

RESEARCH PAPER

Pine needle litter acts as habitat but not as food source for stream invertebrates

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Handling Editor: Eric Chauvet

Funding information

Ministerio de Ciencia y Tecnología del Gobierno de la Provincia de Córdoba, Grant number: GRF 2008; Agencia Nacional de Promoción Científica y Tecnológica, Argentina, Grant number: PICT 1113; Consejo Nacional de Investigaciones Científicas y Técnicas, Argentina

Small streams are ruled by the influence that riparian vegetation has on food webs since it regulates the entry of organic matter and sunlight. The plantation of perennial tree species along banks of grassland streams results in heavy shading of stream bottoms and large inputs of coarse organic matter. In this study, we experimentally analyzed the taxonomic and trophic structure of invertebrate assemblages colonizing pine needles and plastic filaments (mimicking the physical structure of needle accumulations) placed in one afforested and one natural grassland stream from the mountainous region of central Argentina. We studied these two experimental substrates to infer if pine leaves were colonized by invertebrates as food resource or only as a substratum for support and refuge. Coarse mesh litter bags were used to simulate large litter accumulations and four bags of each treatment were randomly removed after 46, 89, and 158 days of exposure. Our study showed that taxonomic and trophic composition of invertebrate assemblages colonizing needles and plastic filaments were mostly similar suggesting that needles constitute mainly a stable habitat but not a trophic subsidy. Richness, diversity, and evenness were lower in assemblages of the afforested stream and differences in taxonomic and trophic structure were also found between afforested and natural grassland streams. We conclude that environmental changes associated to afforestation modified invertebrate assemblages colonizing litter but in the same way for needles and plastic filaments since no evidence was found for supporting that the organic nature of needles makes this substrate different from plastic filaments for invertebrates. Therefore, needles would not be being used as a food resource. Our study highlights the importance of preserving riparian zones mostly unaltered to maintain natural aquatic communities when implementing afforestation programmes in grassland landscapes.

KEYWORDS

aquatic insects, functional feeding groups, invertebrate colonization, organic matter, pine needles

1 | INTRODUCTION

Small streams are commonly heterotrophic ecosystems ruled by the strong influence that riparian vegetation has on food webs since it regulates the entry of organic matter and sunlight (Richardson & Danehy, 2007; Richardson, Bilby, & Bondar, 2005). Allochthonous materials coming from the surrounding landscape (mostly from riparian plants) are known to provide food (e.g., fallen leaves) or to increase habitat heterogeneity due to their low breakdown rates (e.g., twigs and

trunks). Therefore they are rapidly colonized by aquatic biota (Graça et al., 2015). However, small streams surrounded by riparian low profile vegetation (e.g., shrubs, herbs, mosses, or no plants) have food webs mostly based on primary producers (Thompson & Townsend, 2004a; Whiting, Whiles, & Stone, 2011). These unshaded streams are characterized by the presence of biofilm consumers (scrapers) and fine particulate organic matter (FPOM) feeders (collectors), and a paucity of coarse particulate organic matter (CPOM) feeders (shredders) (Gonçalves et al., 2007; Masese et al., 2014; Principe, Gualdoni, Oberto,

Raffaini, & Corigliano, 2010; Wantzen & Wagner, 2006). However, some shredder species are supported by inputs of organic material from riparian grasses and shrubs (Menninger & Palmer, 2007).

Anthropogenic alteration of riparian vegetation has implications in the quality and quantity of organic matter entering to streams (Hladyz et al., 2010; Piccolo & Wipfli, 2002; Snyder, Young, Lemarié, & Smith, 2002). One extreme case occurs when natural grasslands are converted to forested landscapes with no maintenance of riparian zones (Van Wilgen & Richardson, 2012). Such situation is expected to have profound consequences on stream functioning and its biota. For instance, the plantation of rapid growth perennial species along stream banks results in heavy shading of stream bottoms and large inputs and accumulations of CPOM (Giller & ÓHalloran, 2004; Richardson, 2008). As a consequence, these streams shift to heterotrophic conditions affecting biota. Also, there should be a rebalance in primary producer, decomposer, detritivore, and herbivore abundances (Thompson & Townsend, 2004b).

