RESEARCH ARTICLE

Amphibian loss alters periphyton structure and invertebrate growth in montane streams

Alberto Alonso[1] | Silvia Monroy[1] | Jaime Bosch[2,3] | Javier Pérez[1] | Luz Boyero[1,4]

[1]Department of Plant Biology and Ecology, Faculty of Science and Technology, University of the Basque Country (UPV/EHU), Leioa, Spain

[2]IMIB-Research Unit of Biodiversity (CSIC, UO, PA), Oviedo University-Campus Mieres, Mieres, Spain

[3]Centro de Investigación, Seguimiento y Evaluación, Parque Nacional de la Sierra de Guadarrama, Rascafría, Spain

[4]IKERBASQUE, Basque Foundation for Science, Bilbao, Spain

Correspondence
Alberto Alonso
Email: alberto.alonso@ehu.eus

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Abstract

1. Amphibians are declining worldwide due to a combination of stressors such as climate change, invasive species, habitat loss, pollution and emergent diseases. Although their losses are likely to have important ecological consequences on the structure and functioning of freshwater ecosystems, this issue has been scarcely explored.

2. We conducted an experiment in three montane streams—where primary production is the main source of energy and carbon—to assess the effects of amphibian disappearance (i.e. presence or absence of the common midwife toad *Alytes obstetricans*, a common species found in pools of these streams) on several aspects of ecosystem functioning and structure: periphyton biomass and chlorophyll *a* concentration, algal assemblage structure, and growth of macroinvertebrate grazers.

3. We compared four types of experimental enclosures: (i) without macroinvertebrates or amphibians; (ii) with larvae of the caddisfly *Allogamus laureatus*; (iii) with *A. obstetricans* tadpoles; and (iv) with both *A. laureatus* larvae and *A. obstetricans* tadpoles.

4. The absence of tadpoles increased periphyton biomass, but did not cause differences on inorganic sediment accrual. The algal assemblage had a higher diversity in the absence of tadpoles, and their characteristic taxa differed from the assemblages in presence of tadpoles. *A. laureatus* presented higher mass in presence of tadpoles; however, tadpole length was not affected by presence of macroinvertebrates.

5. Our results suggest that presence of tadpoles is a driver of periphyton accrual and assemblage structure, acting as top-down control and with key potential consequences on the functioning of montane stream ecosystems.

KEYWORDS
algae, *Allogamus laureatus*, *Alytes obstetricans*, chlorophyll, tadpoles

1 | INTRODUCTION

Biodiversity is experiencing a dramatic decline, with current extinction rates being equivalent to or higher than those of past mass extinctions (Barnosky et al., 2011; Ceballos et al., 2017). Among the most affected groups of organisms are the amphibians (Collins, 2010; Wake & Vredenburg, 2008), whose dramatic declines worldwide are driven by several factors such as climate change (Blaustein et al., 2010), pollution (Blaustein et al., 2003), habitat loss and fragmentation (Becker et al., 2007; Gallant et al., 2007), and invasive species (Falaschi et al., 2020), including pathogens that are responsible for emergent infectious diseases (Fisher & Garner, 2020; Scheele et al., 2019).

One of the most significant threats to amphibian conservation is the pathogenic fungus *Batrachochytrium dendrobatidis* Longcore, Pessier & D.K. Nichols (Fisher et al., 2009; Fisher & Garner, 2020; Scheele et al., 2019). This fungus (hereafter referred to as Bd) is a generalist pathogen that causes chytridiomycosis, a highly infective disease. Bd infects amphibian epidermal cells and causes great mortality after metamorphosis in sensitive species (Fisher et al., 2009; Garner et al., 2009). The fungus originated in Asia and has recently spread globally due to human activities (O'Hanlon et al., 2018), causing amphibian declines and extinctions in all continents where amphibians are found (Lips, 2016).

Although the loss of amphibian species from freshwater ecosystems can be expected to cause changes in ecosystem structure and functioning, this consequence has been little explored. Only a few studies have been conducted, mainly in tropical streams, where larval amphibian losses have been shown to cause changes in algal (Barnum et al., 2022; Connelly et al., 2008) and macroinvertebrate assemblages (Barnum et al., 2022; Colón-Gaud et al., 2009; Ranvestel et al., 2004), with a resulting simplification of freshwater food webs (Schmidt et al., 2017), a reduction of primary production (Connelly et al., 2008), an increase of periphyton biomass accrual (Connelly et al., 2008; Mallory & Richardson, 2005; Ranvestel et al., 2004) and an alteration of nutrient cycling (Whiles et al., 2013). There are, however, very few relevant studies from freshwater ecosystems in temperate areas, with only one reporting that periphyton accrual was limited by amphibian presence and density (Mallory & Richardson, 2005). Differences in the functioning of freshwater ecosystems at different latitudes is plausible due to often different assemblage composition and diversity, which influence key ecosystem processes (Boyero et al., 2021).

