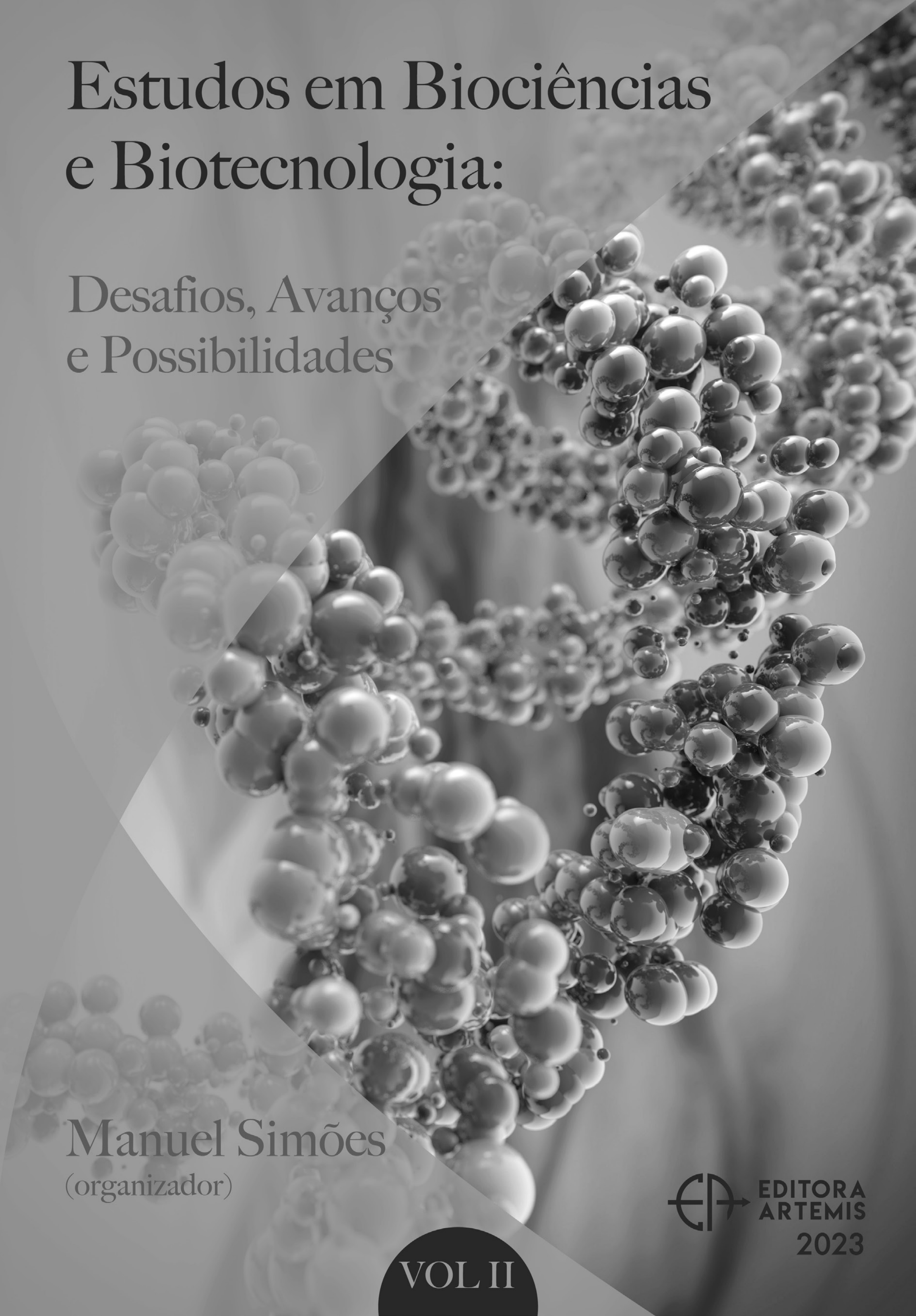
Estudos em Biociências e Biotecnologia:

Desafios, Avanços e Possibilidades

Manuel Simões
(organizador)

EDITORA ARTEMIS
2023

VOL II



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E82 Estudos em biociências e biotecnologia [livro eletrônico] : desafios, avanços e possibilidades: vol. II / Organizador Manuel Simões. – Curitiba, PR: Artemis, 2023.