In the 1970s, Argentine's federal laws promoted afforestation with introduced fast-growing species (primarily pines, *eucalyptus*, and Salicaceae) in vast regions originally occupied by grasslands (Jobbágy, Noretto, Paruelo, & Piñeiro, 2006). Then, large extensions of semiarid grasslands in mountainous landscapes in central Argentina were afforested with pines, which were also planted into riparian zones. This provides a long-term natural experiment where grassland landscapes were completely converted to forests and are now mature monospecific pine stands (~40-year-old). Pine needles have very low decay rates (Webster & Benfield, 1986) due to their thick cuticle and the presence of resins that inhibit microbial colonization and detritivore shredding (Bärlocher & Oertli, 1978) but they are ultimately consumed by aquatic invertebrates in streams draining native coniferous forests (Richardson et al., 2005; Sakai, Fukushima, Naito, Natuhara, & Kato, 2016) and the same is likely to occur (Thompson & Townsend, 2003) or not (Martínez et al., 2013) in streams where this litter is exotic. Additionally, needle accumulations serve as substrate for biofilm development (Mora-Gómez, Freixa, Perujo, & Barral-Fraga, 2016; Romani et al., 2013; Thompson & Townsend, 2004a) and for FPOM trapping (Dangles, Guerold, & Usseglio-Polatera, 2001). Therefore needles may be exploited by different groups of invertebrates and not only by the species that may feed on coarse detritus (Albariño & Balseiro, 2002; Richardson, Shaughnessy, & Harrison, 2004).

Previous studies in the area that focused on riffle habitats found that pine afforestation changes taxonomic and trophic structure of invertebrate assemblages (Márquez, Cibils Martina, Principe, & Albariño, 2015), and that needle breakdown rate is higher in afforested streams than in grassland ones suggesting an effect of invertebrate feeding on the process (Principe, Márquez, Cibils Martina, Jobbágy, & Albariño, 2015). However, the use of the exotic leaf litter has not been studied. To evaluate the use of this novel substrate by invertebrates, a field experiment was designed. Invertebrate colonizing assemblages were compared among two experimental substrates: Pine needles and plastic filaments, mimicking the physical structure of needle accumulations, that were both placed in a grassland and an afforested neighbor stream. We studied these two experimental substrates to infer if pine needles were colonized by invertebrates as a food resource or only as a substratum for support and

refuge. Our aim was to analyze the composition, structure, and temporal dynamics of invertebrate assemblages colonizing two different substrates (needles vs. plastic filaments) in two different streams (grassland vs. afforested). We hypothesized that the environmental changes associated to afforestation modify invertebrate assemblages colonizing litter and that the organic nature of needles make this substrate different from plastic filaments for invertebrates. Then we expected differences in taxonomic composition between grassland and afforested streams and between needle litter and plastic filaments. Low abundance and richness were expected in plastic filaments (compared to needle litter) and in assemblages colonizing litter in the afforested stream (compared to the grassland one). Finally, trophic structure was expected to be different between needles and plastic filaments and between streams with distinct riparian vegetation.

2 | MATERIALS AND METHODS

2.1 | Study area

The study was carried out in two streams of the Ctlamochita river upper basin, Córdoba, Argentina. This river is one of the main tributaries of the Carcaraña river and belongs to La Plata river basin. Headwaters of the Ctlamochita river are located in grasslands of the Comechingones mountains between 800 and 1,500 m a.s.l. The lithology is dominated by granitic rocks but localized patches of metamorphic rocks (gneiss, schist, migmatite) can also be found. Annual precipitation in the region reaches 1,000 mm occurring mostly between spring and the end of summer (Austral region: October–March) (Pasquini, Lecomte, & Depetris, 2004) determining a relatively dry mountainous landscape. Maximum air temperature reaches 34°C in summer (December–March) and decreases up to -5°C in winter (June–September). Grasslands are dominated by *Festuca hieronymi* Hack., *Nassella filiculmis* (Delile) Barkworth, *Schizachyrium condensatum* (Kunth) Nees, and *Eragrostis airoides* Nees (Oggero & Arana, 2012).

Approximately 36,000 ha of the Sierras de los Comechingones in Córdoba (central Argentina) were afforested with pines between 900 and 1,600 m a.s.l. (Plevich, Nuñez, Cantero, Demaestri, & Viale, 2002), which correspond to 15% of upland grasslands in the Comechingones mountains. Currently, the afforested area has diminished as a consequence of harvesting and fire. In the study area, *Pinus elliotii* Engelm is the dominant tree in the plantations followed by *Pinus radiata* D. Don and *Pinus taeda* L. Two first-order streams separated by 1 km from each other and located in sites with similar altitude, slope, exposure to sunlight, and drainage area were selected. One stream drains grasslands, and the neighbor one drains a plantation of *P. elliotii* (Table 1). Previous studies in the area showed that pine afforestation did not alter stream water acidity (Farley, Piñeiro, Palmer, Jobbágy, & Jackson, 2008), and the same has been reported for other regions of the world (Thompson & Townsend, 2004b).

