, architecture and functional capacities (Villeneuve et al. 2010; Wellnitz and Poff 2012). Conversely, an increase in current velocity can lead to an increase in nutrient availability and result in stimulation of algal metabolism and nutrient uptake (Stevenson 1996).

The aim of this study was to test how pine afforestation of grassland headwater streams changes species traits and functional diversity of the epilithic algal community. We hypothesized that afforestation reduces functional diversity given the simplification of periphyton architecture due to the reduction in light availability year round. In contrast, we expected that high light levels in grassland streams result in complex algal communities, with more stalked and filamentous species. However, due to the fact that catchment afforestation may seasonally reduce discharge, intensity of flows and temperature, we hypothesized that hydrological periods would alter functional responses of algal communities depending on vegetation type. At the high water period flood pulses are expected to be more frequent and intense in grassland streams and may negatively affect exposed growth forms; however, this period could benefit sensitive species given a potential higher input of nutrients and temperature. We therefore expected fewer seasonal changes in functional diversity in afforested streams compared to grassland streams.

Materials and methods

Study area

The study was carried out in streams of the Ctlamochita River upper basin, Córdoba, Argentina (Fig. 1). Annual precipitation in the region reaches 725 mm

occurring mostly between spring and the end of summer (October–March) (Cabido et al. 2003), whereas maximum temperature reaches 34 °C in summer (December–March) and decreases up to −5 °C in winter (June–September). Lithology is dominated by granite, but localized patches of metamorphic rocks (gneiss, schist, migmatite) are also present.

Six first-order streams with similar altitude, orientation and drainage area were selected in the Santa Rosa stream sub-basin (which belongs to the Ctlamochita River basin). Three streams drain grasslands, and each one has a neighbor stream that drains plantations of *Pinus elliottii* (afforested streams) (Fig. 1). Drainage area of the study streams ranged from 27 to 142 ha (mean watershed size, grassland 69 ha; forest 86 ha, fully covered by pine plantations) and was located at about 1100 m a.s.l. Grasslands at this altitude are dominated by *Festuca hieronymi*, *Nassella filiculmis*, *Schizachyrium condensatum* and

Eragrostis airoides (Oggero and Arana 2012), and they are primarily used for extensive livestock grazing. Most afforested areas in the region were established in the late 1970s as a result of a tax deferral plan implemented by the Córdoba government. Currently, nearly 36,000 ha of the Sierras de los Comechingones in Córdoba are afforested with exotic pines between 900 and 1600 m a.s.l. (Plevich et al. 2002). In the study area, *Pinus elliottii* is the dominant tree in the plantations followed by *Pinus radiata* and *Pinus taeda*.

Field and laboratory methods

To characterize study streams at each sampling occasion, hydraulic and physicochemical variables were measured. Water temperature and pH were recorded with portable sensors. Depth and current velocity were measured with a Global Flow Probe, and PAR (photosynthetically active radiation) intensity

