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Invasion of temperate riparian forests by *Acacia dealbata* affects macroinvertebrate community structure in streams.

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ABSTRACT

Invasion of temperate riparian forests by *Acacia dealbata* affects macroinvertebrate community structure in streams.

The riparian forest determines many of the stream characteristics such as physicochemical variables and the availability and quality of food resources for aquatic communities. In central Portugal, native mixed deciduous forests are being heavily invaded by *Acacia dealbata*, which is an evergreen and nitrogen-fixing tree species, but effects on stream benthic macroinvertebrate communities are unknown. We assessed invasion effects on macroinvertebrate communities by comparing streams flowing through native forests (native streams) and streams flowing through forests heavily invaded by *A. dealbata* (invaded streams), in central Portugal. We did not find major differences in macroinvertebrate abundance, total taxa richness or diversity between native and invaded streams, due to high variation among native streams. However, taxonomic structure of macroinvertebrate communities differed between stream types, with invaded streams showing lower percentage of sensitive taxa. Macroinvertebrate communities structure based on functional feeding groups also differed between stream types, with a tendency for higher abundance of shredders and scrapers/grazers and lower abundance of filter feeders in native than invaded streams, probably due to altered composition of leaf litter inputs and canopy cover within invaded streams. Our findings suggest that invasion by *A. dealbata* affects the macroinvertebrate community mostly in terms of taxonomic and functional structure. Since *A. dealbata* is rapidly spreading in all southern Europe, this study highlights the importance of enhancing the comprehension of its impact on aquatic communities to identify ecological risks and formulate effective mitigation strategies.

KEYWORDS: Alien vegetation, benthic macroinvertebrates, FFG, forest change, functional structure, litter supply, N-fixing tree

RESUMEN

La invasion de bosques ribereños templados por* Acacia dealbata *afecta la estructura de la comunidad de macroinvertebrados en arroyos.

El bosque ribereño determina muchas de las características de los arroyos, como las variables fisicoquímicas y la disponibilidad y calidad de los recursos para las comunidades acuáticas. En el centro de Portugal, los bosques mixtos autóctonos de hoja caduca están siendo fuertemente invadidos por Acacia dealbata*, un árbol perenne fijador de nitrógeno, pero se desconocen los efectos sobre las comunidades de macroinvertebrados bentónicos de los arroyos. Hemos evaluado los efectos de la invasión sobre las comunidades de macroinvertebrados mediante la comparación entre arroyos que fluyen a través de bosques nativos (arroyos nativos) y arroyos que fluyen a través de bosques invadidos por* A. dealbata *(arroyos invadidos), en el centro de Portugal. No se han encontrado diferencias significativas en la abundancia, riqueza total de taxones y diversidad de macroinvertebrados entre arroyos nativos e invadidos, debido a la alta variación entre arroyos nativos. Sin embargo, la estructura taxonómica de las comunidades de macroinvertebrados difirió entre los dos tipos* *de arroyos, con un menor porcentaje de taxones sensibles en los arroyos invadidos. La estructura de las comunidades basada en grupos de estrategia trófica también difirió entre los tipos de arroyos, con tendencia a una mayor abundancia de trituradores y raspadores/pastadores y una menor abundancia de filtradores en los arroyos nativos en comparación con los arroyos invadidos, probablemente debido a la alteración de la composición de los inputs de hojarasca y de la cobertura de dosel arbóreo en los arroyos invadidos. Nuestros hallazgos sugieren que la invasión por* A. dealbata *afecta principalmente a la comunidad de macroinvertebrados en términos de estructura taxonómica y funcional. Dado que* A. dealbata *se está propagando rápidamente en todo el sur de Europa, este estudio resalta la importancia de mejorar la comprensión de su impacto en las comunidades acuáticas para identificar riesgos ecológicos y formular estrategias de mitigación.*

PALABRAS CLAVES: árbol fijador de nitrógeno, aporte de hojarasca, cambio de bosque, estructura funcional, FFG, macroinvertebrados bentónicos, vegetación exótica.