2.2 | Field experiment

The experiment was carried out between April and November 2012, mostly during the low water period where high biotic activity on

TABLE 1 Characterization of study streams

	Grassland stream	Afforested stream
Coordinates	31°58'S	31°59'S
	64°48'W	64°48'W
Drainage area (ha)	87	89
Altitude (m a.s.l.)	1175	1144
Irradiance ($\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$)	1905 (12)	372 (78)
Wet width (m)	1.40 (0.51)	1.58 (0.17)
Water temperature (°C)	11.4 (1.2)	8.1 (1.1)
pH	8.5	8.0
Conductivity ($\mu\text{S} \cdot \text{cm}^{-1}$)	98	104
TDS ($\text{mg} \cdot \text{L}^{-1}$)	84	93
Carbonate ($\text{mg} \cdot \text{L}^{-1}$)	1.2	0.0
Bicarbonate ($\text{mg} \cdot \text{L}^{-1}$)	44	55
Sulphate ($\text{mg} \cdot \text{L}^{-1}$)	7.9	10.2
Nitrate ($\text{mg} \cdot \text{L}^{-1}$)	1.0	0.0
Nitrite ($\text{mg} \cdot \text{L}^{-1}$)	0.0	0.0
Total hardness ($\text{meq} \cdot \text{L}^{-1}$)	2.3	0.8

Location of streams and values of environmental variables measured during the experimental period. Mean values and standard errors (between brackets) are shown for variables that were measured more than once. See section 2 for details of measurement procedures.

stream resources is expected. Forty-eight plastic litter bags of $23 \times 21 \times 3$ cm and 1 cm mesh size were built to allow the access of large size aquatic macroinvertebrates, including Odonata and Hemiptera. These bags were also prepared with two pieces of fine mesh (1 mm mesh size) on the sides of the bags to prevent the massive loss of the content by the current. Litter bags represented approximately a volume of 1.5 L and a surface area of 0.05 m^2 simulating a relatively large accumulation of needle litter. This amount represents the double of needle litter collected in average by a surber sampler (0.09 m^2 :167.88 g DM of needles, Márquez, own data).

Twenty-four litter bags were filled with 300 g dry mass (DM) pre-conditioned needles (weighed to the nearest 0.01 g). Initial dry mass was estimated for each sample by drying (60°C for 48 hr) and burning (500°C for 10 hr) an independent set of samples to obtain AFDM. Pine needles were obtained from one *P. elliotii* tree to reduce variability and previous to being weighted they were conditioned by placing them during 30 days in containers with water from the same streams in which they were placed later. Water was replaced every two days and an aerator pump was used to maintain proper levels of dissolved oxygen in water. Pre-conditioning was carried out so that needles have extra time underwater and to facilitate the manipulation of needles for bag assemblage. Another set of 24 plastic bags were filled with plastic filaments. We used pieces of black polypropylene filament (22 cm long, 0.086 cm thick, and 0.3 g weight) to simulate the structure provided by pine needle accumulations. Because of the difference in weight between needles (needle weight: 0.12 g) and plastic filaments

of the same length, bags were filled with a similar volume to that occupied by 300 g DM of needles. Both substrates types were placed parallel to the longest side (23 cm) of the bags.

On June 13, 2012, 12 bags of each substrate (needles and plastic filaments) were introduced in shallow pools in a 400 m reach of the grassland and afforested stream. Bags were fixed with 20 cm nails to the streambed in sites with similar conditions of water turbulence and flow (mean current velocity considering both streams: $0.48 \pm 0.11 \text{ m} \cdot \text{s}^{-1}$ and depth of 0.18 ± 0.03 m). Four bags (four replicates) of each substrate (needles and plastic filaments) were randomly removed after 46, 89, and 158 days of exposure in the grassland and afforested stream. Experimental units were placed in individual labeled plastic bags and returned to the laboratory. Physicochemical variables were measured during the experiment to characterize the study streams (Table 1). Stream wet width was measured with a meter tape at four points along the reach. Depth and current velocity were measured with a digital water velocity meter (Global Water flow meter FP101, Sacramento, CA). Water temperature, conductivity and pH were recorded with a portable sensor (Multiparameter PCS TESTR 35 Eutech – Oakton, Vernon Hills, IL). Temperature was measured every 2 weeks during the experimental period. Water samples were also taken for water chemical analyses (Total dissolved solids, carbonate, bicarbonate, sulphate, nitrate, nitrite, total hardness). PAR (photosynthetically active radiation) intensity was measured once during the experimental period with a QSL-2100 Irradiance Sensor (Biospherical Instruments Inc., San Diego, CA) at midday along a 20 m reach in each stream obtaining seven measurements of PAR intensity in each reach.