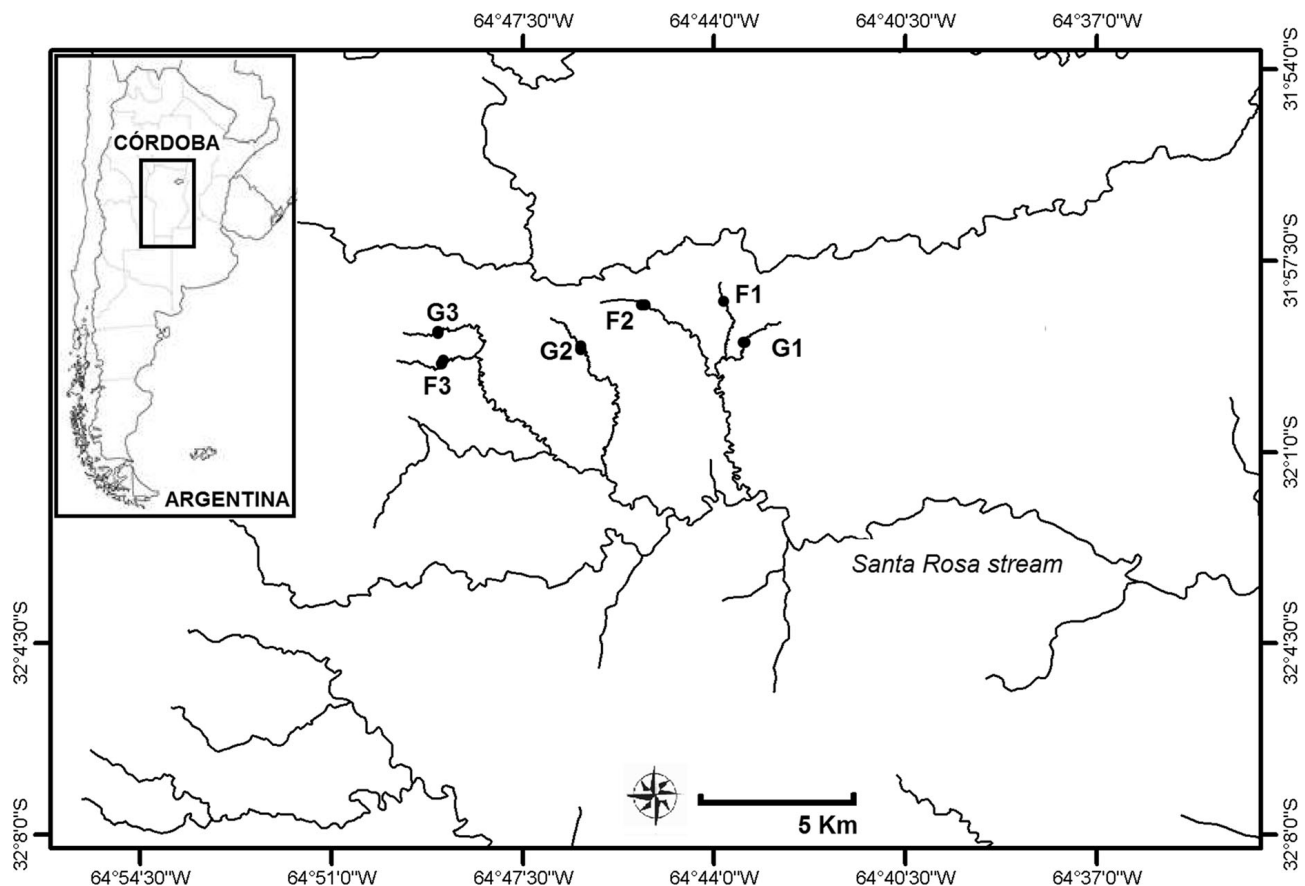


Fig. 1 Paired study streams belonging to Santa Rosa stream sub-basin (Córdoba, Argentina). Three streams drain grasslands (G1, G2 and G3) and the other three streams run through pine plantations (F1, F2 and F3). Sampling points are indicated

was measured with a QSL-2100 Irradiance Sensor (Biospherical Instruments, Inc., San Diego, CA, USA) at midday along a 50-m reach at each stream during the high water period.

Benthic algae samples were collected from four randomly selected cobbles at each stream and hydrological period (July 2008 and February 2009 for low and high water period, respectively). Hydrological periods correspond to austral winter and summer and differed mainly in temperature and in the amount of rains. Cobble surfaces ($\sim 100 \text{ cm}^2$) were scrubbed with a toothbrush. In the laboratory, total sample volume was recorded, and then it was homogenized, fixed with 4 % formalin and was used for species identification and cell density calculations.

The counts were performed by direct methods at $400\times$ magnification with slide and coverslip of $24 \times 50 \text{ mm}$ following transects along the coverslip. The counting unit was an individual cell for unicellular and coenobial organisms, and a cell equivalent of $30 \mu\text{m}$ length for filaments and of $30 \times 30 \mu\text{m}$ area for colonies (Gómez et al. 2009). Soft algae were identified in this count, and diatoms lumped into a single taxonomic category. For diatom species identification, organic matter was previously eliminated using 40 % hydrogen peroxide and permanent slides were prepared according to Hasle (1978), using ZRAX (1,7)[®] as slide mounting medium. At least 600 units (one frustule or two valves) were counted and identified in each sample using a light microscope with $1000\times$ magnification, and abundances were expressed as relative counts. Counts were converted to density of cells per surface area of rocks (number of cells cm^{-2}) based on Villafañe and Reid (1995), accounting for the volume of 0.3 ml used to the counts at each slide. For taxonomic analysis, specific bibliography of each particular group was used and names were updated following Spaulding et al. (2010), Komárek and Hauer (2013) and Guiry and Guiry (2014).

Functional traits: definition

In this study, we propose the use of five biological traits, and their respective categories or modalities, which are shown in Table 1. These morphological traits are functionally relevant for algal individual fitness thus linking the response of individual species to resources and disturbances of their environment.

The assignation of traits to all algal taxa was based on our own observations and on data available in specific bibliography adapted to local species (Passy and Larson 2011; Rimet and Bouchez 2012). Passy (2007) proposed the use of diatom morphological guilds that allow assessing the response of diatoms to nutrient availability and current velocity. Later, Passy and Larson (2011) extended the classification of functional guilds to soft algae, and they also classified species differing in nutrient preferences according to Passy (2008). Rimet and Bouchez (2012) provided tables with metrics obtained from different sources to characterize diatom species according to size, life-forms and ecological guilds. In supplementary material (Online Resource 1), there is a full list of taxa and the assignation to each trait category.

Size was one of the selected traits, given that for example, small species are generally pioneers, tolerant, able to faster colonize substrates, while species with higher biovolumes have higher resources requirements and are expected to be more affected by disturbances. Taxa were assigned to five size classes, consisting of taxa with different biovolumes expressed in Table 1. Species that usually form chains or pseudofilaments were assigned to size class 5, even when the cell biovolume is smaller (e.g., *Fragilaria crotonensis*).

Morphological guild is defined according to the access of taxa to resources (light and nutrients) and vulnerability to drag and dislodgement due to disturbances (e.g., current, grazing). In particular, short-statured algae, either immobile, including adnate, prostrate, erect, and solitary cells, small colonies and coenobia, or slowly moving diatoms, constituted the low-profile guild; tall-statured unicellular, colonial, or filamentous algae extending above the substrate, the high-profile guild; and fast-moving biraphid diatoms or flagellated soft algae, the motile guild. The system functioning is not the same if community is, for example, dominated by low-profile guild or the three guilds are equally represented. Low-profile guild is adapted to high current velocities and to low nutrient concentrations, and the contrary is true for high profile. The motile guild is not tolerant to high current velocities but tolerant to high nutrient concentrations.

