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Colonization and decomposition of litter produced by invasive *Acacia dealbata* and native tree species by stream microbial decomposers

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ABSTRACT

Colonization and decomposition of litter produced by invasive *Acacia dealbata* and native tree species by stream microbial decomposers

Changes in forest composition and litter inputs to streams due to invasion by exotic tree species can affect the functioning of freshwater ecosystems. *Acacia dealbata* is an important invasive tree species in Mediterranean areas, and often replaces the native riparian vegetation. In this study, we assessed the chemical characteristics of three litter types produced by the invasive *Ac. dealbata* (leaflets, flowers and pods) and leaf litter produced by two native tree species with contrasting litter characteristics (*Quercus robur* and *Alnus glutinosa*). We then assessed litter decomposition and associated microbial activity (i.e., overall microbial metabolism as respiration, fungal growth as biomass accumulation, and reproduction by aquatic hyphomycetes as conidial production), and the aquatic hyphomycetes community structure, in laboratory microcosms. In general, *Ac. dealbata* pods supported lower microbial activity and decomposed slower than all other litter types, due to their low nutrient concentrations and high carbon:nutrients molar ratio. *Alnus glutinosa* leaf litter supported high microbial activity and decomposed fast, due to its relatively high nutrient concentrations, low carbon:nutrients molar ratios and low lignin concentration. *Acacia dealbata* leaflets and flowers and *Q. robur* leaf litter generally had similar microbial activity and decomposition rates, intermediate between those of *Ac. dealbata* pods and *Al. glutinosa* leaf litter, likely due to trade-offs between nutrient concentrations and concentrations of structural and secondary compound among litter types. Aquatic hyphomycetes community structure also differed among litter types. For instance, *Ac. dealbata* pods had the lowest species richness per sampling date, but due to high dissimilarity among replicates, total species richness over the incubation period was comparable to that of other litter types. The invasion of native riparian forests by *Ac. dealbata* can affect the quality of litter inputs into streams, potentially affecting the community structure and activity of microbial decomposers, thus altering the functioning of stream ecosystems.

Key words: aquatic hyphomycetes, fruit, pod, flower, leaf

RESUMO

Colonização e decomposição de matéria orgânica produzida pela invasora Acacia dealbata *e espécies arbóreas nativas por decompositores aquáticos*

Mudanças na composição da floresta e na entrada de matéria orgânica nos riachos devido à invasão por espécies arbóreas exóticas podem afetar o funcionamento dos ecossistemas de água doce. Acacia dealbata *é uma importante espécie invasora nas áreas mediterrâneas e frequentemente substitui a vegetação ribeirinha nativa. Neste estudo, avaliámos as características químicas de três tipos de matéria orgânica produzida por* Ac. dealbata *(folíolos, flores e vagens) e folhada produzida por duas espécies arbóreas nativas com características contrastantes (*Quercus robur *e* Alnus glutinosa*). Em seguida, avaliámos a decomposição da matéria orgânica e a atividade microbiana associada (i.e., metabolismo microbiano como respiração, crescimento de fungos como acumulação de biomassa e reprodução de hifomicetes aquáticos como produção de conídios), e a estrutura da comunidade de hifomicetes aquáticos, em microcosmos de laboratório. Em geral, as vagens de* Ac. dealbata *suportaram menor atividade microbiana e decompuseram-se mais lentamente do que todos os outros tipos de matéria orgânica,*

devido às suas baixas concentrações de nutrientes e elevado rácio carbono:nutrientes. A folhada de Al. glutinosa *apresentou alta atividade microbiana e decompôs-se rapidamente, devido às concentrações relativamente altas de nutrientes, ao baixo rácio de carbono:nutrientes e à baixa concentração de lignina. Os folíolos e flores de* Ac. dealbata *e a folhada de* Q. robur *geralmente apresentaram atividade microbiana e taxas de decomposição semelhantes, intermédias entre as apresentadas pelas vagens de* Ac. dealbata *e pela folhada de* Al. glutinosa, *provavelmente devido a compromissos entre as concentrações de nutrientes e as concentrações de compostos estruturais e secundários entre os tipos de matéria orgânica. A estrutura das comunidades de hifomicetes aquáticos também diferiu entre os tipos de matéria orgânica. Por exemplo, as vagens de* Ac. dealbata *suportaram uma baixa riqueza específica por data de amostragem, mas devido à elevada dissimilaridade entre réplicas a riqueza específica total ao longo do período de incubação foi semelhante à dos outros tipos de matéria orgânica. A invasão de vegetação ribeirinha nativa por* Ac. dealbata *pode afetar a qualidade da matéria orgânica que entra nos riachos, potencialmente afetando a estrutura das comunidades e a atividade dos decompositores microbianos, alterando assim o funcionamento dos ecossistemas ribeirinhos.*

Palavras chave: *hifomicetes aquáticos, fruto, vagem, flor, folha*

INTRODUCTION

Invasion by exotic species is a major threat to biodiversity and ecosystem functioning worldwide (Butchart et al., 2010; Vilà et al., 2011; Gallardo et al., 2016). Invasions can affect multiple dimensions of the invaded ecosystem but also those of adjacent ecosystems (Kominoski et al., 2013; Tolkkinen et al., 2020; Ferreira et al., 2021). For instance, temperate forest streams are particularly sensitive to the invasion of riparian forests by exotic plant species (Hladyz et al., 2011; Mineau et al., 2011; Stewart et al., 2018), because they are essentially heterotrophic systems and thus strongly dependent on organic matter (OM) inputs from the terrestrial vegetation (Wallace et al., 1997; Tolkkinen et al., 2020).