Amphibians play a key ecological role in low-order streams of montane areas, where primary production is the main source of energy and carbon (as opposed to streams flowing through forested areas, which are fuelled by allochthonous leaf litter; Swan et al., 2021). In temperate regions, many anuran tadpoles are herbivores that graze on the periphyton growing on mineral or soft substrates, thus tadpoles often interact with grazing macroinvertebrates such as many caddisflies (Cummins & Klug, 1979). Tadpoles can compete with caddisflies for food resources (Colón-Gaud et al., 2010; Kupferberg, 1997), but they can also facilitate the access of algae by smaller caddisflies through the tadpole's removal of sediment (Colón-Gaud et al., 2009, 2010; Ranvestel et al., 2004). The loss of amphibians from these habitats have indirect effects on invertebrates, with possible impacts across the food web (Hocking & Babbitt, 2014) and on fundamental ecosystem processes and structure.

Here, we experimentally quantified how the loss of an amphibian species (*Alytes obstetricans* Laurenti) from montane streams where it was common before its decline due to Bd infection (Bosch et al. 2001, 2018), affected the rate of periphyton accrual, the algal assemblage structure and the growth rates of grazing macroinvertebrates. We conducted an outdoor field experiment using enclosures placed in nine stream pools, which contained tiles previously colonized by periphyton and (i) tadpoles only, (ii) macroinvertebrates only, (iii) both or (iv) none. We hypothesized that (1) amphibian losses would enhance periphyton accrual and sediment accumulation, due to the absence of tadpole consumption and removal through bioturbation (Connelly et al., 2008; Ranvestel et al., 2004); (2) the algal assemblage structure would change toward a higher proportion of larger diatoms as a result of amphibian loss, since large algae are more affected by grazing as they are less likely to intact passage through tadpole gut and therefore to recolonize the substrate after grazing (Connelly et al., 2008; Ranvestel et al., 2004); and (3) caddisfly growth rate would increase in the absence of tadpoles, since tadpoles compete with large macroinvertebrate grazers (Colón-Gaud et al., 2010). Additionally, we expected that (4) the combined effects of grazing by tadpoles and caddisflies on periphyton growth would be lower than expected by their isolated effects (i.e., antagonistic) because competition between them would reduce their grazing efficiency.

2 | MATERIALS AND METHODS

2.1 | Study site and species

The study was conducted in three low-order streams located in the Peñalara Massif (Guadarrama Mountains National Park, Central Spain; Table 1, Figure 1). This is a montane habitat (1800–2430m), with low mean annual precipitation (694mm) and a marked seasonality (temperature annual range: 28°C). Annual mean temperature is 6.6°C, with snowy and cold winters (mean temperature 0.1°C) and warm and dry summers (mean temperature 15°C and precipitation of 109mm) [WorldClim database (Fick & Hijmans, 2017)]. The substrate is mostly granitic and covered by heathlands dominated by *Cytisus oromediterraneus* Riv. Mart. and *Juniperus communis nana* Syme, with isolated *Pinus sylvestris* L. The study sites were close to the treeline and had well oxygenated water, pH ca. 6.5, low conductivity, low nutrient concentration and temperatures ca. 10°C at the time of the study (Table 1). The streams were chosen as they are the two largest streams in the area and the main tributary of one of them, being less likely to dry during the summer than other nearby streams (Bosch et al., 2019).

TABLE 1 Physicochemical characteristics of the study streams during the experimental period (mean ± SE; $n = 6$)

Variable	Stream 1	Stream 2	Stream 3
Latitude (°N)	40.84306	40.83668	40.83563
Longitude (°W)	3.94333	3.95362	3.95333
Altitude (m a. s. l.)	1869	1942	1949
Temperature (°C)	10.01 ± 0.07	11.73 ± 0.09	8.16 ± 0.09
Conductivity ($\mu S\ cm^{-1}$)	11.86 ± 0.66	9.92 ± 0.33	11.90 ± 0.49
pH	6.29 ± 0.12	6.62 ± 0.17	6.43 ± 0.10
Dissolved O_2 ($mg\,L^{-1}$)	8.55 ± 0.17	8.29 ± 0.24	8.89 ± 0.29
% Saturation O_2	100.69 ± 0.41	102.57 ± 0.89	100.64 ± 0.56
Flow ($L\,s^{-1}$)	24.93 ± 17.44	13.55 ± 7.54	6.77 ± 1.68
DIN ($\mu g\,L^{-1}$)	30.84 ± 3.28	28.39 ± 2.84	25.88 ± 3.05
SRP ($\mu g\,L^{-1}$)	5.31 ± 0.57	4.74 ± 1.72	11.34 ± 1.21

Abbreviations: DIN, dissolved inorganic nitrogen; SRP, soluble reactive phosphorus.