Formato: PDF
Requisitos de sistema: Adobe Acrobat Reader
Modo de acesso: World Wide Web
Inclui bibliografia
Edição bilíngue
ISBN 978-65-87396-83-5
DOI 10.37572/EdArt_310523835

1. Ciências biológicas. 2. Biotecnologia. 3. Biomedicina. I.Simões, Manuel.

CDD 574

Elaborado por Maurício Amormino Júnior – CRB6/2422

EDITORA ARTEMIS
2023

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PREFÁCIO

A investigação científica e o desenvolvimento tecnológico têm permitido criar soluções para os mais diversos problemas societais. Contudo, os avanços científicos e tecnológicos não se podem distanciar das abordagens de disseminação relevantes, que permitam que o conhecimento seja disponibilizado de forma criteriosa e compreensível à comunidade académica, às empresas/indústria e ao público em geral.

O segundo volume da edição “Estudos em Biociências e Biotecnologia” é composto por 12 capítulos que descrevem avanços significativos das ciências e tecnologias biológicas aplicadas a diversas áreas de investigação, complementando os trabalhos publicados no primeiro volume. Em particular, este volume, reúne capítulos relacionados com as ciências biológicas nas seguintes áreas/tópicos: biomédica (capítulos 1 e 2); biologia funcional e biotecnologia de plantas (capítulos 3 a 6); produção e proteção de alimentos (capítulos 7 a 9); ambiente e biorrecursos (capítulos 10 a 12).

O leitor deste volume beneficiará de um conjunto de informação inovadora que, além de ser um excelente contributo científico, contribuiu para dar resposta a diversos objetivos de desenvolvimento sustentável estabelecidos pela Assembleia Geral das Nações Unidas.

Manuel Simões

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CAPÍTULO 10

DECOMPOSITION OF THE INVASIVE *ACACIA LONGIFOLIA* IN A PERI-URBAN STREAM

Data de submissão: 14/04/2023
Data de aceite: 29/04/2023

Manuela Abelho
Centre for Functional Ecology
Science for People and the Planet
Associate Laboratory TERRA
Polytechnic Institute of Coimbra
Coimbra Agriculture School
Calçada Martim de Freitas, 3000-456
Coimbra, Portugal
https://orcid.org/0000-0001-7663-3291

ABSTRACT: Biological invasions are a major threat to biodiversity and ecosystem functioning. Invasion by species of acacia results in dense monospecific stands, eventually excluding other plants. Riparian areas provide streams of organic matter in the form of leaf litter, the major source of carbon, nutrients, and energy for these ecosystems. Invasion of riparian areas by species of acacia is a reality worldwide, but few studies assessed the effect of the invasion on stream ecosystem functioning. This study compared decomposition of leaf litter from *Acacia longifolia*, a prominent invader in Portugal, and from the autochthonous alder (*Alnus glutinosa*), a common riparian tree which, like *A. longofilia*, is also a nitrogen-fixer, in a small peri-urban stream of Central Portugal. Under regular current velocity, decomposition rates (-*k*) of alder ranged 0.0351 to 0.0492 day^{-1} while decomposition rates of acacia were low (0.0083-0.0084 day^{-1}). Under strong current velocity which promoted fragmentation, decomposition rates reached 0.0530 day^{-1} for alder and 0.0194 day^{-1} for acacia. Macroinvertebrate colonization, dominated by collector-gatherers, was similar in the species and had no effect on mass loss of acacia. Acacia leaves were recalcitrant; the low mass loss under regular current velocity conditions was due to leaching and to slow microbial decomposition. These results suggest that under a scenario of invasion of the riparian area by *Acacia longifolia*, leaf litter will no longer be the major source of carbon, nutrients, and energy for the stream food webs, thus endangering ecosystem functioning.

KEYWORDS: Acacia. Alder. Ecosystem functioning. Leaf litter. Macroinvertebrates.