Resource requirement is defined in relation to the tolerance or sensitivity of species to nutrients and light limitation, due to low ambient availability or overgrowth (Passy and Larson 2011). Algae requiring high

Table 1 Functional traits and categories defined for epilithic algal communities

Functional traits	Categories or modalities	References
Size	c1 <99 μm^3	Rimet and Bouchez (2012)
	c2 100–299 μm^3	
	c3 300–599 μm^3	
	c4 600–1499 μm^3	
	c5 >1500 μm^3	
Morphological guild	High profile (H)	Passy and Larson (2011), Rimet and Bouchez (2012)
	Low profile (L)	
	Motile (M)	
Resource requirement	Sensitive (S)	Passy and Larson (2011), Passy (2008)
	Tolerant (T)	
Attachment mechanism	Adnate (A)	Modified from Rimet and Bouchez (2012)
	Mucilage pad (P)	
	Mucilage stalk (S)	
	Holdfast (H)	
	Unattached (U)	
Life-form	Unicellular (Un)	Modified from Rimet and Bouchez (2012)
	Colonial (C)	
	Coenobial (Ce)	
	Filamentous (F)	

The assignation of traits to all algal taxa was based on our own observations and on data available in specific bibliography. See Online Resource 1 for more details

resource levels for growth and reproduction are considered sensitive (i.e., meso-eutrophic to hyper-eutrophic forms), while those proliferating under low resource levels are viewed as tolerant (i.e., oligotrophic, mesotrophic and indifferent species).

Attachment mechanism determines the spatial position in biofilm and the ability to withstand disturbances. According to life-forms of Rimet and Bouchez (2012), adnates are those algae firmly attached by their valve face (e.g., *Cocconeis*) or by their girdle view (e.g., *Eunotia*). The mucilage pad category is referred to algae that secrete a small mucilage pad at one end of the valve to attach to the

substrate allowing it to stand upright (e.g., *Diatoma*, *Fragilaria*, *Ulnaria*). Species with mucilage stalk grow attached to surfaces by mucilage stalks, which can be simple (one cell) or can link several cells forming arbuscular “colonies” (e.g., *Achnanthes*, *Cymbella*, *Gomphonema*). In species assigned to holdfast category, filaments are attached by an initial cell (e.g., *Cladophora*, *Oedogonium*). Unattached included species without any specific mechanism of attachment may be because they float (e.g., centric diatoms, coenobia, colonies and filaments of cyanobacteria or zygnematales, represented in periphyton due to spines, weight or mucilage) or move freely (e.g., *Nitzschia*, *Navicula*, *Pinnularia*).

Life-form categories are related to biofilm architecture; the response of a community to environmental factors changes if it is dominated by unicellular forms or filamentous and colonial organisms. Diatoms that usually form chains or rosette or arbuscular aggregates were assigned to colonial category, even when these are not real colonies.

Data analysis

To compare physicochemical variables measured at each stream type and hydrological period, two-way ANOVAs were carried out and one-way ANOVA was used to compare PAR measurements (only recorded at the high water hydroperiod) between grassland and afforested streams.

To compare the proportion of species in the different categories of each trait and the functional diversity indices (see below) between riparian vegetation (grassland and afforested) and hydrological periods (high and low water period), we used general linear mixed models (LMM) in a hierarchical design. We included “catchment”, “stream” and “replicates” as nested random factors, since we collected four cobbles in each stream, and streams corresponded to three different catchments (“stream nested in catchment” and “replicate nested in stream nested in catchment”) and “riparian vegetation” and “hydrological period” were included as fixed factors. Validations of simple assumptions of the models were performed reviewing standardized residuals versus predicted and the normal $Q-Q$ plot of standardized residuals. Specific means were compared to each other using DGC’s (Di Rienzo–Guzman–Casanoves) multiple-comparison test ($\alpha = 0.05$), a hierarchical

method which controls type I error while maintaining acceptable power (Di Rienzo et al. 2002).

Following Berthon et al. (2011), the F value of LMM was used to compare the discrimination power of the categories of traits between riparian vegetation and hydrological period effect, comparable since the degrees of freedom for each trait category were identical. When the F value is high, then the discriminating power of that category is high.

Based on Schleuter et al. (2010), we selected the indices Rao's quadratic entropy FD_Q (Rao 1982) and functional variance FD_σ (Lepš et al. 2006, FD_{var} modified from Mason et al. 2003) to calculate functional diversity with categorical variables. For the calculation of functional diversity indices for each trait and each sample, we used an Excel macro (Lepš et al. 2006). The FD_Q is a generalized form of the Simpson index of taxonomic diversity. The FD_Q index generally reflects the probability that, picking randomly two individuals in a community, they are functionally different (e.g., for single traits, either they have different trait values or different trait categories) (Lepš et al. 2006). To calculate a compound index of functional diversity in terms of multiple traits, we averaged FD_Q calculated by single traits. The mathematical function is:

$$FD_Q = \sum_{i=1}^s \sum_{j=1}^s d_{ij} p_i p_j$$

where p_i is the proportion of i th species in a community; s is the number of species in the community; d_{ij} is the dissimilarity of species i and j ; $d_{ii} = 0$, i.e., dissimilarity of each species to itself is zero.

Functional variance FD_σ has the form:

$$FD_\sigma = \sum_{s \in S_c} \frac{A_s}{A} (X_{ts} - \bar{X}_{ts})^2$$

where S_c set of species present in community c , s species, A_s abundance of species s , A total abundance of all species, X_{ts} average value of trait t in species s and \bar{X}_{ts} is the average of X_{ts} over all species present. If species and/or abundances are clustered around the mean trait value, FD_σ is low; if they are clustered at the trait edges of the community, FD_σ is high.

To compare the functional diversity indices between riparian vegetation and hydrological periods, we used general linear mixed models (LMM) in a hierarchical design, the same as for proportion of species in the different categories of each trait. Statistical analyses were carried out using the R statistical program version 3.0.1 (R Core Team 2013).