INTRODUCTION

Streams and their riparian forests are closely connected ecosystems, linked by the bidirectional flow of matter and energy; e.g., plant litter flows from the riparian forest into the stream where it sustains the detrital food web, and adult aquatic insects flow in the opposite direction to integrate terrestrial food webs (Tolkkinen et al., 2020). The riparian forest determines many of the stream characteristics; e.g., canopy cover influences solar exposure of the stream bed and water temperature (Beschta, 1997), nutrient uptake by and litter inputs from the riparian forest affect the water chemical composition (Dosskey et al., 2010) and the latter also affects the benthic organic matter standing stock (Abelho & Graça, 1996; Molinero & Pozo, 2004), and the supply of branches and logs to streams affects current velocity and depth conditions and changes microhabitat distribution (Gerhard & Reich, 2000). Consequently, the riparian forest influences the instream energetic pathways (autotrophic vs. heterotrophic) and the structure of aquatic communities (Hawkins et al., 1982; Ferreira et al., 2016; Little et al., 2021).

The invasion of native riparian forests by exotic tree species strongly affects forest tree composition and, consequently, the amount, diversity, taxonomic and chemical composition, and seasonality of the litter supply to streams (Lecerf et al., 2007; Hladyz et al., 2011; Mineau et al., 2012). Riparian forest invasion also affects stream characteristics such as water nutrient concentrations; e.g., increase in the cover by nitrogen-fixing species generally leads to increases in dissolved nitrogen concentration in stream water (Goldstein et al., 2009; Atwood et al., 2010; Stewart et al., 2019). Changes in food resources (i.e., litter inputs) and stream water characteristics resulting from forest invasion can potentially affect aquatic communities. For example, the invasion of the Fynbos vegetation by *Acacia mearnsii* De Wild. in South Africa resulted in lower taxa richness (Magoba & Samways, 2010) and altered benthic macroinvertebrate community structure, with lower abundance of cobble-dwelling taxa and higher abundance of particle-feeding taxa in stream sites flowing through invaded than through native vegetation (Lowe et al., 2008). Invasion of temperate deciduous forests by *Lonicera maackii* (Rupr.) Maxim. in the American Midwest also led to changes in benthic macroinvertebrate community structure along an invasion gradient, with the most invaded stream showing the lowest percentage of sensitive taxa (Little et al., 2021). In central and southern Scotland, invasion of riparian areas by exotic plants was associated with a decrease in the diversity and an increase in the abundance of stream benthic macroinvertebrates, and changes in community structure in autumn (but not in spring) (Seeney et al., 2019). Finally, invasion of riparian areas by *Salix fragilis* L. in the northern Patagonian Andes led to changes in stream benthic macroinvertebrate community structure but not in abundance and diversity (Serra et al., 2013). These examples, although not intended to be extensive, show that invasion effects somewhat vary among studies that differ in the native vegetation, invasive species, climate, and potentially also in the extent and duration of the invasion. Still, effects of riparian invasions were pervasive for stream benthic macroinvertebrate community structure. Also, effects of species invasions are likely stronger when native and invasive species show different functional characteristics (Kominoski et al., 2013; Castro-Díez et al., 2014).

In central Portugal, native forests dominated by deciduous tree species (*Quercus* spp., *Castanea sativa* Mill.) are being heavily invaded by *Acacia* species originating from Australia, mainly *A. dealbata* Link.; presently, *Acacia* species cover up to 43% of the area in some small-to-medium sized stream basins (Ferreira et al., 2021; Pereira et al., 2021) (Fig. 1). The invasion success of *Acacia* species is due to its (i) great plasticity that allows colonization of disturbed soils (e.g., stream banks) (Pohlman et al., 2005), (ii) ability to fix atmospheric nitrogen (N) that allows high growth rate in nutrient-poor and acidic soils (Gallagher et al., 2011), (iii) high seed production that confers high spread ability (Lorenzo et al., 2010), and (iv) production of allelopathic compounds that inhibit the germination and growth of competitor species (Lorenzo et al., 2008). In addition to covering large areas (Fig. 1), *A. dealbata* is an evergreen and N-fixing species, traits that markedly differ from those of the dominant native tree species (Lorenzo et al., 2010; Ferreira et al., 2021). Therefore, invasion of native diverse deciduous forests with low representation of N-fixing tree species by *A. dealbata* can potentially affect aquatic communities of adjacent streams (Ferreira et al., 2021).