The amphibian study species was chosen based on its previous abundance and ecological relevance in the study area. *Alytes obstetricans* (Family Alytidae, Order Anura) used to be a frequent species in permanent waters of both ponds and stream pools of the study area, but suffered a dramatic reduction since 1997 due to Bd without recovery to the present (Bosch et al. 2001, 2018). The species has a long-term larval stage that, at high altitudes such as the study area, often prevents metamorphosis during the first year and forces the larvae to remain in the water for several years, reaching large sizes and high numbers in the order of thousands. Other amphibian species at the study area using stream pools are *Salamandra salamandra* Linnaeus and *Rana iberica* Boulenger, both under decline due to Bd and global warming (Bosch et al., 2018).

The macroinvertebrate used was the species *Allogamus laureatus* Navás (Family Limnephilidae, Order Trichoptera), which is a common shredder and grazer in ponds and streams of the study area (Toro et al., 2020). *A. laureatus* present one generation each year, with aerial adults that lay eggs on streams and ponds. Larvae prefer areas with pebbles or organic material accumulations and feed on particulate organic matter and periphyton (Tachet et al., 2010; Toro et al., 2020).

2.2 | Experimental procedure

A 14-day experiment was conducted in June 2021. In each stream, we located 12 enclosures, which were installed in 3 pools per stream, where *Alytes obstetricans* larvae used to be found before their decline due to Bd (J. Bosch, pers. obs.). Enclosures consisted of plastic baskets (0.17 × 0.17 × 0.13 m) closed by a 2-mm glass fibre mesh that allowed water and small invertebrate access but precluded tadpoles and bigger macroinvertebrates from moving in or out. The enclosures had the bottom covered by sediment from the corresponding stream and 6 marble tiles (33.6 cm² surface area) used as standardized substrata for periphyton growth (Figure 1). Tiles were colonized by periphyton, by incubating them for 29 days within a pond located nearby (40.88694°N, 3.88613°W). Control enclosures (C)

only contained tiles, and the other treatments contained either 5 caddisfly larvae (*Allogamus laureatus*; Al), 3 tadpoles (*A. obstetricans*; Ao), or both caddisflies and tadpoles (Al + Ao). Each treatment was replicated three times in each stream (9 replicates in total). In each one of the three pool sections of the stream, we tied 1 replicate per treatment to an iron bar and anchored it to the stream bottom (Figure 1). Field work and experimental protocols were approved by Consejería de Medio Ambiente de la Comunidad de Madrid (ref. 10/045182.9/21).

We collected larvae of *Allogamus laureatus* (hereafter 'caddisflies') of similar size (3.9 ± 0.5 mg) from a nearby stream (40.84167°N, 3.94306°W) in June 2021, and randomly assigned them to the enclosures. We used tadpoles of *Alytes obstetricans* (hereafter 'tadpoles') in development Gosner stage 26 (except for 7 individuals, which ranged from stages 32 to 37) with similar size (snout-to-vent length, SVL ± SE: 20.07 ± 0.361 mm). Tadpoles had been reared in captivity at the 'Centro de Investigación, Seguimiento y Evaluación' (Guadarrama Mountains National Park, Spain). Just before introducing tadpoles and caddisflies into the enclosures, we measured tadpole SVL (0.001 mm precision) using ImageJ software (v. 1.46r).