Results

Physicochemical variables

Grassland and afforested streams did not show marked differences in the measured physicochemical variables (Table 2), except for light intensity, since afforested streams registered 70 % less PAR intensity. The pH was neutral, with similar values for grassland and afforested streams. Mean water velocity was higher in high water period in grassland streams, though not significant. Water temperature showed differences between periods, ranging from 12 to 21 °C in grassland streams and from 10 to 19 °C in afforested streams at low and high water periods, respectively.

Table 2 Physicochemical variables measured at each stream type and hydrological period

	Low water period		High water period		ANOVAs
	Grassland	Afforested	Grassland	Afforested	
Temperature (°C)	12.5 (0.71)	11.33 (1.53)	18.6 (2.02)	18 (1.32)	$F_{1,10(per)} = 45.6, p = 0.0003$
pH	8.51 (0.28)	8.24 (0.14)	7.99 (0.85)	8.16 (0.44)	NS
Current velocity (m s ⁻¹)	0.21 (0.05)	0.23 (0.10)	0.34 (0.06)	0.19 (0.17)	NS
Depth (cm)	8.07 (0.99)	7.51 (1.81)	7.76 (2.74)	4.92 (1.95)	NS
PAR (μmol m ⁻² s ⁻¹)			2028 (169)	533 (353)	$F_{1,5(veg)} = 51.34, p = 0.002$

Mean, standard deviation (in brackets) and two-way ANOVAs results are shown (i.e., F and p values and degrees of freedom for significant factors). PAR values were only analyzed by one-way ANOVA for high water hydrological period

NS no significant difference, *per* factor hydrological period, *veg* factor vegetation

Patterns in functional traits and indices of functional diversity

Total taxonomic richness in the study area, considering grassland and afforested streams at high and low water periods, resulted in 300 taxa. Almost 70 % were diatoms, followed by cyanobacteria (13 %), chlorophytes (9 %) and charophytes (8 %). The most abundant taxa (relative abundance >10 %) included *Gomphonema pumilum* (Grunow) Reichardt and Lange-Bertalot, *Achnantheidium minutissimum* (Kütz- ing) Czarnecki, *Fragilaria capucina* Desmazières, *Cocconeis placentula* Ehrenberg (var. *lineata* and var.

euglypta) in grassland streams and *A. minutissimum*, *C. placentula* and *Karayevia clevei* (Grunow) Bukhti- yarova in afforested streams (Online Resource 1).

Results of LMM for proportions of species in the different categories of each trait are shown in Fig. 2, Table 3 and Online Resource 2. Table 4 and Fig. 4 show functional indices results. Grassland streams were characterized by a similar proportion of species of low and high profile (Fig. 2), and the FD of guilds was higher at the high water period (Fig. 4; Table 4). There was also a higher proportion of colonial and coenobial species (Fig. 2; Table 3) compared to afforested streams. The diversity of life-forms was

Fig. 2 Relative abundance (Pi) of species corresponding to selected functional traits in grassland (G) and afforested streams (F) and low (L) and high (H) water hydrological periods. Mean and standard error are shown. For size classes, two of the five categories are shown (c1 comprises the smaller organisms and c5 the larger ones), for attachment mechanisms, two of the five and for life-forms, two of the four categories. Letters show the results of post hoc tests, mean values with the same letter are not significant different, results for the different categories of each trait are differentiated by uppercase and lowercase letters