At the laboratory, macroinvertebrates were sorted from the remaining leaf and filament material. Leaf remaining dry mass (LRDM) for each needle bag was obtained by drying needles at 60°C for 48 hr and then by burning them at 500°C for 10 hr to obtain AFDM. Invertebrates were identified to the lowest possible taxonomic level with specific keys (Domínguez & Fernández, 2009), counted and assigned to their Functional Feeding Groups (FFG) (gathering collector, filtering collector, scraper, shredder, predator) using available references (Merritt & Cummins, 2006; Merritt, Cummins, & Berg, 2008; Principe et al., 2010; Reynaga & Dos Santos, 2012; Tomanova, Goitia, & Helešić, 2006). Abundance was expressed as individuals per bag.

2.3 | Data analyses

The differences in percentage of dry mass lost (% DM lost) in pine needles bags were tested between grassland and afforested stream by one-way ANOVA for each exposure time (46, 89, and 158 days). Correspondence Analysis (CA) was carried out to analyze the effect of substrate type, exposure time, and riparian vegetation on assemblages colonizing experimental bags ($n=48$; abundance expressed as individuals $\cdot \text{bag}^{-1}$). CA was carried out using CANOCO version 4 (TerBraak & Smilauer, 1999). Differences between the groups defined by CA were tested by Multiresponse Permutation Procedures (MRPP, a non-parametric procedure for testing the hypothesis of no difference between two or more groups of sampling units) using PC-ORD version 5.0 (McCune & Mefford, 1999).

Four structural attributes of the invertebrate assemblages were calculated: total abundance, richness, diversity (calculated as effective number of species: $\exp(H')$ where H' = Shannon index calculated with \ln) (Hill, 1973; Jost, 2006) and evenness index (J'). In addition, rarefaction curves were performed using PAST version 3.13 (Hammer, Harper, & Ryan, 2001) to compare invertebrate richness among the different substrates in the afforested and the grassland stream. This method allows comparison of the number of species expected per site, based on the lowest number of individuals recorded among the sites being compared. To compare structural attributes three-way Linear Models (LM) were used (factors: substrate, vegetation, and exposure time). Validation of normality and homogeneity of variances were performed reviewing the normal Q-Q plot of standardized residuals and standardized residuals versus predicted, respectively. 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 (Di Rienzo et al., 2012) that implements an interface of platform R (R Core Team, 2013) for estimating lineal models. Absolute abundance of the five FFG was also calculated and compared with three-way LM as described for structural attributes.

3 | RESULTS

During the length of the experiment (158 days), pine needle bags lost $48.5\% \pm 5.7$ of their initial DM in the grassland stream (Mean% DM loss after 46 days: $30.6\% \pm 1.9$; 89 days: $35.9\% \pm 2.5$) and $40.5\% \pm 4.9$ in the afforested one (% DM loss after 46 days: $31.9\% \pm 1.5$; 89 days: $35.7\% \pm 4.4$) but the differences between stream types at each exposure time were not significant (one-way ANOVA, $F_{\text{factor, error}}$: 46 days: $F_{1, 6} = 0.23, p = 0.612$; 89 days: $F_{1, 6} < 0.001, p = 0.978$; 158 days: $F_{1, 6} = 0.05, p = 0.83$).

Invertebrate assemblages in experimental bags were different between the afforested and grassland stream (Figure 1) but, interestingly, they did not differ between substrate types. The first axis of the CA ordination mainly separated invertebrate assemblages in bags from streams with different riparian vegetation (40.1% of variance explained by the first two ordination axes; eigenvalues: Axis 1: 0.421, Axis 2: 0.371, Axis 3: 0.256, Axis 4: 0.153). In addition, differences between these groups were significant according to MRPP ($A = 0.011, p < 0.001$).