At the end of the experiment, tiles were collected manually from each enclosure. The surface of all tiles from each treatment was scrubbed with a soft toothbrush in ca. 100 ml of distilled water to collect the associated periphyton. Periphyton was frozen until analyses. In the laboratory, the solution was divided in 3 subsamples. One subsample was vacuum filtered through preincinerated and preweighed GF/F glass fibre filters (0.7-μm); filters were dried (70°C) for 72 h and weighed to quantify dry mass (DM). Afterwards, filters were incinerated (500°C) for 5 h and weighed to estimate periphyton ash-free dry mass (AFDM) and inorganic mass per area unit (g m⁻², Steinman, 2006). The second subsample was filtered (GF/F, 0.7-μm) and used to quantify chlorophyll concentration. Chlorophyll was extracted from filters using a standard method with acetone 90% as solvent (12 h in darkness at 4°C), and samples were sonicated (60 Hz) and centrifuged (2000 rpm) to ensure complete extraction. Then, chlorophyll *a*, *b* and *c* (hereafter Chl-*a*, Chl-*b* and Chl-*c*) concentrations
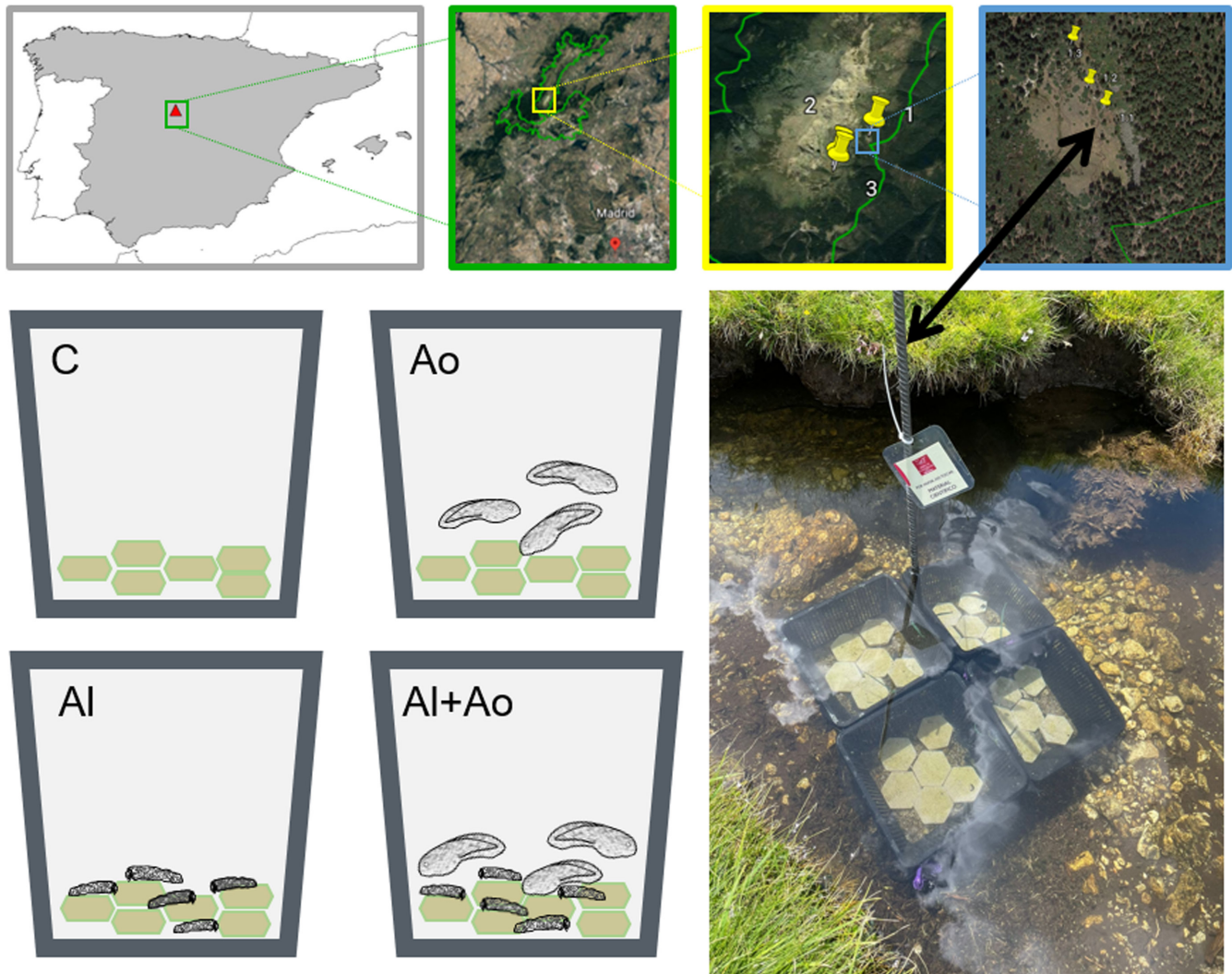
FIGURE 1 Location of Guadarrama Mountains National Park (yellow) in Central Spain and the studied streams (green), with an example of pool location within one of the streams (blue) and a diagram of the experimental design of the different treatments: control (C), presence of caddisflies only (AI), presence of tadpoles only (Ao) and presence of both species (AI+ Ao).