Australian *Acacia* species (Leguminosae: Mimosoideae) are widely distributed across the world, with many species becoming invasive, especially in Mediterranean areas (Souza-Alonso et al., 2017; Ferreira et al., 2021). In Portugal, *Acacia* spp. are serious invaders, occupying more than 17 000 ha (ICNF, 2019; Presidência do Concelho de Ministros, 2019). *Acacia* spp. are nitrogen (N)-fixers (i.e., establish symbiotic relationships with bacteria of the genus *Rizhobia* that fix atmospheric N), generally have fast growth rates and fast maturation, produce large amounts of seeds that are resistant and long lived, and produce allelopathic compounds (Lorenzo et al., 2010; Correia et al., 2016; Souza-Alonso et al., 2017; Ferreira

et al., 2021). These eco-biological characteristics make *Acacia* spp. successful pioneer colonizers of disturbed areas, such as those affected by fires or spates. In these conditions, i.e., when the native vegetation has been eliminated, *Acacia* spp. can quickly colonize large areas and establish (almost) monospecific stands (Lorenzo et al., 2010; Gibson et al., 2011; Aguilera et al., 2015; Souza-Alonso et al., 2017). In central Portugal, *Acacia dealbata* Link. (silver wattle) is often present in the riparian vegetation, in many cases making very dense, monospecific stands (Ferreira et al., 2021). These stands with low species richness, dominated by an evergreen, fast-growing, N-fixing species markedly differ from the native forest, which is composed by a diverse mixture of deciduous species dominated by *Quercus* spp. (oaks) and *Castanea sativa* Mill. (chestnut), with *Alnus glutinosa* (L.) Gaertn. (alder), *Salix* spp. (willow) and *Populus* spp. (poplar) as typical riparian species (Ferreira et al., 2021).

The invasion of the riparian vegetation by *Ac. dealbata* can alter the characteristics of OM inputs to streams given the production of litter with potentially higher concentrations of N and of allelopathic compounds than those of dominant native species as *Quercus* spp. and *C. sativa* that generally have low N concentration; native *Al. glutinosa*, however, is a N-fixer that produces soft, nutrient-rich leaf litter (Lorenzo et al., 2010; Ferreira et al., 2021). *Acacia dealbata* stands can also produce vegetative forms distinct from those

of native species (i.e., very small leaflets compared to the entire leaves of most common native species), and large amounts of reproductive litter (i.e., flowers, pods) (Lorenzo et al., 2010; Ferreira et al., 2021). Changes in the characteristics of the OM inputs to streams can affect aquatic communities and processes (Ferreira et al., 2021).

In streams, benthic microbial communities are prime decomposers of plant OM by directly promoting its softening, incorporating OM carbon into microbial biomass and mineralizing OM carbon (Gulis & Suberkropp, 2003; Baldy et al., 2007). Fungi, mainly aquatic hyphomycetes, are the main microbial decomposers in streams, representing 60–99 % of the total microbial biomass in litter (Baldy et al., 1995; Gulis & Suberkropp, 2003; Pascoal & Cássio, 2004). The activity of microbial decomposers on litter depends on its characteristics, with soft litter, rich in nutrients and poor in structural (e.g., lignin) and secondary compounds (e.g., polyphenols) being colonized and decomposed faster than more recalcitrant litter (Gessner & Chauvet, 1994; Ferreira et al., 2012; Jabiol et al., 2019). The community structure of microbial decomposers also generally differs among litter typologies (e.g., leaves, bark, and wood) and species (Canhoto & Graça, 1996; Gulis, 2001; Ferreira et al., 2006a, 2012; Gonçalves et al., 2007; Ferreira & Graça, 2016; Pereira & Ferreira, 2021) as a result from decomposer species having distinct nutrient requirements and enzymatic capabilities (Zemek et al., 1985; Abdel-Raheem & Ali, 2004; Brosed et al., 2017).

In this study, we compared three types of OM produced by the invasive *Ac. dealbata* (leaflet, flower and pod litter) and leaf litter of two contrasting native tree species (*Quercus robur* L. and *Al. glutinosa*) in terms of litter chemical characteristics, litter decomposition and associated microbial activities and community structure in laboratory microcosms. We hypothesized that (i) the decomposition rates and microbial activities would reflect the litter characteristics and would be higher for litter that is soft and nutrient-rich than for tough and nutrient-poor litter; and (ii) that the structure of the aquatic hyphomycetes community would differ among litter types in response to their chemical characteristics. Although microbial colonization and decomposition of *Ac.*

dealbata leaflets were addressed recently (e.g., Pereira & Ferreira, 2021), this information is still missing for other litter types produced by this invasive species. This study contributes to understand the use of OM derived from *Ac. dealbata* by microbial decomposers in streams flowing through invaded forests.

METHODS

Organic matter

Leaf, flower and pod litter of the exotic invasive species *Ac. dealbata*, and leaf litter of two native species of contrasting characteristics, *Q. robur* and *Al. glutinosa*, were used in this study (Fig. 1). *Acacia dealbata* is an evergreen, fast-growing, N-fixing tree. *Alnus glutinosa* and *Q. robur* are common deciduous European tree species (Graça & Poquet, 2014). *Alnus glutinosa* is a N-fixing species that dominates mature riparian forests and produces soft and N-rich leaf litter, while *Q. robur* dominates in deciduous forests, is often present in the riparian vegetation, and produces litter that is tough and nutrient-poor (Graça & Poquet, 2014).

Leaves and flowers of *Ac. dealbata* were collected by cutting branches from trees located in the right bank of the Mondego River at the city of Coimbra, central Portugal (Pólo II, University of Coimbra; 40° 10' 56.8" N, 8° 24' 37.3" W), in February 2019. Pods of *Ac. dealbata* were collected from the floor under the same trees in August 2019. Leaf litter of *Q. robur* was collected in autumn 2016 and leaf litter of *Al. glutinosa* was collected in autumn 2019, both on the right bank of the Mondego River at the city of Coimbra (Mondego Green Park; 40° 12' 03.0" N, 8° 25' 31.1" W). Leaf litters of native species were collected senescent after natural abscission, showing no signs of damage. All litters were taken to the laboratory, allowed to dry at room temperature in the dark, and stored in paper boxes until used.