DECOMPOSIÇÃO DA INVASORA *ACACIA LONGIFOLIA* NUM RIBEIRO PERI-URBANO

RESUMO: As invasões biológicas constituem uma forte ameaça para a biodiversidade e para o funcionamento dos ecossistemas. A invasão por espécies de acácia resulta em densos grupos monospecíficos, eventualmente excluindo outras plantas. As zonas ripárias providenciam aos ribeiros matéria orgânica sob a forma de folhas mortas, a principal fonte de carbono, nutrientes e energia desses ecossistemas. A invasão das zonas ripárias por espécies de acácia é uma realidade a nível global, mas poucos estudos avaliaram o efeito da invasão no funcionamento do ecossistema dos ribeiros. Este estudo comparou a decomposição de folhas de *Acacia longifolia*, uma invasora proeminente em Portugal e de amieiro (*Alnus glutinosa*), uma árvore ripária comum que, tal como a acácia, é também fixadora de azoto, num pequeno ribeiro periurbano do centro de Portugal. Sob condições de corrente regular, as taxas de decomposição (-k) do amieiro variaram de 0.0351 a 0.0492 dia^{-1} enquanto as taxas de decomposição da acácia foram baixas (0.0083-0.0084 dia^{-1}). Sob condições de forte corrente que promoveu fragmentação, as taxas de decomposição do amieiro atingiram 0.0530 dia^{-1} e da acácia 0.0194 dia^{-1}. A colonização por macroinvertebrados, maioritariamente coletores, foi semelhante nas duas espécies e não teve efeito na perda de massa da acácia. As folhas de acácia demonstraram ser recalcitrantes; a baixa perda de massa deveu-se a lixiviação e a lenta decomposição microbiana. Estes resultados sugerem que num cenário de invasão da zona ripária por *Acacia longifolia*, os detritos foliares já não serão a principal fonte de carbono, nutrientes e energia para as cadeias alimentares aquáticas, ameaçando assim o funcionamento do ecossistema.

PALAVRAS-CHAVE: Acácia. Amieiro. Detritos foliares. Funcionamento do ecossistema. Macroinvertebrados.

1 INTRODUCTION

Invasive alien plants can alter ecosystems, reduce biodiversity, and modify ecosystem functions resulting in negative impacts at the social, and economic levels. Among these species, the genus *Acacia* contains the most prominent invaders worldwide but particularly in Mediterranean areas, spreading in all types of ecosystems, from dunes to riparian areas, and threatening ecosystem functions (Souza-Alonso *et al.*, 2017). Acacias are nitrogen-fixing trees which outcompete autochthonous vegetation (Ferreira et al., 2021). Under disturbed conditions, such as forest fires (Lorenzo et al., 2010), the germination of their immense seed bank contributes for the increase in the number of individuals, eventually excluding other plants and creating dense monospecific stands (Marchante et al., 2003, 2015). Riparian ecosystems are particularly vulnerable to invasion by exotic plant species and many riparian ecosystems in Portugal are already heavily invaded, mainly by *Acacia dealbata* but also by *Acacia longifolia* resulting in the decrease of tree species diversity and in increased concentration of nitrogen in water (Pereira & Ferreira, 2022).

Dead leaves from the riparian vegetation are the basis for the aquatic food webs in many streams, which rely on the decomposition of this allochthonous organic matter as a source of energy (Abelho, 2001). The leaves fall into water and are decomposed through the activity of microbial decomposers – mostly aquatic fungi – and by the feeding activity of detritivore-invertebrates. In temperate zones, this leaf litter is typically composed of a mixture of deciduous species and occurs in autumn (Abelho & Graça, 1998) and aquatic invertebrates synchronize their life cycles with this input of their primary food source (Abelho, 2001).

The replacement of the autochthonous riparian vegetation by exotic species – especially in monoculture - may alter the amount, type, quality, and seasonality of litter inputs, affecting the availability of food for the macroinvertebrate communities and the stream processes (Abelho & Graca, 1996; Pozo et al., 1997; Molinero & Pozo, 2004). The seasonality of litterfall of *Acacia* species is currently unknown in Portugal. Although most acacias are evergreen trees, several studies reported seasonal litterfall of acacia species in the tropics (Carnevale & Lewis, 2001; Barliza & Peláez, 2010; Sugimoto et al., 2013). In Mediterranean zones, Railoun et al. (2021) reported seasonal litterfall for *Acacia mearnsii* in South Africa, with a peak in mid-summer. Thus, the different chemistry of the leaf litter of these invasive species and the different seasonality of the inputs may significantly affect the aquatic food webs and the ability of streams to process leaf litter.

This work compared the decomposition of leaf litter from the invasive *Acacia longifolia* and the autochthonous *Alnus glutinosa*, also a nitrogen-fixing tree, in a peri-urban stream affected by urbanization-related problems. The main objectives were 1) to determine decomposition rates of *Acacia longifolia*, and 2) to assess how the eventual replacement of autochthonous leaf litter (represented by alder) by acacia would affect the ecological functions, already compromised (Isidoro, 2014), of the stream.