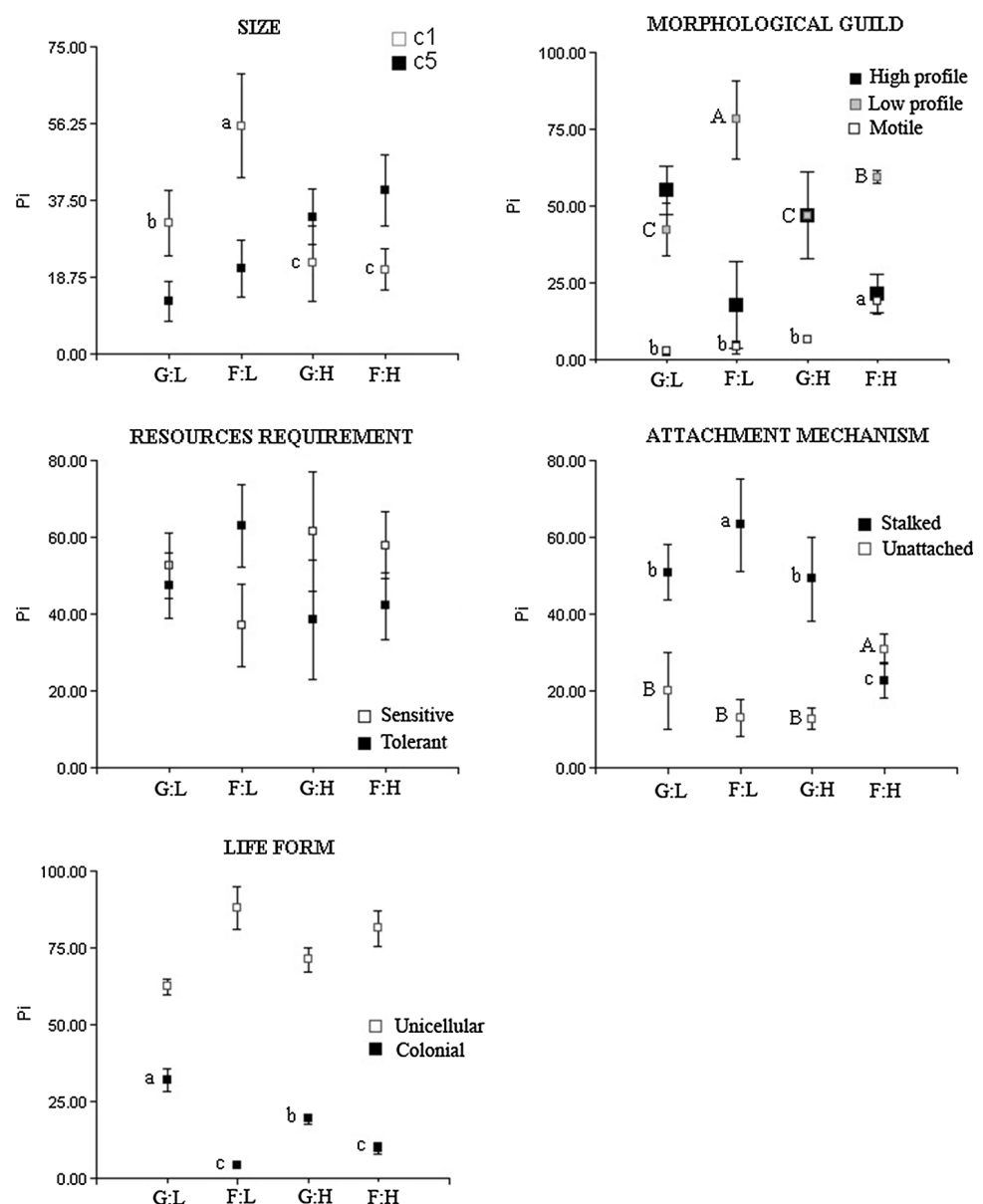


Table 3 Results of comparison of each trait category between grassland (G) and afforested (F) streams and low (L) and high (H) hydrological periods using general linear mixed models in a hierarchical design

Traits categories	$F_{1,22}$	p	Result	Significant effect
<i>Size</i>				
c1	5.18	0.03	FL > GL > FH = GH	Interaction
c2	4.46	0.04	GL > GH > FL = FH	Interaction
c3	7.38	0.01	FH > GH > FL = GL	Interaction
c4	0.78	0.38	GH = FH = GL = FL	None
c5	27.06	<0.0001	H > L	Hydrological period
<i>Morphological guild</i>				
Low profile	6.8	0.01	FL > FH > GH = GL	Interaction
High profile	57.19	<0.0001	G > F	Vegetation
Motile	11.7	0.002	FH > GH = FL = GL	Interaction
<i>Resource requirement</i>				
Sensitive	9.96	0.005	H > L	Hydrological period
	5.13	0.03	G > F	Vegetation
Tolerant	9.95	0.005	L > H	Hydrological period
	5.12	0.03	F > G	Vegetation
<i>Attachment mechanism</i>				
Adnate	9.42	0.006	H > L	Hydrological period
Pad	6.10	0.02	GL = GH = FH > FL	Interaction
Stalked	12.3	0.002	FL > GL = GH > FH	Interaction
Holdfast	6.54	0.02	H > L	Hydrological period
Unattached	4.58	0.04	FH > GL = GH = FL	Interaction
<i>Life-form</i>				
Unicellular	25.24	<0.0001	F > G	Vegetation
Colonial	7.16	0.01	GL > GH > FH = FL	Interaction
Coenobial	8.01	0.01	G > F	Vegetation
Filamentous	0.06	0.80	GH = FH = GL = FL	None

F and p values and the result of post hoc tests are shown only for main factors or interaction factors when significant values were found ($p < 0.05$). Complete results of LMM can be seen in Online Resource 2

higher in grassland streams, and there were differences between periods in the diversity of resource requirements (Fig. 4; Table 4), being higher at the low water period. In contrast, afforested streams showed a higher proportion of low-profile and small species (Fig. 2; Table 3). In afforested streams, higher differences were observed between hydrological periods in the diversity of guilds, with a higher diversity at the high water period (Fig. 4; Table 4). It was also a higher proportion of unicellular forms compared to grassland streams, and stalked species became important in the low water period (Fig. 2; Table 3). In fact, stalked species also predominated during the low water period in grassland streams, but different species performed this guild at each stream, being *Achnanthydium minutissimum* predominant in afforested streams and *Gomphonema pumilum*, *A. minutissimum* and *Cymbella cymbiformis* abundant in grassland streams. Afforested streams at the low water period also

showed a higher proportion of tolerant species (Fig. 2; Table 3).

Interestingly, some traits discriminated significantly between hydrological periods, independently of vegetation type. Size class diversity was higher in the high water period (Fig. 4; Table 4), when a higher proportion of larger species was observed (Table 3). It is worth noting that different species in grassland and afforested streams contributed to the predominance of large-size species in this period, with the presence of some large diatoms in grasslands and cyanobacteria in afforested streams. Furthermore, the proportion of sensitive species was higher in the high water period (Fig. 2; Table 3). The diversity of attachment mechanisms was also higher at the high water period (Fig. 4; Table 4).