Samples of each litter type (n = 3) were ground to pass through a 0.5-mm sieve (Retsch ZM 100 Ultra Centrifugal Mill, Haan, Germany) and the powder was oven-dried (60 °C, 48 h) and analyzed to determine the concentrations of lignin (Goering & Van Soest, 1970), total polyphenols and phosphorus (P) (Bärlocher et al., 2020), car-
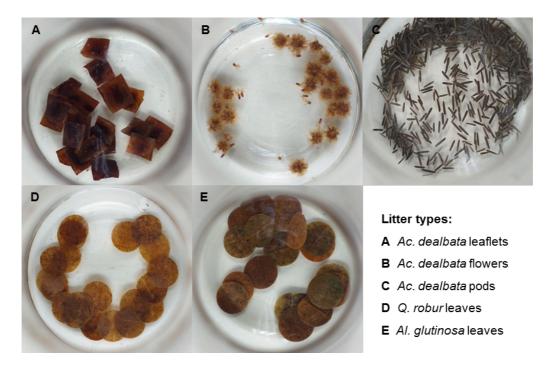
Figure 1. Litter used in the microcosms experiment: pods (A), flowers (B) and leaflets (C) of the invasive species *Acacia dealbata*, and leaf discs of the native species *Quercus robur* (D) and *Alnus glutinosa* (E). *Matéria orgânica utilizada na experiência de microcosmos: vagens (A), flores (B) e folíolos (C) da espécie invasora* Acacia dealbata*, e discos de folhas das espécies nativas* Quercus robur *(D) e* Alnus glutinosa *(E).*

bon (C) and nitrogen (N) (IRMS Thermo Delta V advantage with a Flash EA-1112 series; Thermo Fisher Scientific Inc., Waltham, MA, USA). The results were expressed as percentage of litter dry mass (% DM). Carbon:nutrients molar ratios were also calculated.

Air-dried *Ac. dealbata* leaflets and flowers were detached from the petiole and weighed in groups of 150.3 ± 0.6 mg and 140.0 ± 3.5 mg (mean ± SE; n = 9), respectively. Air-dried *Ac. dealbata* pods were cut into ~ 1-cm^2 squares and weighed in groups of 20 units (161.5 ± 1.3 mg, mean ± SE; n = 9). Leaf litter of *Q. robur* and *Al. glutinosa* were moistened with distilled water to render them soft and allow the extraction of leaf discs with a cork borer (12-mm diameter), avoiding the main vein in the former leaf litter and including a secondary vein in the center of the disc in the latter leaf litter. Discs were weighed in sets of 20 units (*Q. robur*: 88.5 ± 0.2 mg, *Al. glutinosa*: 172.5 ± 4.5 mg, mean ± SE; n = 9).

Experimental design

Samples of the five litter types (*Ac. dealbata* pod squares, flowers and leaflets, *Q. robur* leaf discs, and *Al. glutinosa* leaf discs; n = 9) were distributed in laboratory microcosms consisting of 100-mL Erlenmeyer flasks. The microcosms were kept in an orbital shaker (100–120 rotations/min; GFL 3017, Burgwedel, Germany) under a regime of 12 h light and 12 h dark, with an average temperature of 18 °C and 10 °C in each period, respectively, for the duration of the experiment.

Samples were allowed to be inoculated by microbial decomposers during one week. During this period, the microcosms were supplied with 40 mL of a microbial suspension, renewed daily to avoid the accumulation of leachates and to ensure fresh microbial inoculum (< 1 d old). The microbial suspension was produced in a tank that contained aerated stream water (15 L) and leaf litter from different plant species (dominated by

C. sativa) and at different states of decomposition (~ 0.550 L), which was kept in the same conditions as the microcosms. The water and leaf litter were collected on 11 November 2019 from a local stream (Cerdeira stream, Lousã Mountain, central Portugal; 40° 05' 23.1" N, 8° 12' 05.0" W, 529 m a.s.l.) that flows through a mixed deciduous forest with negligible presence of *Acacia* species and low human activity (Pereira et al., 2021). The stream is circumneutral and has low conductivity and nutrient concentrations (Gulis et al., 2006; Ferreira et al., 2016a; Pereira et al., 2021). At the sampling date, the stream water had 156.45 µg/L of dissolved inorganic N (colorimetric method, AA3 Bran + Luebbe autoanalyzer; SEAL Analytical, Norderstedt, Germany) and 16.19 µg/L of soluble reactive P (ascorbic acid method). After the microbial inoculation period (day 0), microcosms (5 litter types × 3 sampling dates × 3 replicates = 45 microcosms) were filled with 40 mL of filtered stream water, renewed twice a week. After 17, 38 and 73 days, three replicates of each litter were sacrificed to determine microbial respiration rates, fungal biomass, aquatic hyphomycetes conidial production, and litter mass remaining (see below).

Microbial respiration rates

In order to determine microbial metabolism, microbial respiration rates were estimated from subsamples of sacrificed microcosms: five leaf discs, five pod squares or a portion of approximately ¼ of the total mass of flower and leaflet samples. A closed six-channel dissolved oxygen (O_2) measuring system (Strathkelvin 929 System, North Lanarkshire, UK) connected to a computer was used (Bärlocher et al., 2020). The O_2 electrodes were calibrated at 13.5 °C with a solution of sodium sulfite in 0.01 M sodium borate (0 % O_2) and with O_2-saturated filtered stream water (100 % O_2). The subsamples were incubated for 1 h in chambers containing 3 mL of O_2-saturated filtered stream water. Additional chambers without subsamples were used as controls. After incubation, the subsamples were removed from the chambers and deposited in pre-weighed aluminum cups, oven-dried (60 °C, 48 h), and weighed (± 0.1 mg) to determine subsample DM. Microbial respiration rates were calculated as the difference

in O_2 concentration in the chambers with subsamples and in control chambers, at the beginning and at the end of a 20-min interval during which O_2 consumption was linear, corrected for the chamber volume, time and DM of the subsamples. Results were expressed in mg O_2 g^{-1} litter DM h^{-1}.