In this study we compared the benthic macroinvertebrate communities between streams flowing through native mixed deciduous forests and streams flowing through forests heavily invaded by *A. dealbata*, in central Portugal, to test the hypotheses that riparian forest invasion would negatively affect macroinvertebrate abundance, taxa richness (total and of sensitive taxa), and diversity, as well as change community taxonomic structure. Community structure in terms of functional feeding groups was also hypothesized to differ between stream types, especially for shredders that strongly depend on allochthonous leaf litter and have their life cycles synchronized with the autumnal leaf litterfall in native forests. Since *A. dealbata* is widely naturalized in all southern Europe (Sheppard et al., 2006; Lorenzo et al., 2010), this study contributes with relevant information that can inform about the cross-ecosystem effects of riparian invasion on stream communities in Mediterranean countries.

METHODS

Study area and streams

This study took place in Serra da Lousã, central Portugal, where invasive *Acacia* species (mainly

Figure 1. Image of a valley (São João stream) in the study area in Serra da Lousã, central Portugal, in February 2019, depicting heavy invasion by *A. dealbata*. *A. dealbata* is easily recognizable by its bright yellow flowers. *Imagen de un valle (arroyo São João) en el área de estudio en la Serra da Lousã, centro de Portugal, en febrero del 2019, que muestra una fuerte invasión de* A. dealbata*.* A. dealbata *es fácilmente reconocible por sus flores de color amarillo brillante.*

Acacia dealbata Link.) cover large areas, replacing native shrub communities and native deciduous forests dominated by *Quercus* spp. and *Castanea sativa* Mill. (Ferreira et al., 2021; Pereira et al., 2021). Six small streams (< 4 m wide, basin area ≤ 6 km^2) where selected: three streams flow through native mixed deciduous forests (native streams: Maior, Cerdeira and Candal) and the other three streams flow through forests heavily invaded by *A. dealbata* (cover in the riparian area: 94 – 100%) (invaded streams: Sotão, Fiscal and Piedade) (Table 1). All streams have low conductivity and circumneutral pH, and the substrate is composed mainly of schist gravel and cobbles, with coarse sand in depositional areas (Pereira & Ferreira, 2021).

Stream water was characterized regarding dissolved nutrient concentrations on three dates (3 January, 14 January and 11 February 2019) preceding the macroinvertebrate sampling (26 February 2019). Stream water was collected, filtered in the field through fiberglass filters (47 mm diameter, 0.7 µm pore size; Whatman GF/F, GE Healthcare UK Limited, Little Chalfont, UK) and distributed by two acid-washed flasks for determination of soluble reactive phosphorus (SRP) concentration (ascorbic acid method; APHA, 1995) and of nitrate, nitrite and ammonia concentrations (colorimetric method; AA3 Bran + Luebbe autoanalyzer; SEAL Analytical, Norderstedt, Germany). Dissolved inorganic nitrogen (DIN) concentrations were estimated as the sum of the concentrations of nitrate-N, nitrite-N and ammonia-N.

Benthic macroinvertebrate communities

Benthic macroinvertebrates were sampled from all streams on 26 February 2019 using a semi-quantitative method. Three samples were taken over a representative 50-m section in each stream by disturbing (kicking) the substrate and allowing the current to move the dislodged macroinvertebrates into a hand net (30 × 30 cm opening, 0.5 mm mesh size) that was placed just downstream of the operator (the same across streams); kicking was done from downstream to upstream for a standard distance and time (1 m for 1 minute)

Table 1. Location, basin area, *Acacia dealbata* cover in the riparian area, and water nutrient concentrations (mean ± SD, n = 3 between 3 January and 11 February 2019) of streams flowing through native forests (native streams) and of streams flowing through forests heavily invaded by *A. dealbata* (invaded streams) in Serra da Lousã, central Portugal. Comparison between stream types was done by t-test considering α = 0.050, and *p*-values are shown (significant values are highlighted in bold). *Localización, área de la cuenca,* A. dealbata *en la zona de ribera, y concentraciones de nutrientes en el agua (media ± DE, n = 3 entre el 3 de enero y el 11 de febrero del 2019) de arroyos que fluyen a través de bosques nativos (arroyos nativos) y de arroyos que fluyen a través de bosques fuertemente invadidos por* A. dealbata *(arroyos invadidos) en la Sierra de Lousã, centro de Portugal. La comparación entre tipos de arroyos se realizó mediante t-test considerando α = 0.050, y se muestran los* p-*valores (los valores significativos se resaltan en negrita).*