A total of 64 taxa of aquatic invertebrates colonizing needle bags were found in the grassland stream. At 46 days the non-biting midges Pentaneurini, the caddisfly *Hydroptila* spp. and the mayfly *Caenis* spp. were the dominant taxa (supporting material). This mayfly became the most abundant taxon at 89 days. It is worth noting the increase in abundances of the riffle beetle *Austrelmis* spp. and the sludge worms Naidinae. The caddisfly *Hydroptila* spp. showed an important decrease. Toward the end of the experiment, assemblages were characterized by high abundance of non-biting midges, being *Pseudochironomus* spp. the most abundant taxa. In the afforested stream, 58 taxa were found in needle bags. These bags were early colonized by non-biting midges,

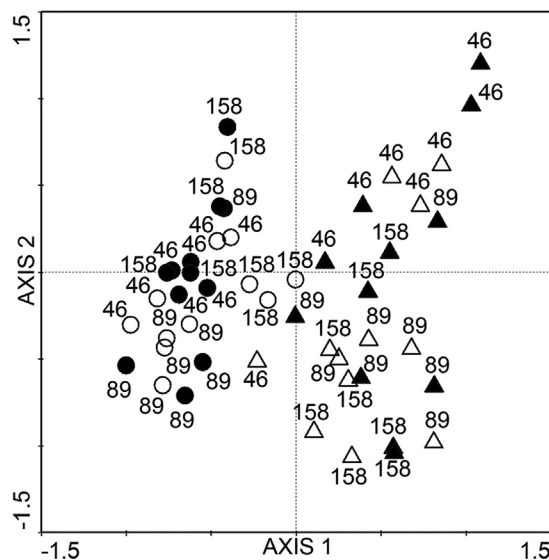


FIGURE 1 Correspondence analysis of invertebrate assemblages in 48 experimental bags with different substrates: needles (black symbols) and plastic filaments (white symbols) placed in a grassland stream (circles) and in an afforested stream (triangles). Numbers correspond to exposure time (46, 89, and 158 days). Abundance: $\text{Ind} \cdot \text{bag}^{-1}$

being *Corynoneura* spp. the dominant taxa (supporting material) and from day 89, needle bags were dominated by Naidinae.

Plastic filament bags were also colonized by a rich fauna of aquatic invertebrates (supporting material). In the grassland stream, 69 taxa were found and the mayfly *Caenis* spp. was the dominant taxon until day 89. At 158 days, an important shift in composition was observed; the non-biting midges *Pseudochironomus* spp. and *Cricotopus* spp. together with Naidinae dominated, while *Caenis* spp. did strongly reduce its abundance. In the afforested stream, colonization of plastic filaments took place differently (supporting material). A total of 52 taxa were found in plastic filament bags of this stream. At day 46, *Corynoneura* spp. resulted the dominant taxa and *Americabaetis* spp. and *Parametrioctenus* spp. were present in high abundances. From day 89, Naidinae became the most abundant taxa in filament bags similarly to what happened in needle litter bags.

Rarefaction analysis differentiated two main groups of assemblages colonizing bags with similar taxa richness of invertebrates (Figure 2). In general, bags exposed in the grassland stream presented higher values of taxonomic richness than those placed in the afforested one, but no differentiation between substrate types was observed. Filament bags exposed 158 days in grassland reached the greatest value of richness (39), whereas needle bags in the afforested stream after the same exposition time exhibited the lowest value of taxa richness (20).

Structural attributes differed mainly among bags placed in streams with different riparian vegetation (three factor LMs, Table 2). Total richness, diversity, and evenness were lower in assemblages of the afforested stream (A) than in the grassland one (G) (DGC post hoc test: $G > A$. Mean values \pm EE, total richness: $G = 32.46 \pm 1.31$, $A = 24.63 \pm 1.07$; diversity: $G = 13.78 \pm 0.75$, $A = 8.43 \pm 0.57$;

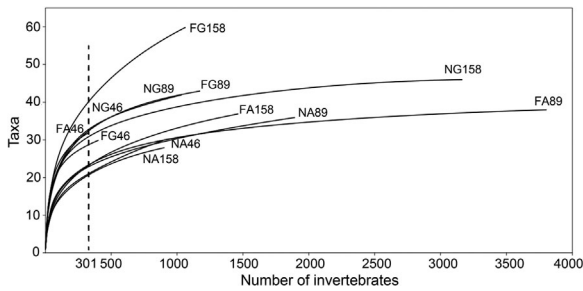


FIGURE 2 Rarefaction curves based on the number of individuals of invertebrate assemblages in needle (N) and filament bags (F) placed in a grassland (G) and in an afforested (A) stream with different exposure time (46, 89, and 158 days). The minimum number of individuals is indicated with a dashed line

evenness: $G = 0.75 \pm 0.02$, $A = 0.65 \pm 0.02$). Invertebrate total abundance showed a significant interaction between vegetation and exposure time (Table 2) with the highest abundance in bags that remained 89 and 158 days at the grassland stream (G) and in bags with 89 days of exposure at the afforested stream (A) (DGC post hoc test: $G158 = A89 = G89 > A158 = A46 = G46$. Mean values \pm EE, $G158 = 2126 \pm 592$, $A89 = 1442 \pm 492$, $G89 = 1124 \pm 175$, $A158 = 648 \pm 164$, $A46 = 618 \pm 262$, $G46 = 393 \pm 62$).