(μg Chl m^{-2}) were assessed spectrophotometrically, by measuring absorbance at 750, 665, 647 and 630 nm, respectively (Steinman, 2006). The three types of Chl were assessed because they are indicative of the biomass of total algae, green algae and diatoms, respectively. The biofilm autotrophic index, which shows the ratio between autotrophic and heterotrophic organisms in biofilm, was calculated as AFDM divided by Chl-a concentration (Steinman, 2006). The third subsample was preserved with acidic Lugol's solution (0.4%) and used to characterize the periphyton algal assemblage. Taxonomic identification to the lowest level possible (genus) and cell counting was performed using an optical microscope and a Neubauer chamber at ×200 magnification following Bellinger and Sigee (2015). Due to the difficulty of counting cells of *Coelosphaerium*, cell number per colony was determined by counting the cells of 8 colonies from different samples at ×1000 magnification (19.875 ± 2.240 cells per colony), and all counted colonies were multiplied by the average cell number (Godo et al., 2011). For each sample, abundance (cell m^{-2}), taxon

richness (number of taxa per enclosure) and Shannon's diversity index were calculated.

At the end of the experiment, the caddisflies that we had previously introduced were recollected and preserved in ethanol 70%. In the laboratory, they were dried (72 h, 70°C) and weighed to quantify final DM (mg). Initial DM was estimated based on the mean DM of each species obtained from 30 extra larvae which were collected at the beginning of the experiment. Tadpoles were collected and measured using ImageJ. Tadpole growth was calculated as the difference between final and initial SVL divided by initial SVL.

2.3 | Data analysis

Differences in algal biomass, Chl-a, Chl-b and Chl-c concentrations, the autotrophic index, inorganic mass, algal abundance, richness and Shannon's diversity index, caddisfly mass and tadpole growth

among treatments (C, Al, Ao and Al+Ao) were examined with linear models (*lmer* function, 'LMERTEST' R package, Kuznetsova et al., 2015), with treatment as fixed factor, stream as random factor and pool as random factor nested within stream. Significant differences among treatments (α = 0.05) were analysed with marginal means and effect sizes (*emmeans* and *eff_size* functions of the 'EMMEANS' R package, Lenth et al., 2018). The influence of water flow on those variables was examined with likelihood ratio tests to show the significance of the random factors (*ranova* function of the 'LMERTEST' R package, Kuznetsova et al., 2015), since flow was the main factor differing among streams. Differences in algal assemblage structure among treatments and streams were explored with permutational multivariate analysis of variance based on the Bray–Curtis similarity index (*adonis* function of the 'VEGAN' R package, Oksanen et al., 2007). In order to identify the most representative taxa of each assemblage, an indicator value index (*multipatt* function of the 'INDICSPECIES' R package, De Cáceres, 2013) was used. All statistical analyses were performed with R version 4.1.2.

3 | RESULTS

Periphyton biomass varied significantly depending on treatment, with higher values in control (C, mean±SE: 0.43±0.07) than in presence of tadpoles (Ao, 0.29±0.02) or both species (Al+Ao, 0.28±0.03; Table S1, Figure 2A, Figure S1). Chl-*a* concentration showed a similar pattern (Ao, 6234.83±1865.77 µg m^{-2}; C, 10526.06±3058.30 µg m^{-2}; Table S1, Figure 2B, Figure S1) but, in this case, it did not present significant differences. Chl-*b* and Chl-*c* concentrations were not significantly different across treatment, although there seemed to be a trend in Chl-*c* concentration, which tended to be higher in Al (232.47±84.58 µg m^{-2}) than in the other treatments (Table S1, Figure 2C,D, Figure S1). The autotrophic index (Table S1, Figure 2E, Figure S1) and inorganic mass (Table S1, Figure 2F, Figure S1) did not vary among treatments. All of these variables, except Chl-*c* concentration, were significantly affected by stream but not by pool within stream (Table S2).

Algal abundance did not present statistical differences; however, it tended to be higher in Al (682738.2±167284.7 cell m^{-2}) than in Ao (297532.9±91568.5 cell m^{-2}) and Al+Ao (337028.9±43036.8 cell m^{-2}; Table S1, Figure 2G, Figure S1). Taxon richness did not differ significantly either, but it also tended to be higher in treatments where tadpoles were absent (C, 11.88±0.68 taxa; Al, 12.13±0.42 taxa) than in the ones where they were present (Ao, 9.88±1.06 taxa; Al+Ao, 10.5±0.51 taxa; Table S1, Figure S1). Shannon's diversity showed a similar pattern but in this case: being significantly higher in C (0.97±0.03) and Al (0.97±0.05) than in Ao (0.81±0.05; Table S1, Figure 2H, Figure S1). Algal abundance and richness were significantly affected by stream, but not Shannon's diversity (Table S2). Algal assemblage structure varied among streams but it was not significantly affected by treatment (Table 2). However, the indicator value index, which indicates which taxa characterize a treatment by presenting higher abundance than in other treatments, showed that

Synedra spp. Ehrenberg was characteristic of C, and *Monoraphidium* spp. Komárková-Legnerová was common in C and Al. The assemblage in all treatments was, in general, dominated by *Navicula* spp. Bory, *Scenedesmus* spp. Meyen and *Gomphonema* spp. Ehrenberg (Table S3), with *Oscillatoria* spp. Vaucher ex Gomont also being common in the smallest and coldest site (stream 3).