Stream	Latitude (N)	Longitude (W)	Altitude (m a.s.l)	Basin area (km^2)[1]	Riparian *Acacia dealbata* (%)[1,2]	DIN (µg N/L)[3]	SRP (µg P/L)[4]
Native streams							
Maior	40°07'53.3"	8°11'40.7"	195	6.05	1	25 ± 4	9 ± 6
Cerdeira	40°05'23.1"	8°12'05.0"	529	3.37	1	22 ± 2	8 ± 5
Candal	40°04'54.1"	8°12'16.6"	634	0.98	0	20 ± 2	7 ± 2
Invaded streams							
Sotão	40°07'54.1"	8°09'08.3"	373	1.14	94	76 ± 28	23 ± 7
Fiscal	40°06'40.2"	8°13'35.1"	329	1.24	100	60 ± 17	14 ± 2
Piedade	40°05'52.6"	8°14'11.5"	250	2.15	100	47 ± 8	9 ± 5
Average							
Native streams			453	3.47	1	23 ± 3	8 ± 1
Invaded streams			317	1.51	98	61 ± 15	15 ± 7
t-test (p-value)			0.380	0.262	**<0.001**	**0.012**	0.139

[1] from Pereira et al. (2021); [2] the riparian area was defined as an area 50 m wide on each stream bank and 250 m long upstream from the sampling point; [3] DIN, dissolved inorganic nitrogen; [4] SRP, soluble reactive phosphorus

and samples were collected moving from downstream to upstream in the stream section. Benthic samples were placed in plastic bags with stream water and transported to the laboratory in cold boxes. In the laboratory, samples were preserved with formaldehyde (final concentration of 4%) and stored in the dark until processed. Samples were rinsed thoroughly with tap water on top of a series of sieves (2 cm, 1 cm, and 0.5 mm mesh) to fractionate the sample and facilitate subsequent macroinvertebrate sorting. After sorting, macroinvertebrates were kept in vials with 80% ethanol. Macroinvertebrates were then identified at the lowest taxonomic level possible (Tachet et al., 2000; Vieira-Lanero, 2000) and counted under a stereo microscope (Leica, M80, Wetzlar, Germany). Macroinvertebrates were also grouped by functional feeding groups (FFG): predators, shredders, grazers/scrapers, gatherers/collectors, and filter feeders (Tachet et al., 2000).

Data analysis

Altitude, basin area, and water nutrient concentrations (SRP and DIN; average of the three dates) were compared between stream types (native vs. invaded, n = 3) by student's t-test (α = 0.050). Macroinvertebrate abundance, total taxa richness, Ephemeroptera + Plecoptera + Trichoptera (EPT, known as sensitive taxa) taxa richness, diversity indices, and abundance of FFG (average of the three samples) were also compared between stream types (native vs. invaded, n = 3) by student's t-test (α = 0.050). Two diversity indices were used: the Margalef species richness index (d), which is sensitive to abundant species (Morris et al., 2014), has its maximum value when all individuals belong to different species and its minimum when all individuals belong to the same species, i.e., a higher value indicates higher diversity; and the Pielou species evenness index, where a higher value indicates higher uniformity in the distribution of individuals by species and therefore higher diversity (Pielou, 1996).

Macroinvertebrate communities (taxonomic and FFG matrices) were compared between stream types (native vs. invaded) by analysis of similarity (ANOSIM, α = 0.050) based on a Bray-Curtis similarity matrix of log-transformed abundance data; visual representation of the similarity among communities was achieved by non-metric multidimensional scaling (nMDS; considered valid if stress level < 0.20). The taxa or FFGs responsible for distinguishing between stream types were identified by performing similarity percentage (SIMPER) analysis, which examined the contribution of each taxon or FFG to the mean dissimilarity between stream types.