Fungal biomass

Another set of subsamples from sacrificed microcosm was used for determination of ergosterol concentration as a fungal biomass surrogate (Gessner & Chauvet, 1993). Subsamples (five leaf discs, five pod squares or a portion of approximately ¼ of the total mass of flower and leaflet samples) were frozen (– 20 °C), lyophilized overnight (Lablyo Mini, Frozen in Time Ltd, North Yorkshire, UK), and weighed (± 0.1 mg) do determine subsample DM. Ergosterol was extracted by incubating the subsamples in alkaline methanol (8 g KOH/L) for 30 min at 80 °C, purified by solid phase extraction (Waters Sep-Pak Vac RC tC18 cartridges; Waters Corp., Milford, USA), eluted with isopropanol, and quantified by high-performance liquid chromatography (HPLC) by measuring absorbance at 282 nm (Bärlocher et al., 2020). The HPLC equipment (Dionex DX-120, Sunnyvale, CA, USA) was supplied with a Thermo Scientific Syncronis C18 column (250 × 4 mm, 5-µm particle size; Thermo, Waltham, MA, USA), and HPLC-grade methanol was used as the mobile phase (1.4 mL/min, 33 °C). Ergosterol concentrations were converted into fungal biomass assuming 5.5 µg ergosterol/mg fungal DM (Gessner & Chauvet, 1993). Results were expressed as mg fungal biomass/g litter DM.

Aquatic hyphomycetes conidial production

The reproductive activity and species richness of aquatic hyphomycetes were determined from conidial production. At each sampling date, the conidial suspensions in the microcosms were poured into graduated flasks with addition of 2 mL of 37 % formalin, and completed up to 45 mL with distilled water. The flasks were stored in the dark until processed. When preparing filters for conidia identification and counting, 100 µL of Triton X-100 (0.5 %) were added to the suspen-

sion, gently mixed with a magnetic stirring bar, and aliquots of the suspension were slowly filtered through cellulose nitrate filters (25-mm diameter, 5-μm pore size; Sartorius Stedim Biotech GmbH, Goettingen, Germany). The filters were stained with Trypan Blue (0.05 %) in 60 % lactic acid and conidia were observed and counted with an optical microscope at 200× magnification (Bärlocher et al., 2020). Aquatic hyphomycetes sporulation rates were expressed as number of conidia mg^{-1} litter DM d^{-1} and species richness as the number of species/sample.

Litter decomposition

The remaining subsamples of each sacrificed microcosm (after removal of subsamples for determination of microbial respiration and fungal biomass) were placed in pre-weighed cups, oven-dried (60 °C, 48 h), and weighed (± 0.1 mg) to determine subsample DM. The DM values of all subsamples from each microcosm were added together to estimate total DM remaining. Results were expressed as percentage of initial litter DM: (DM remaining / initial DM) × 100. Initial DM of each sample on day 0 (i.e., after the microbial inoculation period) was obtained by multiplying the initial air-dry mass of each sample by a conversion factor between air-dry mass and oven-dry

mass after the microbial inoculation period (i.e., taking into account mass loss due to leaching) obtained from an extra set of three samples for each litter type that were prepared as the experimental samples, but oven-dried (60 °C, 48 h) and weighed (± 0.1 mg) after the microbial inoculation period.

Data analysis

Initial lignin, total polyphenols, P, C and N concentrations, and C:P and C:N molar ratios, were compared among the five litter types using one-way analysis of variance (ANOVA), followed by Tukey's honest significant difference (HSD) test when significant differences were detected.

Decomposition rates were estimated for the five litter types assuming a negative exponential decay, by linear regression of the ln-transformed fraction of DM remaining against time (days). The ln-transformed fraction of DM remaining was compared among litter types using analysis of covariance (ANCOVA, time as covariate), followed by Tukey's HSD test for post hoc comparisons.

Microbial variables (i.e., microbial respiration rates, fungal biomass, and aquatic hyphomycetes sporulation rates and species richness) were compared among litter types and sampling dates by two-way ANOVA, followed by the Tukey's HSD test when significant differences were detected.

Table 1. Chemical characteristics of invasive (*Acacia dealbata* pods, flowers and leaflets) and native (*Quercus robur* and *Alnus glutinosa* leaves) litter used in the microcosms experiment. Values are means ± SE (n = 3). Litter types with the same letter do not significantly differ (one-way ANOVA followed by Tukey's HSD test, *p* > 0.050). *Características químicas da matéria orgânica da espécie invasora (vagens, flores e folíolos de* Acacia dealbata*) e das espécies nativas (folhas de* Quercus robur *e* Alnus glutinosa*) utilizada no experimento de microcosmos. Os valores são médias ± SE (n = 3). Tipos de matéria orgânica com a mesma letra não diferem significativamente (ANOVA de uma via seguida pelo teste de Tukey HSD,* p *> 0.050).*

Litter characteristic	*Ac. dealbata* pods	*Ac. dealbata* flowers	*Ac. dealbata* leaflets	*Q. robur* leaves	*Al. glutinosa* leaves
Lignin (%DM)	32.83 ± 1.63 [b]	51.65 ± 0.85 [a]	51.13 ± 0.89 [a]	31.62 ± 0.98 [b]	37.16 ± 2.40 [b]
Polyphenol (%DM)	9.28 ± 0.20 [bc]	12.51 ± 0.45 [a]	8.78 ± 0.89 [bc]	6.78 ± 0.38 [c]	10.17 ± 0.73 [ab]
Carbon (%DM)	44.98 ± 0.23 [b]	49.52 ± 0.46 [a]	48.71 ± 0.20 [a]	42.48 ± 0.23 [c]	45.41 ± 0.05 [b]
Phosphorus (%DM)	0.019 ± 0.001 [d]	0.06 ± 0.004 [bc]	0.111 ± 0.006 [a]	0.044 ± 0.007 [c]	0.076 ± 0.005 [b]
Nitrogen (%DM)	0.47 ± 0.01 [d]	1.13 ± 0.19 [bc]	2.41 ± 0.04 [a]	0.83 ± 0.02 [cd]	1.46 ± 0.03 [b]
Carbon:Phosphorus*	6151 ± 281 [a]	2150 ± 171 [bc]	1139 ± 73 [c]	2592 ± 345 [b]	1559 ± 95 [c]
Carbon:Nitrogen*	111.68 ± 1.77 [a]	54.12 ± 9.34 [bc]	23.61 ± 0.52 [d]	59.81 ± 1.34 [b]	36.29 ± 0.77 [cd]

DM, dry mass; *molar ratio.