The highest F value was observed for high-profile guild ($F = 57.19$, Fig. 3), that discriminated between riparian vegetation (Table 3), likewise unicellular life-

Table 4 Results of comparison of indices of functional diversity FD_Q and FD_σ between grassland and afforested streams (factor vegetation) and low and high water periods (factor hydrological period) using general linear mixed models in a hierarchical design

Trait	VS	FD_Q	FD_σ
Size	Veg	$F_{1,22} = 0.01, p = 0.92$	$F_{1,22} = 0.93, p = 0.34$
	Per	$F_{1,22} = 12.12, p = 0.002$	$F_{1,22} = 0.99, p = 0.33$
	Veg \times per	$F_{1,22} = 0.74, p = 0.39$	$F_{1,22} = 3.21, p = 0.08^*$
Morphological guild	Veg	$F_{1,22} = 1.48, p = 0.24$	$F_{1,22} = 7.15, p = 0.01$
	Per	$F_{1,22} = 26.63, p < 0.001$	$F_{1,22} = 47.44, p < 0.001$
	Veg \times per	$F_{1,22} = 6.04, p = 0.02$	$F_{1,22} = 9.56, p = 0.005$
Resource requirement	Veg	$F_{1,22} = 3.66, p = 0.07$	$F_{1,22} = 2.66, p = 0.12$
	Per	$F_{1,22} = 1.35, p = 0.26$	$F_{1,22} = 1.47, p = 0.24$
	Veg \times per	$F_{1,22} = 14.43, p = 0.001$	$F_{1,22} = 13.2, p = 0.001$
Attachment mechanism	Veg	$F_{1,22} = 1.58, p = 0.22$	$F_{1,22} = 1.5, p = 0.23$
	Per	$F_{1,22} = 8.92, p = 0.007$	$F_{1,22} = 18.9, p < 0.001$
	Veg \times per	$F_{1,22} = 3.57, p = 0.07^*$	$F_{1,22} = 3.55, p = 0.07^*$
Life-form	Veg	$F_{1,22} = 27.12, p < 0.001$	$F_{1,22} = 6.99, p = 0.01$
	Per	$F_{1,22} = 1.45, p = 0.24$	$F_{1,22} = 2.08, p = 0.16$
	Veg \times per	$F_{1,22} = 2.18, p = 0.15$	$F_{1,22} = 0.31, p = 0.58$
FD_Q mean	Veg	$F_{1,22} = 5.17, p = 0.03$	
	Per	$F_{1,22} = 13.45, p = 0.001$	
	Veg \times per	$F_{1,22} = 6.35, p = 0.02$	

Significant results for each analysis are in bold. The symbol (\bullet) points a marginally significant difference. Post hoc results when interaction is significant can be seen in Fig. 4

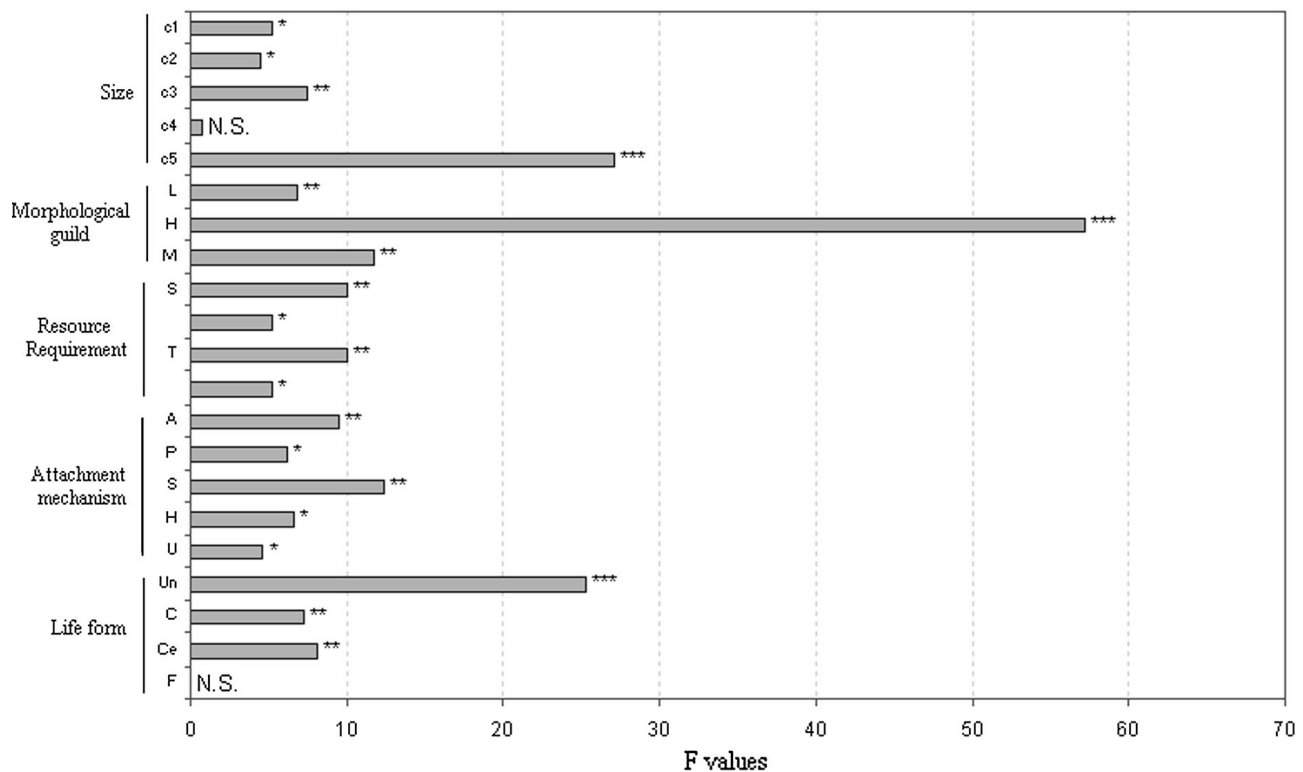


Fig. 3 Discrimination power of the different traits categories for riparian vegetation and hydrological period as indicated by the F values of LMM. See Table 3 for references of traits categories. NS no significant difference, $*p < 0.05$, $**p < 0.01$, $***p < 0.001$