2 METHODOLOGY

2.1 STUDY SITE

The experiment was carried out in Ribeira dos Covões (Coimbra, Portugal: (40°13° N, 8°27° W), a 4th order peri-urban stream with a length of 5 km and a drainage area of 6.2 km^2. The stream is affected by several urban syndrome problems, including hydrological (Ferreira et al., 2016), due to channelization, altered geomorphology of the channel, removal and/or replacement of the natural riparian vegetation, among others, which result in impoverished macroinvertebrate communities and a deficient ecological condition (Isidoro, 2014).

The first experiment (Serra & Abelho, 2018) was carried out in the autumn of 2013 at two sites separated by approximately 800 m which differed in current velocity. The second experiment (Couceiro & Abelho, 2015) was carried out in the spring 2014 in the first site of 2013. On each sampling occasion, measurements of temperature and conductivity were taken before sampling. After sampling, mean width (at five transepts), mean depth (at each margin and the center of each transept), and surface velocity were measured to determine discharge (Table 1).

In the 2013 experiment, the experiment coincided with a period of heavy rain, which increased current velocity and discharge during the whole experiment (Table 1) and resulted in the loss of some experimental bags. On day 28 in site 2, current velocity and discharge reached 1.15 m s^{-1} and 0.62 m^3 s^{-1} leading to the loss of all remaining bags.

Table 1. Physic-chemical parameters of the study stream during the two study periods (Mean ± SE).

Experiment	**Temperature (ºC)**	**Conductivity (µS cm^{-1})**	**Depth (cm)**	**Velocity (m s^{-1})**	**Discharge (m^3 s^{-1})**
2013 Site 1	14.40 ± 0.63	331.25 ± 9.68	13.60 ± 3.07	0.48 ± 0.06	0.297 ± 0.055
2014 Site 2	14.38 ± 0.72	348.75 ± 9.00	29.40 ± 2.17	0.68 ± 0.16	0.343 ± 0.093
2014	13.54 ± 1.08	220.34 ± 29.90	16.82 ± 0.95	0.40 ± 0.02	0.127 ± 0.013

2.2 EXPERIMENTAL PROCEDURES

Leaves from acacia (*Acacia longifolia* (Andrews) Willd.) were picked green while leaves of alder (*Alnus glutinosa* (L.) Gaertn.) were picked senescent from the trees in Autumn. The leaves were air-dried in the laboratory and weighed in groups (2.005 g ± 0.008 SE in the experiment of 2013 and 2.486 g ± 0.001 SE in the experiment of 2014) to form replicate samples of each species. On the day of starting the experiment, the leaves were sprayed with distilled water to soften and avoid damage and enclosed in mesh bags. The bags were tied in groups of two (alder-acacia; experiment 2013) or four (acacia fine mesh - acacia coarse mesh - alder fine mesh – alder coarse mesh; experiment 2014). Three additional samples of each species, correspondent to day 0, were oven-dried (60ºC, 72 hours) and weighed to determine humidity. A humidity coefficient for each species (oven-dry mass/air-dry mass) was multiplied by the air-dry mass of each sample to obtain initial oven-dry mass of the experimental samples.

In the experiment of 2013, decomposition was assessed in coarse (5 mm) mesh bags in two sites, with 12 bags of each species per site, and sampling was carried out after 7, 14, 28, and 42 days of immersion in the stream. In the experiment of 2014, decomposition was assessed in fine (0.5 mm) and coarse (5 mm) mesh bags at one site, with a total of 21 bags of each species and mesh size, and sampling was carried out after 2, 9, 16, 30, 48, 58, and 72 days of immersion in the stream.

On day 0 the ropes connecting the bags were tied to structures at the stream margins so that the bags were deployed transversally at the streambed in three transepts.

Three groups, one from each transept, were randomly collected on each sampling day and were transported isolated to the laboratory in an icebox.

In the laboratory, the bags were opened, and each leaf was gently washed with tap water over a 0.5 mm screen to detach debris and macroinvertebrates. The leaves were collected, and oven dried as above to obtain the percentage dry mass remaining (final oven-dry mass/initial oven-dry mass*100).

The macroinvertebrates retained by the screen were picked with forceps and stored in 75% alcohol until processing. Identification was carried out to the lowest possible level under a binocular microscope using the key from Tachet et al. (2010). In the experiment of 2014, the individuals in each sample were allocated to pre-weighed small aluminum tins and oven-dried (60°C, 72 hours) to determine dry biomass. The macroinvertebrates were classified into functional feeding groups (shredders, collector-gatherers, collector-filterers, scrapers, or predators) according to Merritt & Cummins (1996) and the community was characterized in terms of abundance, biomass, richness, diversity (H', Shannon-Wiener diversity index), and proportion of functional feeding groups.