Student's t-tests were performed using Excel (Microsoft Office 2019) and diversity indices and multivariate analyses were performed using PRIMER 6 (v6.1.16) & PERMANOVA+ (v1.0.6; Primer-E Ltd, Plymouth, UK) software.

RESULTS

Study streams

Mean dissolved nutrient concentrations were 1.9× (SRP) and 2.7× (DIN) higher in invaded than in native streams, but the difference was significant only for DIN (Table 1). Still, DIN concentrations were in the oligotrophic range in all streams (< 77 μg/L) (Table 1).

Benthic macroinvertebrate communities

In total, benthic samples retrieved 8599 macroinvertebrates distributed by 84 taxa (Table S1, see Supplementary information at https://www.limnetica.net/en/limnetica). Mayflies (Ephemeroptera: 32% individuals, 11 taxa), caddisflies (Trichoptera: 17% individuals, 22 taxa), worms (Oligochaeta: 16% individuals, 5 taxa), true flies (Diptera: 13% individuals, 16 taxa), and stoneflies (Plecoptera: 12% individuals, 8 taxa) were the most represented groups across streams (Table S1).

Mean abundance was 2.5× higher in native than in invaded streams, but differences were non-significant due to high variation (up to 5.3×) among native streams (Table 2). A similar pattern was observed for total taxa richness, which was 20% higher in native than in invaded streams, with differences being non-significant due to high variation (25%) among native streams (Table 2). However, stream types significantly differed in EPT taxa richness, which was 47% higher in

Table 2. Benthic macroinvertebrate community parameters (mean ± SE, n = 3) in streams flowing through native forests (native streams) and in streams flowing through forests heavily invaded by *A. dealbata* (invaded streams) in Serra da Lousã, central Portugal, in February 2019. Comparison between stream types was done by t-test considering α = 0.050, and *p*-values are shown (significant values are highlighted in bold). *Parámetros de la comunidad de macroinvertebrados bentónicos (media ± DE, n = 3) en arroyos que fluyen a través de bosques nativos (arroyos nativos) y en arroyos que fluyen a través de bosques fuertemente invadidos por* A. dealbata *(arroyos invadidos) en la Sierra de Lousã, centro de Portugal, en febrero del 2019. La comparación entre tipos de arroyos se realizó mediante t-test considerando α = 0.050, y se muestran los* p*-valores (los valores significativos se resaltan en negrita).*

Stream	Abundance (no. ind./sample)	Total taxa richness (no. taxa/sample)	EPT taxa richness (no. taxa/sample)	Margalef index (d)	Pielou index (J')
Native streams					
Maior	728 ± 134	40 ± 1	23 ± 1	5.91 ± 0.26	0.74 ± 0.00
Cerdeira	1115 ± 150	40 ± 5	22 ± 1	5.63 ± 0.73	0.68 ± 0.09
Candal	210 ± 77	32 ± 4	20 ± 2	5.92 ± 0.41	0.83 ± 0.04
Invaded streams					
Sotão	366 ± 27	35 ± 1	14 ± 2	5.71 ± 0.26	0.75 ± 0.02
Fiscal	238 ± 28	30 ± 0	15 ± 2	5.32 ± 0.12	0.76 ± 0.02
Piedade	209 ± 82	30 ± 5	17 ± 3	5.56 ± 0.49	0.81 ± 0.05
Average					
Native streams	684 ± 22	37 ± 1	22 ± 1	5.82 ± 0.14	0.75 ± 0.02
Invaded streams	271 ± 18	31 ± 1	15 ± 1	5.53 ± 0.11	0.77 ± 0.01
t-test (*p*-value)	0.196	0.132	**0.010**	0.121	0.694

EPT, Ephemeroptera + Plecoptera + Trichoptera (see Table S1)

native than invaded streams (Table 2). Diversity indices did not significantly differ between stream types (Table 2).