Caddisfly mass was significantly higher in the presence of tadpoles (Al, 0.70±0.26 mg; Al+Ao, 1.20±0.39 mg; Table S1, Figure 3A). However, tadpole length was not significantly affected by the presence of caddisflies in the enclosures (Ao, 6.11±0.97; Al+Ao, 8.61±1.3; Table S1, Figure 3B). Neither caddisfly mass nor tadpole length were affected by stream or pond (Table S2).

4 | DISCUSSION

Our experiment showed that presence or absence of tadpoles can alter stream ecosystem structure through changes in primary producer assemblages. Periphyton biomass was higher in the absence of tadpoles than in their presence, and Chl-*a* concentration showed a similar but nonsignificant trend. This agrees with results from other studies in tropical and temperate streams, where tadpole presence has been shown to reduce periphyton accrual due to grazing and bioturbation (Connelly et al., 2008; Flecker et al., 1999; Mallory & Richardson, 2005; Ranvestel et al., 2004). In our study, the reduction in periphyton biomass was more likely caused by consumption, since we did not find any differences in sediment accrual among treatments without consumers and the ones containing tadpoles or caddisflies, and the effect of stream (mainly driven by flow) affected most periphyton related variables. This suggests that stream flow was low but enough to overshadow any potential effect of tadpoles on sediment accumulation, and hence any change in bioturbation when tadpoles disappeared, contrasting with other studies where sediment accrual was reduced by tadpole presence (Barnum et al., 2022; Mallory & Richardson, 2005; Ranvestel et al., 2004). These differences can be due to the different conditions of neotropical streams and montane temperate streams, since differences in flow and tadpole densities between riffles and pools within the same stream have can determined the effects of tadpole loss on bioturbation (Barnum et al., 2022).