The coefficient of variation (in percentage) was calculated for aquatic hyphomycetes species richness per sampling date, and averaged across sampling dates for each litter type, to quantify the variation in species richness among samples. Aquatic hyphomycetes communities, in terms of relative species contribution to total conidial production (log(x+1)-transformed), were compared among litter types and sampling dates by analysis of similarity (ANOSIM), based on a Bray-Curtis similarity matrix; visual representation of the similarity among communities is given by hierarchical cluster analysis.

Data normality was checked by Shapiro-Wilk's test and homogeneity of variances by Levene's test and data were transformed when necessary (information given in statistical tables). Analyses were made using STATISTICA 7 (StatSoft, Inc., Tulsa, OK, USA), with the exception of ANOSIM and cluster analysis, which were performed using PRIMER 6 (Primer-E Ltd, Plymouth, UK).

RESULTS

Litter characterization

The litter significantly differed in initial chemical characteristics (Table 1). Lignin concentration

Table 2. Exponential decomposition rate (*k*; mean ± SE) of invasive (*Acacia dealbata* pods, flowers and leaflets) and native (*Quercus robur* and *Alnus glutinosa* leaves) litter incubated in laboratory microcosms for up to 73 days. Litter types with the same letter do not significantly differ (ANCOVA followed by Tukey's HSD test, *p* > 0.050). *Taxa de decomposição exponencial (*k*; média ± SE) de matéria orgânica da espécie invasora (vagens, flores e folíolos de* Acacia dealbata*) e das espécies nativas (folhas de* Quercus robur *e* Alnus glutinosa*) incubada em microcosmos de laboratório por até 73 dias. Tipos de matéria orgânica com a mesma letra não diferem significativamente (ANCOVA seguida pelo teste de Tukey HSD,* p > 0.050*).*

Litter type	*k* (/d)	*p*
Ac. dealbata pods	0.0013 ± 0.0001 [a]	<0.001
Ac. dealbata flowers	0.0026 ± 0.0007 [a]	0.007
Ac. dealbata leaflets	0.0036 ± 0.0008 [ab]	0.001
Q. robur leaves	0.0038 ± 0.0012 [ab]	0.013
Al. glutinosa leaves	0.0083 ± 0.0015 [b]	0.001

was highest for *Ac. dealbata* flowers and leaflets compared with the other three litter types. Polyphenols concentration was highest for *Ac. dealbata* flowers and *Al. glutinosa* leaves, followed by *Ac. dealbata* pods and leaflets, and lowest for *Q. robur* leaves. Carbon concentration was highest for *Ac. dealbata* flowers and leaflets, followed by *Ac. dealbata* pods and *Al. glutinosa* leaves, and lowest for *Q. robur* leaves. Phosphorus and N concentrations were highest for *Ac. dealbata* leaflets, followed by *Al. glutino-*

Table 3. Summary table for ANCOVA made on dry mass remaining, and two-way ANOVAs made on microbial respiration rate, fungal biomass, aquatic hyphomycetes sporulation rate and species richness associated with invasive (*Acacia dealbata* pods, flowers and leaflets) and native (*Quercus robur* and *Alnus glutinosa* leaves) litter incubated in laboratory microcosms for 17, 38 and 73 days. *Tabela de resumo para ANCOVA feita com base na massa seca remanescente e ANOVAs de duas vias feitas com base na taxa de respiração microbiana, biomassa fúngica, taxa de esporulação e riqueza de espécies de hifomicetes aquáticos associadas a matéria orgânica da espécie invasora (vagens, flores e folíolos de* Acacia dealbata*) e das espécies nativas (folhas de* Quercus robur *e* Alnus glutinosa*) incubada em microcosmos de laboratório por 17, 38 e 73 dias.*

Source of variation	df	F	*p*
Dry mass remaining[1]			
Litter	4	4.345	0.005
Time	1	10.290	0.003
Respiration rate[2]			
Litter	4	20.216	<0.001
Time	2	1.957	0.159
Litter × Time	8	3.528	0.006
Fungal biomass[2]			
Litter	4	57.860	<0.001
Time	2	13.973	<0.001
Litter × Time	8	1.664	0.149
Sporulation rate[3]			
Litter	4	58.852	<0.001
Time	2	10.159	<0.001
Litter × Time	8	3.016	0.013
Species richness[3]			
Litter	4	0.403	<0.001
Time	2	0.001	0.943
Litter × Time	8	0.047	0.020

[1]ln(x)-transformed; [2]log(x)-transformed [3]log (x+1)-transformed

sa leaves, intermediate for *Ac. dealbata* flowers and *Q. robur* leaves, and lowest for *Ac. dealbata* pods. Conversely, the C:nutrient molar ratios (C:P and C:N) were highest for *Ac. dealbata* pods, followed by *Q. robur* leaves, intermediated for *Ac. dealbata* flowers and *Al. glutinosa* leaves, and lowest for *Ac. dealbata* leaflets.