Macroinvertebrate community structure (based on the taxonomic matrix) significantly differed between native and invaded streams (ANOSIM, R = 0.47, $p = 0.001$) (Fig. 2). The dissimilarity of macroinvertebrate communities between stream types was moderate (SIMPER analysis, cumulative dissimilarity = 61%). The taxa that contributed the most to differences between native and invaded streams (contribution to dissimilarity > 2.5%) were *Amphinemura* sp., *Habroleptoides* sp., *Chloroperla* sp., and *Rithrogenea* sp., which were more abundant in native streams, and *Ephemera* sp. Simuliidae, and *Paraleptophlebia* sp., which were more abundant in invaded streams (Table 3).

Abundance of FFGs (predators, shredders, grazers/scrapers, gatherers/collectors, and filter feeders) did not significantly differ between native and invaded streams (t-tests, $p \geq 0.111$; Fig. 3A). Still, macroinvertebrate communities (based on the FFG matrix) significantly differed between native and invaded streams (ANOSIM, R = 0.25, $p = 0.012$), owing to the slightly higher relative contribution of shredders and scrapers/grazers in native streams and of filter feeders in invaded streams (Fig. 3B), but dissimilarity of macroinvertebrate communities between stream types was low (SIMPER analysis, cumulative dissimilarity = 16%).

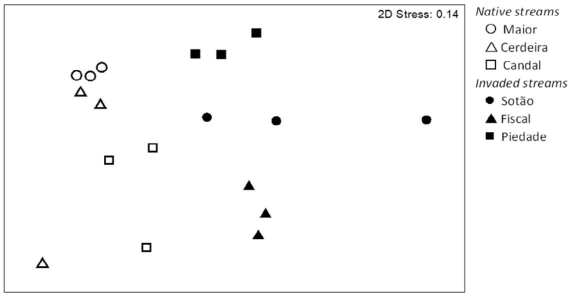

Figure 2. Ordination (nMDS based on Bray-Curtis similarity of log-transformed abundance data) of benthic macroinvertebrate communities in streams flowing through native forests (native streams) and in streams flowing through forests heavily invaded by *A. dealbata* (invaded streams) in Serra da Lousã, central Portugal, in February 2019. *Ordenación (nMDS basada en la similitud Bray-Curtis de datos de abundancia transformados por logaritmo) de las comunidades de macroinvertebrados bentónicos en arroyos que fluyen por bosques nativos (arroyos nativos) y en arroyos que fluyen por bosques fuertemente invadidos por* A. dealbata *(arroyos invadidos) en la Sierra de Lousã, centro de Portugal, en febrero del 2019.*

Table 3. Result of the similarity percentage (SIMPER) analysis, performed on benthic macroinvertebrate communities, showing the contribution (%) of each taxon to the dissimilarity between streams flowing through native forests (native streams) and streams flowing through forests heavily invaded by *A. dealbata* (invaded streams) in Serra da Lousã, central Portugal, in February 2019. *Resultado del análisis de porcentaje de similitud (SIMPER), realizado en comunidades de macroinvertebrados bentónicos, mostrando la contribución (%) de cada taxon a la disimilitud entre arroyos que fluyen por bosques nativos (arroyos nativos) y arroyos que fluyen por bosques fuertemente invadidos por* A. dealbata *(arroyos invadidos) en la Sierra de Lousã, centro de Portugal, en febrero del 2019.*