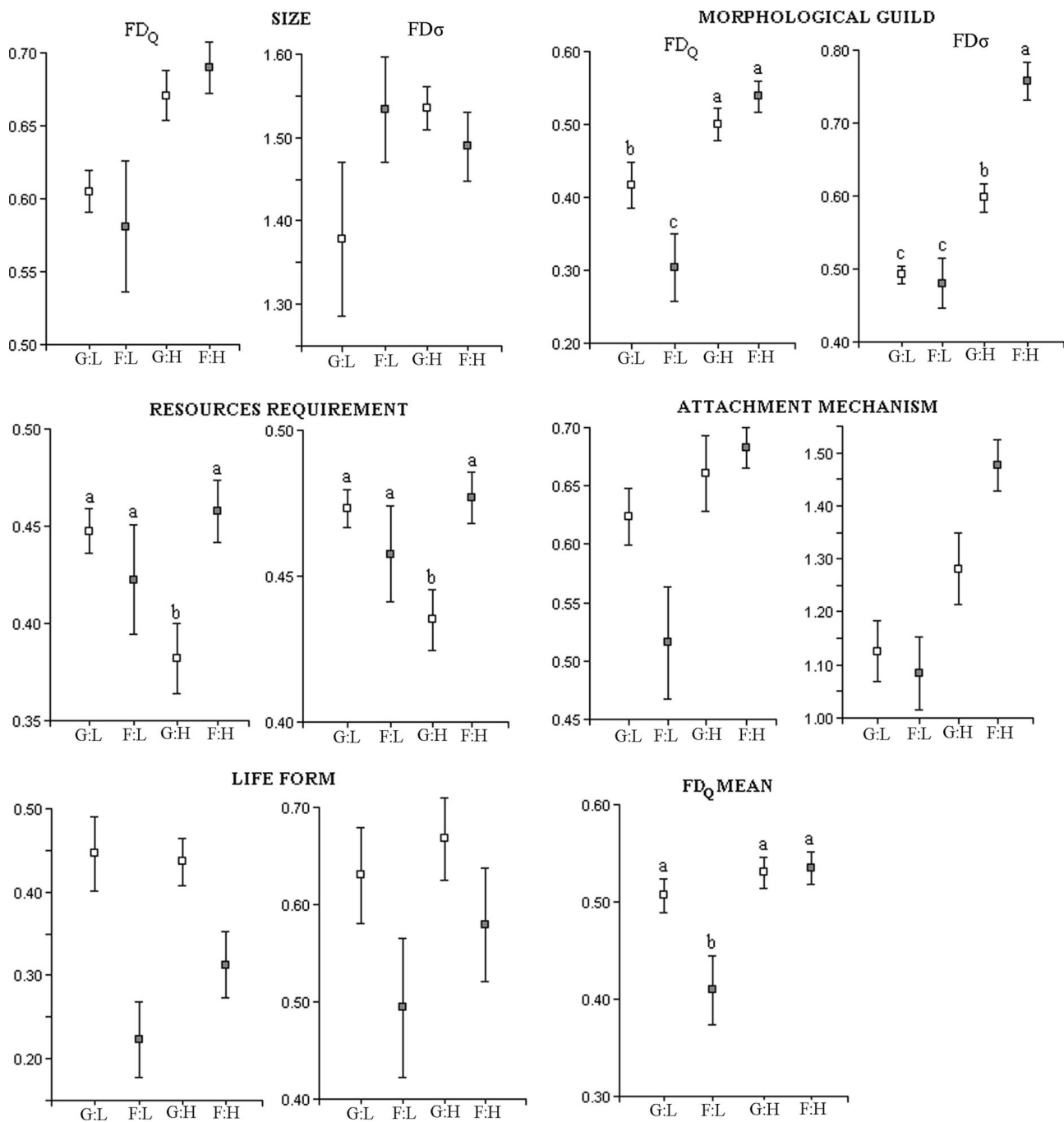


Fig. 4 Indices of functional diversity FD_Q and FD_σ for each trait in grassland (open, G) and afforested streams (filled, F) and low (L) and high (H) water hydrological periods. Letters show

the results of post hoc tests ($p < 0.05$), mean values with the same letter are not significantly different

form ($F = 25.24$). Size class c5 also showed a high F value ($F = 27.06$, Fig. 3; Table 3) but detected differences between hydrological periods. Most trait categories showed a significant effect of one or both factors (Table 3); 47 % (nine out of 19 trait

categories) were sensitive to riparian vegetation but depending on the hydrological period (significant interaction), while five out of 19 categories discriminated between vegetation types and five out of 19 reflected changes between hydrological periods.

For most traits, both indices (FDQ and $FD\sigma$) showed the same pattern, except for size classes (Table 4). FDQ mean showed that at the low water period, grassland streams had a higher functional diversity than afforested streams, but no difference was observed at high waters (Fig. 4; Table 4). Besides, FDQ mean was similarly higher between hydroperiods in grassland streams (Fig. 4).