The analysis of algal assemblage structure showed significant differences among streams independently of experimental treatments, possibly in relation to differences in physicochemistry or other stream characteristics (Mallory & Richardson, 2005). Despite the fact that neither algal assemblage structure nor abundance nor richness presented statistically significant differences, they showed strong trends. Abundance and richness tended to be higher in presence of only caddisflies than in treatments with tadpoles, possibly due to the more efficient grazing activity of tadpoles compared to macroinvertebrates (Colón-Gaud et al., 2010). The trend of higher Chl-*c* concentration in presence of caddisflies alone suggests that tadpole presence mostly reduced the abundance of diatoms, for several reasons: (1) these algae are more easily removed by bioturbation (Barnum et al., 2022; Ranvestel et al., 2004); (2) they are preferred over
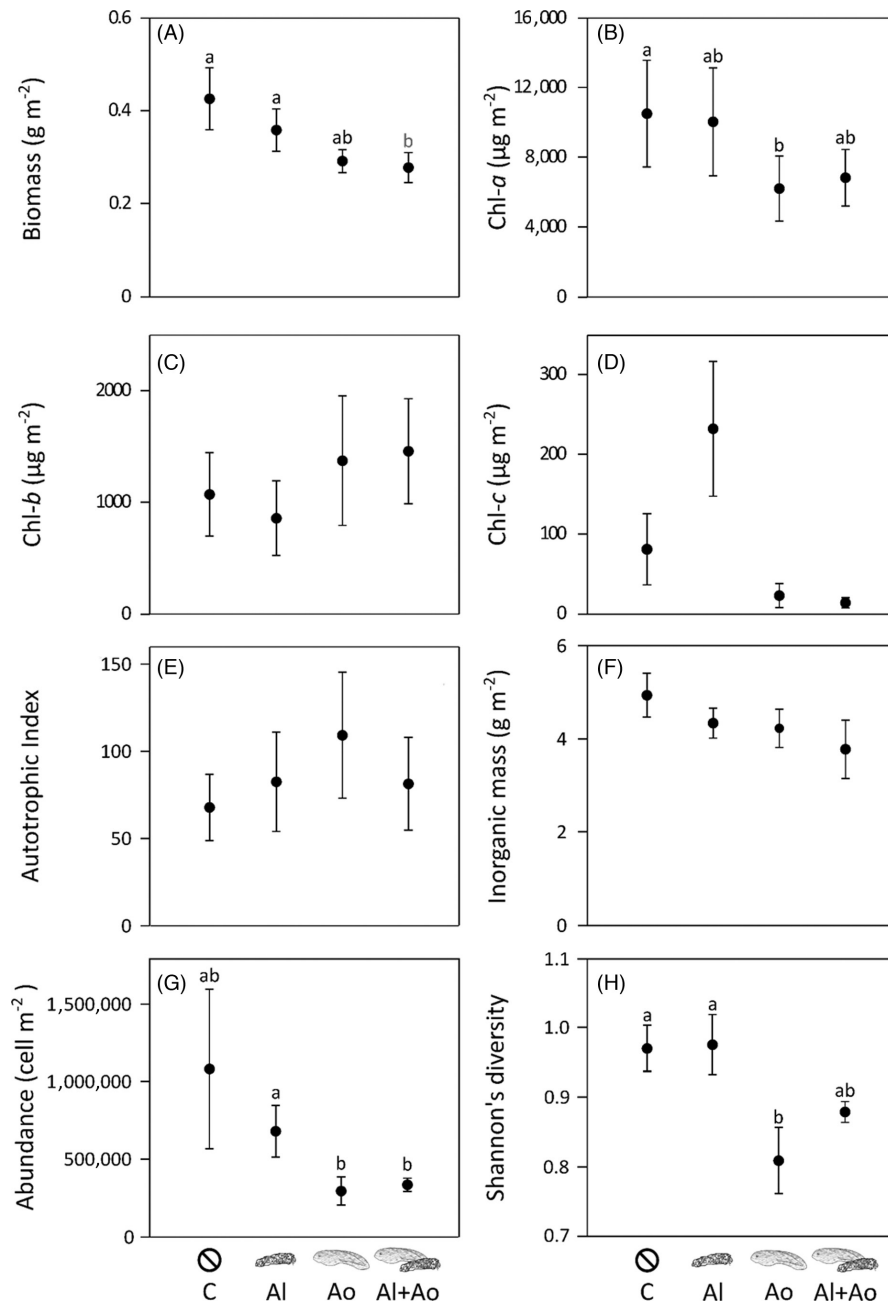
FIGURE 2 Periphyton biomass (g m^{-2}; a), Chl-*a* (b), Chl-*b* (c) and Chl-*c* (d) concentrations (µg m^{-2}), autotrophic index (e), inorganic mass (g m^{-2}; f), algal abundance (cell m^{-2}; g) and Shannon's diversity index (h) in the control (c), presence of caddisflies (AI), presence of tadpoles (Ao) and presence of both species (AI+Ao). Circles represent means and whiskers standard errors. Different letters indicate significant differences among treatments.

| Factor	df	F	R²	p	Sign. paired comparisons
Treatment	3	1.46	0.1314	0.085	
Stream	1	3.55	0.1068	0.002	Stream 1 vs. Stream 2; Stream 1 vs. Stream 3; Stream 2 vs. Stream 3
Treatment × Stream	3	1.12	0.1011	0.313	

Abbreviations: df, degrees of freedom; F, F-statistic; p, p-value; R², adjusted R².

TABLE 2 Results of PERMANOVAs exploring the effects of treatment (control, presence of caddisflies, presence of tadpoles and presence of both species), stream and their interaction, as well as significant paired comparisons, on algal assemblage structure. Stress = 0.1471433

filamentous green algae (Kupferberg, 1997); and (3) due to their tendency to present a larger size than other algae such as green algae or cyanobacteria, they are better assimilated by tadpoles and less likely to be intact after passage though tadpole gut, and therefore, less prone to recolonize the grazed area (Connelly et al., 2008; Peterson & Boulton, 1999; Steinman, 1996). This view is supported by the differences in Shannon's diversity and the indicator taxa that we found in both treatments without tadpoles. The control was characterized by