Taxa	Native average abundance	Invaded average abundance	Average dissimilarity	Dissimilarity/SD	Contribution %	Cumulative %
Amphinemura sp.	3.49	0.20	2.60	3.32	4.29	4.29
Habroleptoides sp.	3.20	0.00	2.32	1.35	3.84	8.12
Chloroperla sp.	2.76	0.00	2.13	2.75	3.51	11.63
Ephemera sp.	0.15	2.60	2.03	3.09	3.36	14.99
Simuliidae	0.75	2.40	1.64	1.37	2.72	17.71
Rithrogenea sp.	2.42	0.63	1.64	1.50	2.71	20.41
Paraleptophlebia sp.	1.26	1.60	1.58	1.12	2.61	23.03
Ecdyonurus sp.	2.94	1.53	1.52	1.66	2.50	25.53
Acentrella sinaica	2.85	1.96	1.39	1.33	2.30	27.83
Lepidostoma hirtum	2.13	0.88	1.38	1.45	2.28	30.11
Larcasia partita	2.15	1.02	1.37	1.49	2.26	32.37
Baetis sp.	2.25	0.99	1.33	1.45	2.20	34.58
Polycelis spp.	1.49	1.36	1.30	1.19	2.15	36.73
Epeorus sp.	1.77	0.77	1.30	1.27	2.14	38.87
Duguesia spp.	1.50	0.80	1.29	1.12	2.13	41.00
Beraea sp.	1.82	0.38	1.29	1.19	2.13	43.13
Hydrocyphon sp.	1.23	1.14	1.19	1.33	1.96	45.09
Heptagenia sp.	1.55	0.18	1.18	1.52	1.95	47.04
Lumbriculidae	2.73	3.17	1.16	1.24	1.91	48.96
Diplectrona felix	1.23	0.92	1.05	1.08	1.74	50.69
Hydropsyche sp.	2.41	2.30	1.02	1.28	1.68	52.37
Protonemura sp.	0.86	1.45	0.91	1.29	1.50	53.87
Thremma sp.	1.50	0.48	0.90	1.46	1.49	55.36
Tanytarsini	2.61	2.28	0.90	1.58	1.49	56.85
Sphaerium sp.	0.20	1.19	0.88	1.45	1.45	58.30
Orthocladiinae	1.89	1.60	0.85	1.48	1.41	59.71
Leuctra sp.	0.76	0.91	0.85	1.15	1.40	61.11

DISCUSSION

In this study we assessed the effects of the invasion of native mixed deciduous forests by *A. dealbata* on stream benthic macroinvertebrate communities in central Portugal. Following the pattern found in the literature (see examples in the Introduction), invasion effects were more evident for macroinvertebrate community structure than for abundance, total richness or diversity indices.

In accordance with our hypothesis, macroinvertebrate community structure differed between stream types, which agrees with other studies addressing invasion effects on benthic macroinvertebrate communities (Lowe et al., 2008; Serra et al., 2013; Seeney et al., 2019; Little et al., 2021). In particular, we found higher EPT taxa richness and higher number of EPT taxa as indicators for streams flowing through native than invaded forests, as also seen in the case of *L. maacki* (Little et

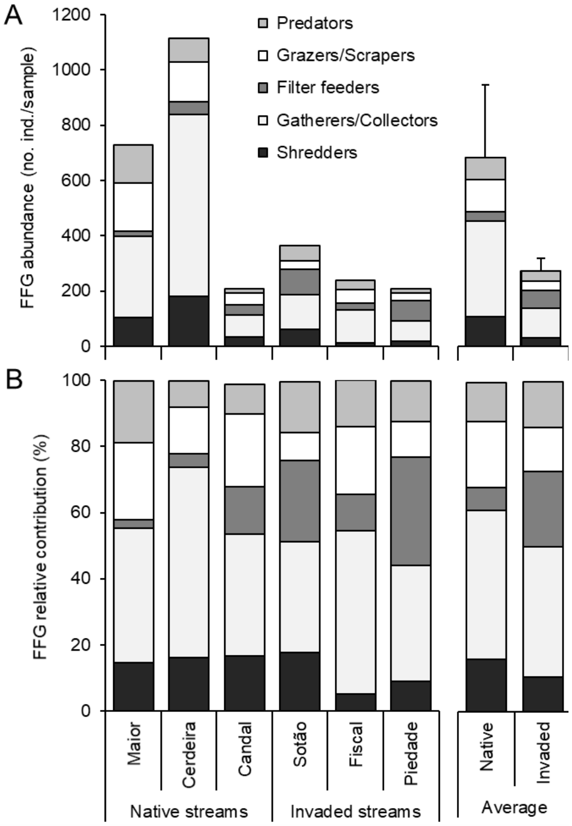

Figure 3. Benthic macroinvertebrates functional feeding groups (FFG) abundance (A) and relative contribution (B) in streams flowing through native forests (native streams) and in streams flowing through forests heavily invaded by *A. dealbata* (invaded streams) in Serra da Lousã, central Portugal, in February 2019. *Abundancia (A) y contribución relativa (B) de grupos funcionales de alimentación (FFG) de macroinvertebrados bentónicos en arroyos que fluyen por bosques nativos (arroyos nativos) y en arroyos que fluyen por bosques fuertemente invadidos por* A. dealbata *(arroyos invadidos) en la Sierra de Lousã, centro de Portugal, en febrero del 2019.*

al., 2021) and *A. mearnsii* (Magoba & Samways, 2010) invasions. EPT orders are known to have many taxa that are sensitive to environmental perturbations (Fore, 1996). Concurrently, invaded sites had higher contribution of generalist taxa, such as Simuliidae and Sphaeridae (Fore, 1996; Tampo et al., 2021).