LMs showed the effect of riparian vegetation and exposure time on the trophic structure of colonizing assemblages, but only scrapers exhibited differences between substrate types (Table 2). The abundance of filtering collectors, predators, and shredders were significantly reduced in the experimental bags placed in the afforested stream, regardless of the substrate type (DGC post hoc test, $p < 0.05$) (Figure 3). Scrapers also varied in relation to riparian vegetation type being more abundant in bags of the grassland stream. They were the only FFG that showed differences between substrate types being more abundant in needle bags. In relation to exposure time, the abundance of filtering collectors was higher at 158 days, predators were more abundant from day 89 and shredders had higher abundance at day 89 (Figure 3). Gathering collector abundance showed interaction between two factors (vegetation \times exposure time). These invertebrates had high abundance in bags exposed 158 days in the grassland stream and in those exposed 89 day in the afforested stream.

4 | DISCUSSION

Our study showed that substrate identity (organic vs. artificial) did not have influence on the taxonomic structure of colonizing assemblages, contrary to what was expected. However, and in agreement with our expectations, substrates exposed in the afforested stream were characterized by lower richness, diversity, and evenness than those placed in the reference grassland stream. Moreover, taxonomic composition also changed in the afforested stream in comparison with the grassland one. Nevertheless, invertebrate abundance did not exhibit a clear pattern of variation since the differences between

TABLE 2 Results of three factors linear models (LM) used to compare assemblage attributes and trophic structure of assemblages colonizing experimental bags

Assemblage attributes	Vegetation		Substrate		Exposure time		Vegetation \times Substrate		Vegetation \times Exposure time		Vegetation \times Substrate \times Exposure time	
	F	p	F	p	F	p	F	p	F	p	F	p
Total abundance	2.40	0.129	3.53	0.068	9.70	<0.001	1.19	0.282	3.35	0.046	0.67	0.516
Taxonomic richness	23.14	<0.001	0.04	0.839	0.95	0.395	2.20	0.146	2.59	0.089	1.33	0.277
Diversity (exp ^{Shannon H'})	33.78	<0.001	1.96	0.169	2.97	0.064	0.17	0.680	0.67	0.517	0.77	0.553
Evenness (Shannon J')	12.52	0.001	1.83	0.184	4.59	0.016	0.62	0.435	0.12	0.883	1.01	0.374
Trophic structure (FFG abundance)												
Predators	52.27	<0.001	3.99	0.053	8.75	<0.001	1.10	0.302	2.37	0.108	1.03	0.367
Gathering collectors	0.13	0.724	3.20	0.082	10.18	<0.001	1.57	0.218	4.74	0.014	0.37	0.696
Filtering collectors	7.07	0.011	0.82	0.370	15.25	<0.001	1.99	0.167	0.08	0.924	1.12	0.336
Shredders	6.30	0.016	0.95	0.335	7.35	0.002	0.76	0.390	0.91	0.411	0.43	0.652
Scrapers	39.28	<0.001	7.19	0.011	0.54	0.586	0.10	0.755	1.31	0.281	1.13	0.333

Abundance data were expressed as individuals \cdot bag⁻¹ (factors: vegetation, substrate, and exposure time). Df (factor: error); vegetation (1:3,6), substrate (1:3,6), exposure time (1:3,6), vegetation \times substrate (1:3,6), vegetation \times exposure time (2:3,6), and vegetation \times substrate \times exposure time (2:3,6). Significant values ($p < 0.05$) are shown in bold.