2.3 STATISTICAL ANALYSIS

Percentage dry mass remaining on each sampling day was used to calculate the exponential decay rates (-*k*) of each species in each mesh size: $M_t=M_i*e^{-k}$ where M_t is dry mass remaining at a given time, M_i is initial dry mass, and *e* is Euler's number or the basis of the natural logarithm.

The slopes of the natural logarithm of dry mass remaining along time were compared with two-factor analysis of covariance (ANCOVA; ZAR, 2010) to test for the effect of species and site (2013 experiment) and for the effect of species and mesh (2014 experiment) on mass loss.

Coarse mesh bags access decomposition due to the activity of microbial decomposers and macroinvertebrates while fine-mesh bags exclude access of macroinvertebrates to the leaves and thus assess decomposition due to microbial activity. Thus, the difference between coarse and fine mesh bags is due to fragmentation, either by the feeding activity of macroinvertebrates or physical abrasion by the stream current.

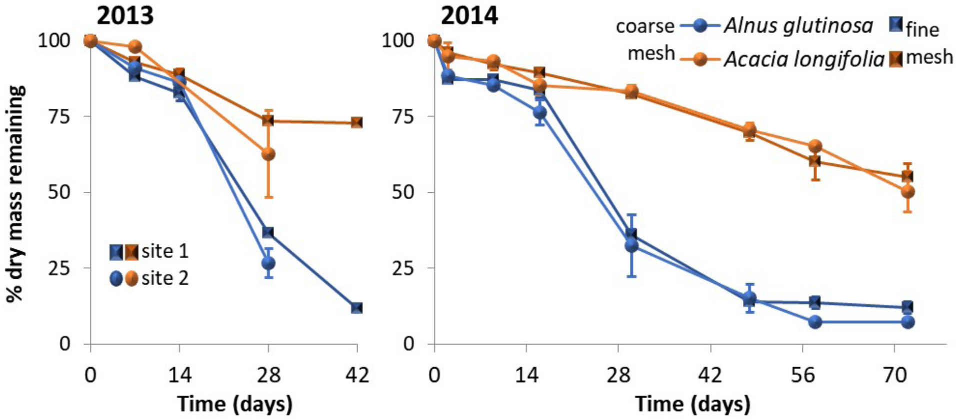
Analysis of variance (ANOVA; ZAR, 2010) was used to test for the effect of species and site on macroinvertebrate abundance and richness in the 2013 experiment and for the effect of species on macroinvertebrate abundance, richness, and biomass in the 2014 experiment. After testing for parametric assumptions, data from the 2013 experiment was heteroscedastic (after Levine's test) and was transformed with the square root transformation, $x' = \sqrt{x + 3/8}$ (Zar, 2010).

3 RESULTS

3.1 MASS LOSS AND DECOMPOSITION RATES

In the two experiments, alder lost more mass than acacia, both at the two sites and in the fine and coarse mesh bags (Figure 1), and the difference between the two species started already during the leaching period. In the 2013 experiment, after 7 days, alder had lost 10.5 ± 0.8% while acacia lost only 5.9 ± 1.7% of the initial mass. By day 21 alder had already lost 50% of initial mass at both sites while acacia reached the end of the experiment with 63 and 77% of initial mass remaining, respectively in site 1 and in site 2 (Figure 1, left). In the 2014 experiment, on day 2 alder had lost 12.0 ± 0.5% while acacia lost only 4.7 ± 2.5% of initial mass. By day 28, alder had already lost more than 50% of initial mass both in fine and coarse mesh bags while by day 72, acacia still had 50% and 55% of dry mass remaining in coarse and fine mesh bags, respectively (Figure 1, right).

Figure 1. Dry mass remaining (mean ± SE) of alder and acacia leaf litter in the two sites of the 2013 experiment (left) and in the fine and coarse mesh bags of the 2014 experiment (right).



Accordingly, decomposition rates were significantly faster for alder than for acacia (Table 2). In the 2013 experiment, breakdown rates of alder were faster than breakdown rates of acacia in site 1 (Table 2). There was no significant effect of site and no interaction between the factors ($F_{1,39}$ = 0.41, p = 0.52). In the 2014 experiment, there was no significant difference in the decomposition rate of fine and coarse mesh bags of each species and no interaction between the factors ($F_{1,87}$ = 0.02, p = 0.94). There was no effect of fragmentation on mass loss of acacia, i.e., decomposition rates were equal in coarse and fine mesh bags and the effect on mass loss of alder was low ($-k$ = 0.0064 day^{-1}).