Macroinvertebrate community structure differed between stream types also when macroinvertebrate FFGs were considered. Shredders strongly depend on allochthonous leaf litter as a food resource (Wallace et al., 1997). In this sense, vegetation changes can produce important variations in the distribution of FFG (Balderas et al., 2016). As seen in Mancilla et al. (2009), in central Chile, streams flowing through basins with less than 20% native vegetation cover had significantly higher number of gatherers and lower number of shredders than streams flowing through basins with higher percentage of native vegetation. Shredders transform coarse organic resources into fine particulate organic matter, thus supporting secondary consumers (Cummins et al., 1973; Patrick, 2013), such as gatherers/collectors and filter feeders (Shepard & Minshall, 1984). Alterations in forest composition that result in decreased quality of leaf litter inputs to streams and augmented canopy cover can lead to decreases in litter decomposition rates and algal production, diminishing resources availability and inducing shifts in consumer assemblages, as found in the case of invasion of deciduous riparian forests by *Rhododendron ponticum* L. in Western Ireland (Hladyz et al., 2011). Similar changes resulting from the invasion of deciduous forests by *A. dealbata* can explain the tendency for the lower abundance of grazers/scrapers and higher abundance of filter feeders in invaded streams.

Contrary to anticipated, no significant differences were found in macroinvertebrate abundance, total taxa richness, and diversity between streams flowing through native forests and streams flowing through forests heavily invaded by *A. dealbata*, which was mainly attributed to high variation among native streams. Native streams may have differed more in current velocity or in the composition of plant litter input than invaded streams that had their riparian vegetation dominated by *A. dealbata* (Pereira et al., 2021).

Our data suggests that invasion of native deciduous forests by *A. dealbata* may affect community taxonomic and functional (FFG) structure, although it may not strongly affect macroinvertebrate communities in terms of total abundance, total richness, and diversity. However, we recall that benthic macroinvertebrate sampling was performed only once, in winter. Benthic macroinvertebrate communities can exhibit natural seasonal variation, induced by various factors such as food availability, temperature, and photoperiod, which may moderate invasion effects (Álvarez-Cabria et al., 2011; Seeney et al., 2019). Additionally, the use of a unique sampling time did not allow assessing successional changes occurring over time during the invasion process, shading temporal dynamics and the impact of different percentages of *Acacia* cover. Moreover, lack of pre-invasion data makes it challenging to establish a cause-and-effect relationship between the invasion and the observed changes in community structure. Finally, the reduced number of streams used may have contributed to the high variability within stream types (especially among native streams), precluding the identification of clearer invasion effects.

Future studies should consider a larger number of streams to take into consideration the natural variability among native streams and the different invasion degrees in invaded streams; this could allow establishing correlations between the degree of invasion and macroinvertebrate metrics (Ferreira et al., 2016). Also, benthic macroinvertebrate sampling should be carried out seasonally to take into consideration macroinvertebrate life cycles and litter inputs phenology to streams, which may moderate invasion effects on benthic macroinvertebrates (Seeney et al., 2019). Additionally, since invasion by *A. dealbata* is proceeding at high rates (500 ha/year between 2005 and 2015, and likely faster since 2017 owing to large-scale wild fires; ICNF, 2019; Ferreira et al., 2021), pre-invasion information may (soon) become available for streams that integrate the official bioassessment scheme and that were sampled before invasion (early 2000's) and that now flow through forests which became invaded; this would allow before-after comparisons. Finally, since *A. dealbata* is invasive in Mediterranean countries, collaborative assessments should also be considered for a large scale understanding of invasion impacts on stream communities. A better understanding of invasion impacts on streams could help identify most vulnerable ecosystems and envisage mitigation measures.

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