FIGURE 3 Caddisfly mass (mg; a) and tadpole increase in length (proportion of SVL; b) in treatments with caddisflies (Al), tadpoles (Ao) and both species (Al+Ao). Circles represent means, whiskers standard errors and arrows estimated initial caddisfly mass. Different letters indicate significant differences among treatments.
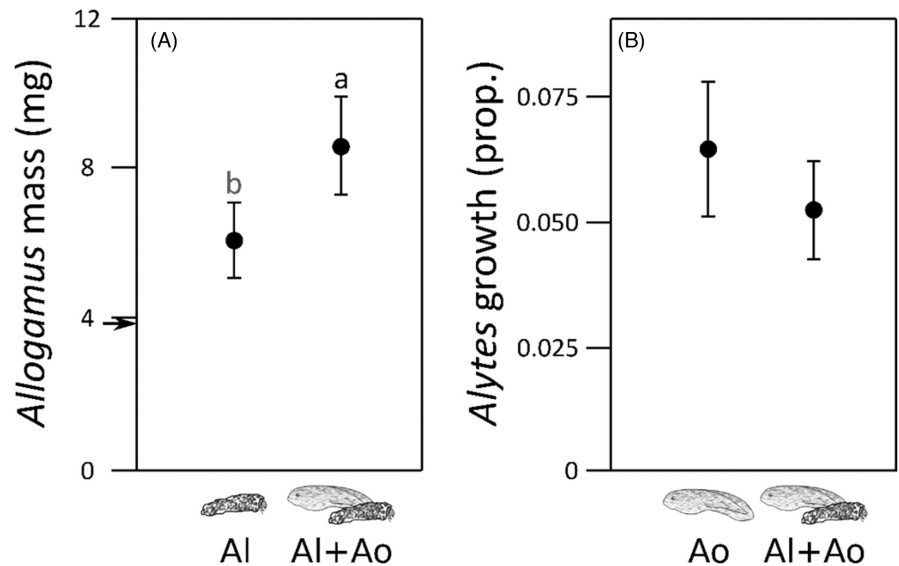
Synedra, which is a large diatom, known to be a preferred resource for tadpoles and to be efficiently digested by them (Connelly et al., 2008; Peterson & Boulton, 1999; Peterson & Jones, 2003); and both treatments without tadpoles (control and only caddisflies) were characterized by *Monoraphidium*, which is a green alga whose abundance has previously been found to be affected by grazing (Tarkowska-Kukuryk et al., 2020) and, therefore, potentially more affected by tadpole presence. Unlike other studies showing that grazer presence increased periphyton diversity by opening patches where new colonizers can settle, reducing dominance of stronger competitive species and allowing the persistence of rarer taxa (Dunck et al., 2018; Hillebrand, 2009), we found the highest diversity in absence of tadpoles.

Tadpoles did not increase their length much during the experiment, regardless of the presence or absence of caddisflies, with no variation among treatments, as it could be expected due to the short duration of the experiment relative to the life cycle of *Alytes obstetricans* (Garriga et al., 2017; Scheidt & Uthleb, 2005). On the other hand, caddisflies almost doubled their body mass in both treatments, with greater growth in presence of tadpoles. This all suggests that large grazers such as *Allogamus laureatus* do not directly compete with tadpoles, contrasting with studies in tropical streams where larger grazers were benefited by tadpole losses (Colón-Gaud et al., 2010). Instead, caddisflies seemed to benefit from tadpole activity, as has been observed for small grazers in tropical streams (Colón-Gaud et al., 2010; Ranvestel et al., 2004). This may be due to facilitating access to underlying algal resources caused by tadpole grazing, and to the more generalist feeding of *A. laureatus*, which allows them to feed also on fine particulate organic matter produced by tadpoles or drawn by the current (Kupferberg, 1997; Ranvestel et al., 2004).

5 | CONCLUSIONS

Amphibian losses have been shown to alter ecosystem functioning through changes in periphyton and invertebrate assemblages

in tropical streams (Colón-Gaud et al., 2010; Connelly et al., 2008; Ranvestel et al., 2004). Here, we have shown that ongoing amphibian declines have similar effects in temperate montane streams, causing an increase in periphyton biomass, a reduction in macroinvertebrate biomass and changes in algal assemblage structure in the short term, effects that might be expected to become more apparent over larger temporal scales (Connelly et al., 2008). Our results are highly relevant, since amphibians are suffering dramatic declines globally (Collins, 2010), and that could lead to further alterations of ecosystem structure and functioning in those ecosystems presenting stable amphibian populations. Therefore, it is important to continue studying the role that amphibians play in ecosystems in order to better predict the ecological effects of their disappearance, and to improve protection measures that avoid their extinction.

Ambiente de la Comunidad de Madrid extended permits for field work and approved experimental protocols (ref. 10/045182.9/21).

CONFLICT OF INTEREST

DATA AVAILABILITY STATEMENT

ORCID

Alberto Alonso https://orcid.org/0000-0002-9953-420X

Silvia Monroy https://orcid.org/0000-0002-7430-1776

Jaime Bosch https://orcid.org/0000-0002-0099-7934

Javier Pérez https://orcid.org/0000-0001-6305-4151

Luz Boyero https://orcid.org/0000-0001-7366-9299

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SUPPORTING INFORMATION

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