Table 2 Decomposition rates (-k day^{-1}) and 95% confidence limits (CL) of alder and acacia leaf litter in the two sites of the 2013 experiment and in the fine and coarse mesh bags of the 2014 experiment. Decomposition rates with the same superscript letter are not significantly different after Tukey test. ns: nonsignificant regression.

					ANOVA	
	Species	Site/ mesh	N	-k day^{-1} ± 95% CL	Species effect	Site/mesh effect
2013	Alder	Site 1	13	0.0530 ± 0.0111 ^{a}	$F_{1,39} = 7.70$ $p < 0.05$	$F_{1,39} = 0.23$ $p = 0.63$
		Site 2	12	0.0492 ± 0.0171 ^{a}		
	Acacia	Site 1	11	0.0083 ± 0.0019 ^{b}		
		Site 2	7	ns 0.0195 ± 0.0217 ^{a}		
2014	Alder	Fine	22	0.0351 ± 0.0044 ^{a}	$F_{1,87} = 17.31$ $p < 0.001$	$F_{1,87} = 0.01$ $p = 0.91$
		Coarse	22	0.0415 ± 0.0059 ^{a}		
	Acacia	Fine	23	0.0084 ± 0.0014 ^{b}		
		Coarse	24	0.0084 ± 0.0017 ^{b}		

3.2 MACROINVERTEBRATE COLONIZATION

Macroinvertebrate colonization was similar in alder and acacia leaf litter (Table 3). In the experiment of 2013, there were oscillations in colonization due to the floods; peak colonization at site 1 was achieved on day 42 for both species while on site 2 colonization of alder increased until the end of the experiment and colonization of acacia peaked on day 7 decreasing afterwards (Figure 2, left). There was a significantly higher colonization of both species in site 1 than in site 2 (Table 3; Figure 2, left). In the experiment of 2014, peak colonization of both species occurred on day 9 for biomass and on day 16 for abundance (Figure 2, right) decreasing steeply afterwards. Richness and diversity per sampling day were also similar in the two leaf species (Figure 3, left). The macroinvertebrate community was dominated by collector-gatherers in both species, although the proportions changed between sites and between the two experiments (Figure 3, right).

Table 3 Results of the ANOVA comparing the effect of species and site (2013 experiment) or species (2014 experiment) on macroinvertebrate colonization of leaf litter of alder and acacia in the coarse mesh bags.

	Effect	**Abundance**	**Richness**	**Biomass**
2013	Species	$F_{1,37} = 0.06$, $p = 0.82$	$F_{1,37} = 0.01$, $p = 0.92$	---
	Site	$F_{1,37} = 4.76$, $p < 0.05$	$F_{1,37} = 7.05$, $p < 0.05$	---
	Interaction	$F_{1,37} = 1.09$, $p = 0.30$	$F_{1,37} = 0.22$, $p = 0.64$	---
2014	Species	$F_{1,37} = 0.62$, $p = 0.44$	$F_{1,37} = 0.26$, $p = 0.61$	$F_{1,37} = 0.82$, $p = 0.37$

Figure 2. Abundance and biomass (mean ± SE) of the macroinvertebrates colonizing alder and acacia leaf litter in the coarse mesh bags of the two sites in the 2013 experiment (left) and in the 2014 experiment (right).