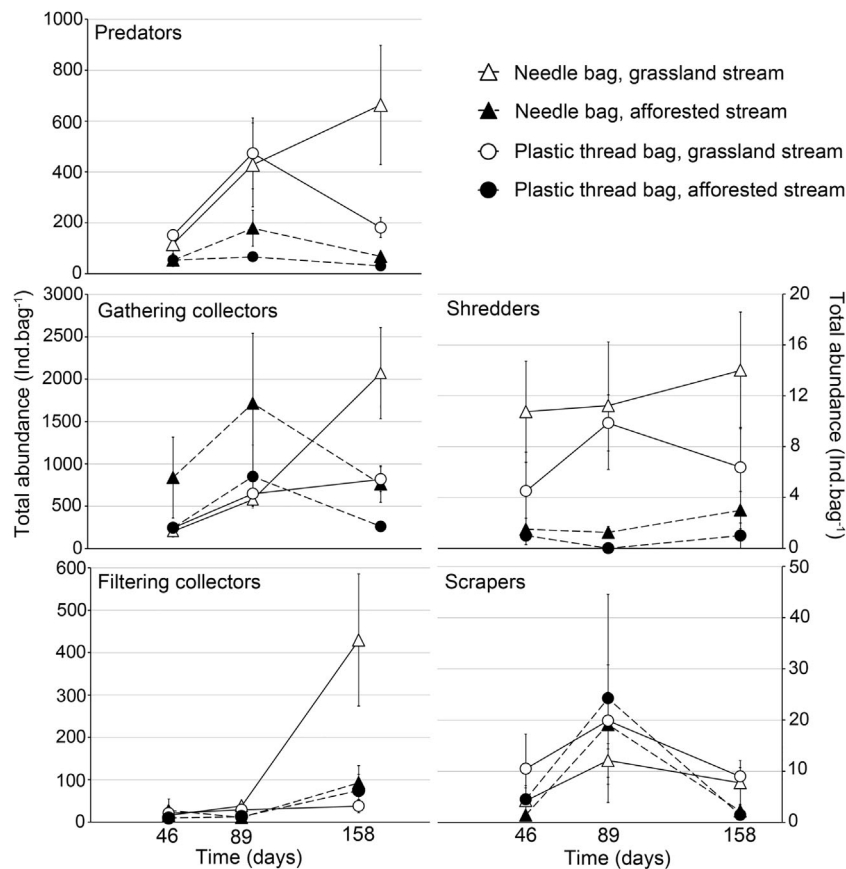


FIGURE 3 Abundance of Functional Feeding Groups in pine needle litter and plastic filament bags placed in one grassland and one afforested neighbour stream ($\text{ind} \cdot \text{bag}^{-1}$, mean \pm S.E.)

grassland and afforested stream varied in relation to exposure time. Several authors have reported modifications in aquatic invertebrate communities after clear felling of native forests (including riparian zones) for commercial afforestation (Death, Baillie, & Fransen, 2003; Miserendino & Masi, 2010; Richardson, 2008; Thompson & Townsend, 2005) but few studies have accounted for changes in stream invertebrate assemblages due to grassland afforestation (Thompson & Townsend, 2003). Contrary to our results, Thompson and Townsend (2003) reported lower invertebrate abundance in pine afforested streams and did not find differences in taxonomic richness. On the other hand, a previous research work in our study area showed that richness of invertebrate assemblages living in riffles of afforested streams was lower than in grasslands, and indicator species (taxa from riffles with specificity for afforested streams) were also heavily reduced (Márquez et al., 2015). Here, we reveal a similar pattern for the assemblages colonizing needle litter and artificial filaments. Overall, the previous and the present study in headwater streams of Córdoba Province suggest that pine afforestation of grassland catchments results in an impoverished pool of stream invertebrates with low affinity for needle detritus.

Our results also showed that substrate identity contained in experimental bags did not have influence on the trophic structure of invertebrate assemblages. Similarly, field experiments carried out in Chile did not find differences between detritivorous invertebrates colonizing pine needles and plastic substratum, indicating that pine

litter is very difficult to assimilate (Valdovinos, 2001). Although some studies show that pine needles ultimately become conditioned and are potentially available for detritivores (Campos and González, 2009; Sakai et al., 2016; Thompson & Townsend, 2003), their thick cuticle and resins make them little palatable and hard to decompose (Bärlocher & Oertli, 1978; Webster & Benfield, 1986) representing mostly a stable habitat (Hisabae, Sone, & Inoue, 2011). Additionally, it has been demonstrated that, in accumulations of slowly processed leaves, retained FPOM strongly determines the abundance and richness of colonizing invertebrates (Dangles et al., 2001). Thus, pine needles in our study streams (and in the same way plastic filaments) may act as a FPOM filter and offer shelter and substratum for invertebrates but they may not be exploited as a direct trophic subsidy.