Litter decomposition

Litter mass decreased over the incubation period for all litter types and after 73 days litter mass remaining varied between 57 % and 92 % of initial mass for *Al. glutinosa* leaves and *Ac. dealbata* pods, respectively (Fig. 2A). Consequently, exponential decomposition rates varied in the opposite direction, between 0.0013/d and 0.0083/d for *Ac. dealbata* pods and *Al. glutinosa* leaves, respectively (Table 2). Litter decomposition was significantly affected by litter type (Table 3), being significantly slower for *Ac. dealbata* pods and flowers than for *Al. glutinosa* leaves (Tukey's HSD, p = 0.003 and 0.029, respectively) (Table 2). Interestingly, decomposition rates of *Ac. dealbata* litter (pods, flowers and leaflets) did not significantly differ, despite the 2.1 to 2.8-fold higher decomposition rates of leaflets and flowers, respectively, compared with pods (Table 2). In addition, decomposition rates of leaf litter (*Ac. dealbata* leaflets and *Al. glutinosa* and *Q. robur* leaves) did not significantly differ among tree species, despite the 2.2 to 2.3-fold higher decomposition rate of *Al. glutinosa* leaves (Table 2).
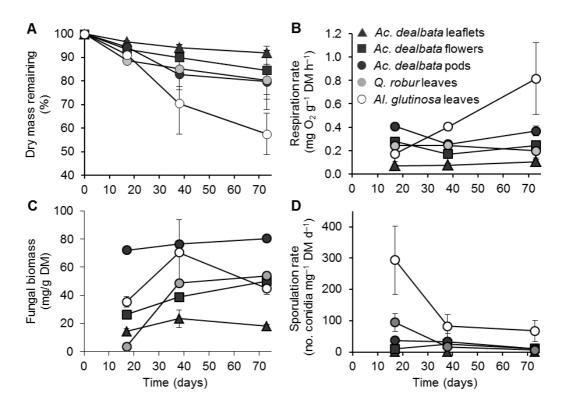

Figure 2. Dry mass remaining (A), microbial respiration rate (B), fungal biomass (C) and aquatic hyphomycetes sporulation rate (D) on invasive (*Acacia dealbata* pods, flowers and leaflets) and native (*Quercus robur* and *Alnus glutinosa*) leaves) litter incubated in laboratory microcosms for 17, 38 and 73 days (note that three pod samples did not produce conidia, two on day 38 and one on day 73). Values are means ± SE (n = 3). *Massa seca remanescente (A), taxa de respiração microbiana (B), biomassa fúngica (C) e taxa de esporulação de hifomicetes aquáticos (D) em matéria orgânica da espécie invasora (vagens, flores e folíolos de* Acacia dealbata*) e das espécies nativas (folhas de* Quercus robur *e* Alnus glutinosa*) incubada em microcosmos de laboratório por 17, 38 e 73 dias (notar que três amostras de vagens não produziram conídios, duas no dia 38 e uma no dia 73). Os valores são médias ± SE (n = 3).*

Microbial respiration rates

Microbial respiration rates were generally stable over the incubation period, except for *Al. glutinosa* leaves where they increased over time (Fig. 2B; Table 3, litter × time interaction). Maximum respiration rates varied between 0.1 and 0.8 mg O_2 g^{-1} DM h^{-1} for *Ac. dealbata* pods and *Al. glutinosa* leaves, respectively (Fig. 2B). Respiration rates were significantly affected by litter and the litter × time interaction (Table 3), being significantly lower for *Ac. dealbata* pods than for all other litter types on most dates and lower for *Q. robur* than for *Al. glutinosa* leaves at day 73 (Tukey's HSD, $p \leq 0.045$) (Fig. 2B).

Fungal biomass

Fungal biomass increased over the incubation time for *Ac. dealbata* flowers and leaflets and *Q.*
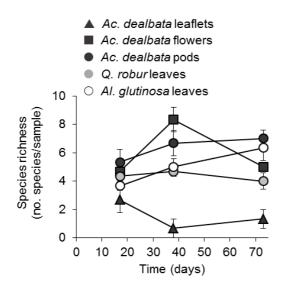

Figure 3. Aquatic hyphomycetes species richness associated with invasive (*Acacia dealbata* pods, flowers and leaflets) and native (*Quercus robur* and *Alnus glutinosa* leaves) litter incubated in laboratory microcosms for 17, 38 and 73 days (note that three pod samples did not produce conidia, two on day 38 and one on day 73). Values are means ± SE (n = 3). *Riqueza específica de hifomicetes aquáticos associados a matéria orgânica da espécie invasora (vagens, flores e folíolos de* Acacia dealbata) *e das espécies nativas (folhas de* Quercus robur *e* Alnus glutinosa) *incubada em microcosmos de laboratório por 17, 38 e 73 dias (notar que três amostras de vagens não produziram conídios, duas no dia 38 e uma no dia 73). Os valores são médias ± SE (n = 3).*

robur leaves, while it attained a peak by day 38 for *Ac. dealbata* pods (smoother) and *Al. glutinosa* leaves (sharper) (Fig. 2C; Table 3, time). Maximum fungal biomass varied between 23 and 80 mg/g DM for *Ac. dealbata* pods and leaflets, respectively (Fig. 2C). Fungal biomass was affected by litter (Table 3), being significantly higher for *Ac. dealbata* leaflets and significantly lower for *Ac. dealbata* pods than for all other litter types (Tukey's HSD, $p < 0.001$) (Fig. 2C).

Aquatic hyphomycetes sporulation rates

Sporulation rates by aquatic hyphomycetes were stable over the incubation period, except for *Q. robur* leaves where sporulation rates where significantly lower at day 73 than at day 17 (Tukey's HSD, $p = 0.004$) (Fig. 2D; Table 3, litter × time interaction). Maximum sporulation rates varied between 0.1 and 293 conidia mg^{-1} DM d^{-1} for *Ac. dealbata* pods and *Al. glutinosa* leaves, respectively. Sporulation rates were affected by litter and the litter × time interaction (Table 3), being significantly lower for *Ac. dealbata* pods than for all other litter types at most dates (Tukey's HSD, $p \leq 0.018$), lower for *Ac. dealbata* flowers than for *Al. glutinosa* leaves at day 17 ($p < 0.001$), lower for *Ac. dealbata* flowers than for *Q. robur* leaves at day 17 ($p = 0.020$), and lower for *Q. robur* leaves than for *Al. glutinosa* leaves at day 73 ($p = 0.032$) (Fig. 2D).