Discussion

Afforestation of grassland streams influenced the functional diversity of epilithic communities, but hydrological period also imposed variation. We expected that afforestation would reduce functional diversity through the simplification of periphyton architecture that would follow a reduction in light availability (Hudon and Bourget 1983; Wellnitz and Ward 1998). Mean values of FD_Q , the most integrative index of our measured functional traits, supported this hypothesis at the low water period, when afforested streams showed lower functional diversity than grassland streams. Contrary to our expectations, there were no differences between riparian vegetation types during the high water period. Hence, hydrological period had an important influence on the functional responses of algal communities under different riparian vegetations, in addition to seasonal changes in functional diversity in afforested streams. Differences between riparian vegetation types at the low water period could respond to lower scouring disturbances in afforested streams, highlighting the effect of the reduction in resources availability. At the high water period, environmental factors such as discharge and temperature may be more important than differences in riparian vegetation.

Potential mechanisms explaining patterns

Given that this study was designed as a natural experiment to compare algal communities of pine-afforested streams with reference grassland streams, there are multiple interacting factors that may be influencing algal responses. However, two non-exclusive mechanisms potentially explain responses on the functional traits we studied. First, in relation to a reduction in light intensity, afforested streams

contained a greater proportion of small, low-profile species, which can colonize substrate faster and tolerate the low light availability (Lange et al. 2011; Stenger-Kovács et al. 2013). Mason et al. (2005) proposed that higher functional diversity indicates a high degree of niche differentiation and thus low competition. Hence, ecosystem functioning, in a broad sense, is enhanced because of a more efficient resource use. In this sense, the higher functional diversity for resource requirements and for guilds (FD_g) in afforested streams compared to grasslands in the high water period could be reflecting a more efficient use of scarce resources, such as sunlight. In grassland streams, higher light intensity may have facilitated the coexistence of different growth forms (Passy and Larson 2011) and would allow the development of a more architecturally complex community, yet more vulnerable to disturbances (Hudon and Bourget 1983; Lange et al. 2011) as we observed by the higher diversity of life-forms and the similar proportion of low and high-profile species.

A second potential explanation is that higher water velocity and discharge in the high water period may have stimulated algal metabolism and nutrient uptake (Stevenson 1996) and thus allowed the presence of larger and/or nutrient-sensitive species. These species can obtain a beneficial spatial position in the over-story, where resource acquisition is favoured (Larson and Passy 2012). It is worth noting that different species in grassland and afforested streams contributed to the predominance of larger species in this period, with the presence of some large diatoms in grasslands and cyanobacteria in afforested streams, each of which has different tolerance to stream conditions. This is similar to the results of Bourassa and Cattaneo (2000), who found higher proportion of cyanobacteria in shaded channels. The greatest proportion of sensitive and larger algae in the high water period suggests a higher availability of resources in this period (Larson and Passy 2012). Besides, afforested streams at the low water period showed a higher proportion of tolerant species that can grow and reproduce when resources are scarce (Passy and Larson 2011).

Effects attributed to hydrological alteration by afforestation cannot be fully discriminated from other seasonal factors such as temperature or nutrients. However, a previous study in the area found that water chemistry was similar between grassland and

afforested streams and that nutrient content was low and similar in both stream types during two successive years (on six dates covering fall, winter and spring) (Farley et al. 2008). Water temperature could be acting in the same way as current velocity, accelerating biofilm formation and stimulating metabolism (Díaz Villanueva et al. 2011; Romaní et al. 2014). In our study, although water temperature did not differ between stream types, it was higher during the high water period, which coincided with summer.

Pine plantations in the study area reduce discharge in afforested streams during the summer tree-growing season (Jobbágy et al. 2013), and catchment afforestation likely moderates flood intensity and frequency as well. We therefore expected seasonal differences in functional diversity to be more pronounced in grassland streams. Interestingly, most of our measured traits showed higher diversity at the high water period and differences between periods for both stream types. This suggests that higher water temperature, discharge, day length and light intensity may greatly reduce the differences between stream types, thereby increasing the functional diversity of both algal communities. We expected that flooding in grassland streams would affect exposed growth forms, limiting or even resetting community development (Villeneuve et al. 2010). In contrast, we found a higher diversity of attachment mechanisms at the high water period and greater life-form diversity in grassland streams, suggesting a coexistence of strategies to withstand stress produced by more frequent floods. Tuji (2000) reported an increase in relative abundance of stalked species in environments with patchy perturbations; however, stalked proportion decreased in afforested streams we studied at the high water period and the abundance of the other categories of this trait increased. Differences between hydrological periods demonstrate the key role of flow in the physical structure of communities (see Lamb and Lowe 1987; Poff et al. 1997; Villeneuve et al. 2010; Hart et al. 2013). Further research is needed to disentangle effects of flow and current velocity to distinguish their effects on physiognomy.

Concluding remarks

We found traits that could be used for monitoring changes in streams produced by afforestation, as well

as for monitoring other freshwater ecosystems. Most trait categories reflected the effect of changes to riparian vegetation and hydrological period. Some trait categories were more sensitive to variations in hydrological period than to afforestation, e.g., size class c5. Nevertheless, the categories unicellular life-form and high-profile guild can be used to distinguish changes in riparian vegetation. Both FD_Q and FD_σ indices reflected the responses of algal communities, showing the same pattern for most traits. FD_Q mean showed a similar trend to that observed for some of the FD_Q on single traits, summarizing information provided by single traits. Several methods have been described for the calculation of the functional diversity of a community, but FD_Q is the most common multivariate index of functional diversity (Schleuter et al. 2010). Our work contributes to the general knowledge of how changes produced by afforestation affect community structure and functioning by modifying the distribution of species traits. Despite the fact that traits not applied here may also be suitable for evaluating strategies of periphytic species, those selected did respond to the changes we were evaluating and could be used as tools for management and monitoring programs.

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