Allochthonous CPOM is the main source of energy to forest headwater streams. In many of these streams, especially those from temperate regions, a diverse and abundant shredder fauna inhabits (Boyero et al., 2012; Graça et al., 2015). In our study area, where several catchments are almost completely covered by ~40-year-old implanted pine forests, invertebrate communities might have responded to such landscape conversion by shifting the trophic structure typical of grassland streams. Indeed, in our afforested stream trophic structure became altered. Abundance of shredders, scrapers, filtering collectors, and predators were lower in litter bags from the afforested stream whereas gathering collectors showed higher

abundance. Similarly, low abundance of shredders and high abundance of gathering collectors were found in breakdown experiments with low quality leaf litter of native plants in Brazilian Cerrado streams (Alvim, Medeiros, & Rezende, 2014; Ligeiro, Moretti, & Callisto, 2010; Moretti, Ligeiro, & Callisto, 2007). This colonization pattern seems characteristic of regions where the original set of shredder species is poor (França, Medeiros, Rosa, & Callisto, 2006; Ligeiro et al., 2010; Tomanova et al., 2006).

Grassland streams are known to base their food webs on primary production, mostly periphyton, exploited by grazer-scraper invertebrates (Dodds, Gido, Whiles, Daniels, & Grudzinski, 2015; Thompson & Townsend, 2004a; Wallace & Eggert, 2009). Accordingly, we found higher abundance of scrapers in the grassland stream suggesting higher periphyton development associated to the unshaded condition of these streams. Otherwise, although autochthonous production is the main source of energy in unshaded streams, some authors demonstrated that riparian inputs from herbs and grasses may constitute important resources promoting strong aquatic-terrestrial linkages (Menninger & Palmer, 2007). These authors indicated that freshwater amphipods and tipulid larvae have an important role on the biotic processing of herb and grass detritus. These taxa were also found in needle litter bags in our grassland stream so they may potentially be feeding on this novel resource. Although further analysis of gut content will allow confirming if some invertebrates are able to feed on needle litter, the results of our study mainly support that this novel resource would not be being used as a food resource. Eventually, some invertebrates could exploit periphyton growing on needles, but our study showed that most of the invertebrates may be using fine particulate organic matter, which is certainly retained by needles packs, since abundance of gathering collectors increased in the afforested stream but most of the other functional groups decreased.

There is a global need for planted forest areas to counterbalance natural forest area reduction and to cover human demands (D'Annunzio, Sandker, Finegold, & Min, 2015). Forestry best management practices such as maintaining riparian strips to buffer timber harvesting, in order to preserve water quality and biota, have become the norm in the past 50 years (McConigley et al., 2015; McGinley et al., 2012; Richardson, Naiman, & Bisson, 2012), at least in developed countries. However, when riparian zones have low profile plant communities, managing decisions might be less clear (Lyons, Thimble, & Paine, 2000) and are infrequently addressed in forestry protocols and policies (Blumenfeld, Lu, Christophersen, & Coates, 2009; Mátyás & Sun, 2014). Our working hypothesis was partially corroborated since results support that environmental changes associated to afforestation modified invertebrate assemblages colonizing litter but in the same way for needles and plastic filaments with no evidence for supporting that the organic nature of needles makes this substrate different from plastic filaments for invertebrates. Our study showed that taxonomic and trophic composition of invertebrate assemblages colonizing needles and plastic filaments were mostly similar suggesting that needles constitute mainly a stable habitat but not a trophic subsidy. Moreover, invertebrates assemblages associated to litter on the streambed were different between afforested and natural grassland streams. These results highlight the importance of

preserving stream margins mostly unaltered to maintain natural aquatic communities when implementing afforestation programs in grassland landscapes.

ACKNOWLEDGMENTS

We especially thank to J. Zanotto Arpellino, M. Abraham, and J. Ison for their assistance in the field. Prof. M. Almirón provided language assistance, and two anonymous reviewers greatly improved the quality of the manuscript. We are also greatly thankful to the reviewers for their kind but thorough comments and suggestions that radically improved the manuscript. This research has received financial support from the Agencia Nacional de Promoción Científica y Tecnológica, Argentina (ANPCYT, PICT 1113) and the Ministerio de Ciencia y Tecnología del Gobierno de la Provincia de Córdoba (MCYT, GRF 2008). J. Márquez and L. Cibils Martina are supported by fellowships from the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET, Argentina).

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How to cite this article: Márquez JA, Principe RE, Martina LC, Albariño RJ. Pine needle litter acts as habitat but not as food source for stream invertebrates. *Internat Rev Hydrobiol.* 2017;102:29–37. <https://doi.org/10.1002/iroh.201601856>