Aquatic hyphomycetes communities

Aquatic hyphomycetes species richness showed different dynamics over time across litter types: it increased over time for *Ac. dealbata* leaflets and *Al. glutinosa* leaves, it attained a peak by day 38 for *Ac. dealbata* flowers, it remained stable for *Q. robur* leaves, and it decrease for *Ac. dealbata* pods (Fig. 3; Table 3, litter × time interaction). Maximum aquatic hyphomycetes species richness per sampling date varied between 3 and 8 species for *Ac. dealbata* pods and flowers, respectively (Fig. 3). Species richness was affected by litter and the litter × time interaction (Table 3), being significantly lower for *Ac. dealbata* pods than for all other litter types at days 38 and 73 (Tukey's HSD, $p < 0.001$) (Fig. 3). Although *Ac.*

dealbata pods had the lowest species richness per sampling date, dissimilarity among samples was high (Table 4), and total species richness across sampling dates was thus fairly high (10 species, compared with 6 – 12 for the other litter types; Table 5). A total of 15 aquatic hyphomycetes species were identified across litter types (Table 5).

Aquatic hyphomycetes community structure significantly differed among litter types and time (ANOSIM, R = 0.69 and $p < 0.001$ for both factors) (Table 5). In particular, communities on *Ac. dealbata* pods markedly differed from communities on the other litter types (Fig. 4). In general, the most common aquatic hyphomycetes species across litter types were *Articulospora tetracladia*, *Flagellospora curvula* and *Tetrachaetum elegans*. *Tricladium patulum* was found only on *Ac. dealbata* pods at day 17 and *Clavatospora longibrachiata* was found only on *Ac. dealbata* leaflets at day 38 (Table 5).

Table 4. Coefficient of variation (CV) of aquatic hyphomycetes species richness associated with invasive (*Acacia dealbata* pods, flowers and leaflets) and native (*Quercus robur* and *Alnus glutinosa* leaves) litter incubated in laboratory microcosms for up to 73 days. Values are means ± SE (n = 3). *Coeficiente de variação (CV) da riqueza específica de hifomicetes aquáticos associados a matéria orgânica da espécie invasora (vagens, flores e folíolos de* Acacia dealbata*) e das espécies nativas (folhas de* Quercus robur *e* Alnus glutinosa*) incubada em microcosmos de laboratório por até 73 dias. Os valores são médias ± SE (n = 3).*

Litter type	CV (%)
Ac. dealbata pods	106 ± 35
Ac. dealbata flowers	10 ± 5
Ac. dealbata leaflets	22 ± 4
Q. robur leaves	17 ± 4
Al. glutinosa leaves	20 ± 2

Table 5. Relative contribution (%) of aquatic hyphomycete species to total conidial production on invasive (*Acacia dealbata* pods, flowers and leaflets) and native (*Quercus robur* and *Alnus glutinosa* leaves) litter incubated in laboratory microcosms for 17, 38 and 73 days (note that three pod samples did not produce conidia, two on day 38 and one on day 73). Values are means (n = 3). *Contribuição relativa (%) de espécies de hifomicetes aquáticos para a produção total de conídios em matéria orgânica da espécie invasora (vagens, flores e folíolos de* Acacia dealbata*) e das espécies nativas (folhas de* Quercus robur *e* Alnus glutinosa*) incubada em microcosmos de laboratório por 17, 38 e 73 dias (notar que três amostras de vagens não produziram conídios, duas no dia 38 e uma no dia 73). Os valores são médias (n = 3).*

Aquatic hyphomycetes species	*Ac. dealbata* pods			*Ac. dealbata* flowers			*Ac. dealbata* leaflets			*Q. robur* leaves			*Al. glutinosa* leaves		
	13	38	73	13	38	73	13	38	73	13	38	73	13	38	73
Alatospora acuminata Ingold			10.0				0.2	0.4	34.5	0.8	12.9	14.1		3.5	1.5
Alatospora pulchella Marvanová	6.7				0.8	1.0									
Anguillospora filiformis Greath.			25.0	1.5	0.6	0.7			0.2	0.6	0.6	1.9	0.3	4.6	6.6
Articulospora tetracladia Ingold	6.7		40.0	16.5	4.3	2.2	23.2	68.6	7.8	24.8	13.9	6.9	37.3	60.3	44.8
Clavatospora longibrachiata (Ingold) Sv. Nilsson ex Marvanová & Sv. Nilsson					0.7										
Dimorphospora foliicola Tubaki				1.4	0.2			6.8	0.7		1.1	2.5			0.8
Flagellospora curvula Ingold	18.1		25.0	61.0	24.3	40.3	21.0	16.6	21.0	22.3	63.6	74.5	18.1	4.8	39.6
Hydrocina chaetocladia Scheur*		50.0			0.2		0.2		0.3						0.2
Lemonniera aquatica De Wild.	4.8						0.2	0.4	0.3						
Lunulospora curvula Ingold				1.5	1.0		0.2	4.0	1.0					0.2	0.5
Margaritispora aquatica Ingold/*Goniopila monticola* (Dyko) Marvanová & Descals							0.6	1.1	0.3						2.8
Tetrachaetum elegans Ingold	23.8	50.0		19.5	60.6	4.3	54.4	1.6		51.6	8.0		44.3	26.5	3.2
Tricladium attenuatum Iqbal				1.4					0.7						
Tricladium patulum Marvanová	33.3														
Triscelophorus acuminatus Nawawi	6.7			5.3		50.5		0.6	33.3						
Species richness (no. species/treatment/date)	7	2	4	5	11	7	8	9	11	5	6	5	4	6	9
Species richness (no. species/treatment)		10			11			12			6			9	