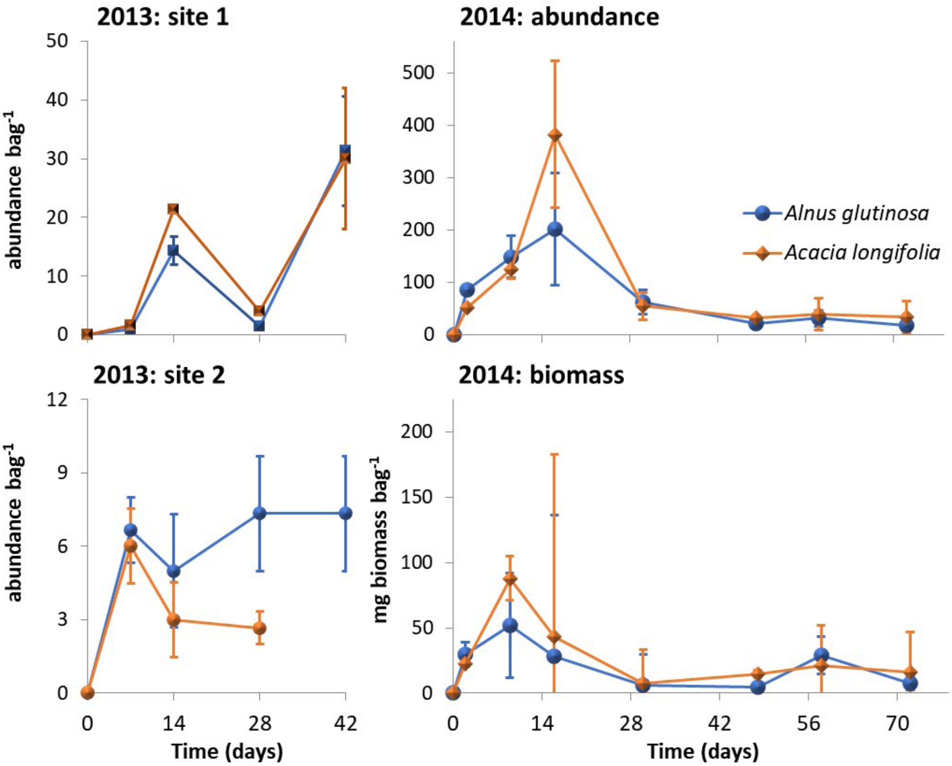
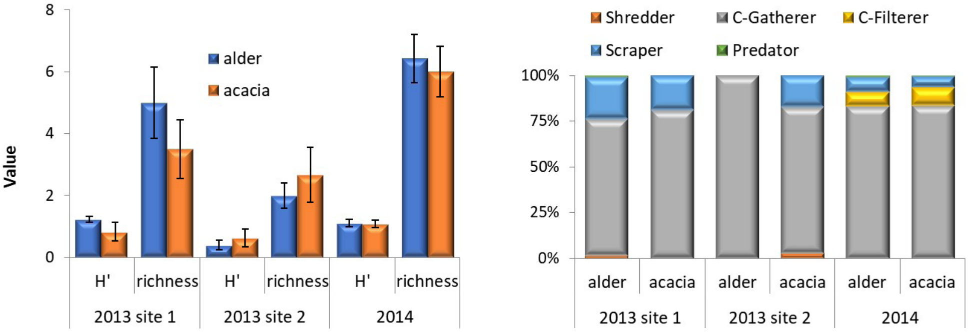


Figure 3. Taxa richness, Shannon-Wiener diversity index (mean ± SE; left) and proportion of functional feeding groups (C-G=collector-gatherer, C-F=collector-filterer; right) of the macroinvertebrates colonizing alder and acacia leaf litter in the coarse mesh bags of the two sites in the 2013 experiment and in the 2014 experiment.



4 DISCUSSION

Although the ecological status of the stream is compromised (Isidoro, 2014), the aquatic communities were able to process leaf litter from the autochthonous species. Alder leaf litter is rich in nitrogen, soft, and fast-decomposing due to both microbial activity and feeding preference by leaf-shredding invertebrates (Abelho, 2001). Under regular current velocity conditions, most mass loss of alder was due to microbial-driven decomposition, as shown by the small difference between coarse and fine mesh bags. Other works have shown higher aquatic hyphomycete sporulation rates and species richness in alder when compared to acacia species, resulting in faster decomposition rates (Pereira et al., 2021; Pereira & Ferreira, 2021). In this stream the macroinvertebrate community was dominated by collector-gatherers, among which the family Chironomidae constituted 43-86% of all invertebrates colonizing alder leaves. Although chironomids are not typical shredders, they can use leaf litter of riparian vegetation - especially from alder - as a food source and many chironomids in streams may be able to feed on fast decomposing leaves (Callisto et al., 2007). Thus, the feeding activity of non-typical shredders was probably the cause for the additional mass loss in coarse mesh bags when compared to the fine mesh bags under regular current velocity.

On the other hand, acacia leaf litter is also rich in nitrogen, but the leaves are tough. Although chironomids constituted 43-74% of all macroinvertebrates colonizing acacia leaves there was no mass loss due to leaf-shredding activity, probably due to leaf cuticle and the tough leaves (Callisto et al., 2007). Thus, mass loss of acacia was totally driven by microbial decomposition (Ferreira et al., 2021), but the effect of microbial activity was low probably due to low aquatic hyphomycete sporulation rates and species richness, as shown for other species of acacia (Pereira et al., 2021; Pereira & Ferreira, 2021). The acacia leaves were recalcitrant to decomposition and the highest mass loss occurred due to fragmentation under strong current velocity and high discharge.

In conclusion, in a future scenario of invasion of the riparian area by *Acacia longifolia*, leaf litter may no longer be the major source of carbon, nutrients, and energy for the stream food web, thus endangering the functioning of the ecosystem.

5 ACKNOWLEDGEMENTS

The author thanks two former students, Filipe Serra and Denise Couceiro, for the field and laboratory work.

APPENDIX

List of taxa, functional feeding groups (FFG: C-G: collector-gatherer, C-F: collector-filterer, P: predator, Scr: scraper, Shr: shredder), and abundance of the macroinvertebrates colonizing leaf litter of alder and acacia in the coarse mesh bags in the experiments of 2013 and 2014. Contributions ≥10% of the total are highlighted in bold.

2013 experiment			**Site 1**		**Site 2**	
FFG	**Taxa**		**alder**	**acacia**	**alder**	**acacia**
C-G	Oligochaeta	Lumbricidae	0	0	4	0
C-G		Lumbriculidae	**(13%) 18**	**(16%) 21**	6	2
C-G		Naididae	5	4	0	0
C-G		Tubificidae	1	0	0	0
Scr	Gastropoda	Hydrobiidae	**(21%) 29**	**(18%) 24**	0	6
Scr	Ephemeroptera	Leptophlebiidae	1	0	0	0
Scr	Coleoptera	Dryopidae	1	0	0	0
P		Laccophilinae	1	0	0	0
C-G	Diptera	Chironomidae	**(57%) 77**	**(62%) 81**	**(86%) 68**	**(74%) 26**
C-G		Stratiomyidae	0	0	1	0
Shr		Tipulidae	2	0	0	1
Total abundance			**135**	**130**	**79**	**35**

2014 experiment				
FFG	**Taxa**		**alder**	**acacia**
C-G	Oligochaeta	Lumbricidae	2	2
C-G		Lumbriculidae	25	1
C-G		Naididae	**(27%) 464**	**(33%) 712**
C-G		Tubificidae	**(11%) 195**	170
Scr	Gastropoda	Hydrobiidae	50	61
Scr		Lymnaea	1	0
Scr		Physidae	0	1
P	Arachnida	Hydracarina	3	4
Scr	Ephemeroptera	Baetidae	90	69
Shr	Plecoptera	Nemoura	1	0
P	Trichoptera	Hydroptilidae	0	2
C-F		Hydropsychidae	0	1
P	Diptera	Ceratopogonidae	8	1
P		Chaoboridae	0	1
C-G		Chironomidae	**(43%) 739**	**(43%) 928**
C-G		Dixidae	2	1
P		Limoniidae	1	0
C-G		Psychodidae	0	1
C-F		Simulidae	131	**(10%) 206**
Total abundance			**1712**	**2161**

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SOBRE O ORGANIZADOR

Manuel Simões é licenciado em Engenharia Biológica e doutorado em Engenharia Química e Biológica. Atualmente é Professor Associado com Agregação e Pró-Diretor da Faculdade de Engenharia da Universidade do Porto (FEUP), e investigador sénior do Laboratório de Engenharia de Processos, Ambiente, Biotecnologia e Energia (LEPABE) do Departamento de Engenharia Química da FEUP. Nos últimos anos esteve envolvido em 10 projetos nacionais (5 como investigador principal) e 6 projetos europeus. Foi membro do comité de gestão da ação COST BACFOODNET (Rede Europeia para Mitigação da Colonização e Persistência Bacteriana em Alimentos e Ambientes de Processamento de Alimentos) e esteve envolvido em outras 2 ações: iPROMEDAI e MUTALIG. Manuel Simões tem mais de 190 artigos publicados em revistas indexadas no Journal of Citation Reports, 4 livros (1 como autor e 3 como editor) e mais de 40 capítulos em livros. Ele é Editor Associado para o jornal Biofouling - The Journal of Bioadhesion and Biofilm Research (o periódico mais antigo sobre pesquisa em biofilme), Editor Associado para o jornal Frontiers in Microbiology e Section Editor-in-Chief para o jornal Antibiotics. Seus principais interesses de pesquisa estão focados nos mecanismos de formação de biofilme e seu controlo com agentes antimicrobianos, particularmente usando novas moléculas antimicrobianas, e no uso de microalgas para tratamento de efluentes. É um dos investigadores mais citados do mundo (top 1%), tendo sido distinguido nos últimos dois anos no índice Essential Science Indicators, um dos mais prestigiados indicadores da qualidade de investigação.

Identificação SCOPUS: 55608338000; Nº orcid: 0000-0002-3355-4398

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