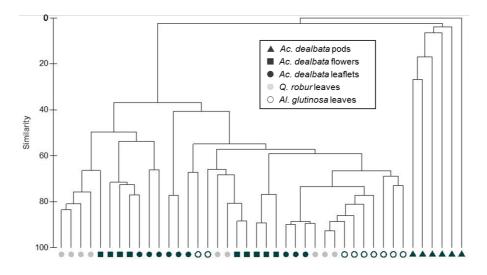*Syn. *Tricladium chaetocladium* Ingold

Figure 4. Hierarchical cluster analysis based on Bray-Curtis similarity index of aquatic hyphomycete communities on invasive (*Acacia dealbata* pods, flowers and leaflets) and native (*Quercus robur* and *Alnus glutinosa* leaves) litter incubated in laboratory microcosms for up to 73 days (only samples with conidial production are shown; three pod samples did not produce conidia, two on day 38 and one on day 73). *Análise de agrupamento hierárquico baseada no índice de similaridade de Bray-Curtis de comunidades de hifomicetes aquáticos em matéria orgânica da espécie invasora (vagens, flores e folíolos de* Acacia dealbata*) e das espécies nativas (folhas de* Quercus robur *e* Alnus glutinosa*) incubada em microcosmos de laboratório por até 73 dias (apenas amostras com produção de conídios são mostradas; três amostras de vagens não produziram conídios, duas no dia 38 e uma no dia 73).*

DISCUSSION

The invasion of native forests by *Ac. dealbata* occurs in many regions of the world (Ferreira et al., 2021). Invasion effects on soil characteristics, soil organisms and vegetation have been widely studied (e.g., see reviews by Le Maitre et al., 2011; Lorenzo & Rodríguez-Echeverría, 2015; Souza-Alonso et al., 2017). However, its effects on forest streams structure and functioning have been little addressed so far (Pereira et al., 2021; Pereira & Ferreira, 2022). Here, we show differences in chemical characteristics when considering the distinct litter types produced by *Ac. dealbata* (pods, flowers and leaflets) and when considering leaf litter produced by different tree species (*Ac. dealbata*, *Q. robur* and *Al. glutinosa*). Differences in litter characteristics across litter types resulted in differences in decomposers activities and litter decomposition, as well as in the structure of the aquatic hyphomycetes communities, but these differences were more pronounced across *Ac. dealbata* litter types than across leaf species.

Litter characteristics influenced microbial activity and decomposition

Overall, *Ac. dealbata* pods supported lower microbial activity and decomposed slower than all other litter types. Pods had the lowest nutrient concentrations (N and P) and the highest C:nutrient molar ratios compared with all other litter types, which likely made them less attractive for microbial decomposers, despite their relatively low lignin concentration. Litter nutrient concentrations (and C:nutrient molar ratios) are of major importance in controlling microbial colonization and activity under oligotrophic conditions, i.e., when the availability of dissolved inorganic nutrients is low (Gulis & Suberkropp, 2003; Lecerf & Chauvet, 2008; Arroita et al., 2012). In these conditions, decomposers need to rely on the litter as a source of nutrients, which likely requires a high energetic investment for enzyme production at the expense of growth (low accumulation of fungal biomass) and reproduction (low sporulation rates) (Gulis & Suberkropp, 2003). In addition, pods can be categorized as woody substrates,

like bark, and previous studies have shown that this type of material tends to decompose slowly in streams (Mackensen et al., 2003; Spänhoff & Gessner, 2004; Gonçalves et al., 2007; Gulis et al., 2008). Instream decomposition of pods has not received much attention, despite their likely significant contribution to litter inputs to streams in invaded forests, especially during late spring and summer (Lorenzo et al., 2010; Ferreira et al., 2021). Pod inputs to streams have not yet been measured, but quantification of pod contribution to the annual litterfall of other *Acacia* species in Australia has found values as high as 35.5 % (Milton & Moll, 1982). Also, quantification of pod contribution to the annual litterfall of another invasive legume species in riparian areas in the Iberian Peninsula (*Robinia pseudoacacia* L., black locust) found values as high as 15 % (Medina-Villar et al., 2015). In addition to pods, other types of fruits can make important contributions to litter inputs to streams as found in an Hawaiian stream where fruits (mainly *Psidium guajava* L., guava and *Mangifera indica* L., mango) represented up to 28 % of the daily litter input (Larned, 2000; Larned et al., 2001). Therefore, fruit inputs can make significant contributions to stream energetic budgets, especially in tropical regions where streams may receive large inputs of succulent fruits (Covich, 2000; Larned, 2000; Larned et al., 2001), but also in temperate regions where fruit inputs may occur in seasons of low leaf litter inputs such as in spring and summer (Molinero & Pozo, 2004; Medina-Villar et al., 2015). Fruits of invasive species may, however, have negative effects (e.g., increased mortality) on aquatic biota as shown for Hawaiian stream macroinvertebrates exposed to *Lonicera maackii* (Rupr.) Maxim. (amur honeysuckle) berries (Custer et al., 2017).

In contrast, *Al. glutinosa* leaves tended to support high microbial activity and fast decomposition, as a result of its relatively high nutrient concentrations (N and P), low C:nutrient molar ratios and low lignin concentration. *Alnus glutinosa* leaves are known to be of high quality for microbial decomposers and to decompose faster than those of other common riparian tree species (Ferreira et al., 2012; Pereira et al., 2016, 2021). Although *Al. glutinosa* leaves decomposed 2.2

and 2.3-fold faster than *Q. robur* leaves and *Ac. dealbata* leaflets, respectively, no significant difference was found among leaf litters, in contrast with a previous study (Pereira & Ferreira, 2021), which was likely due to the high variation associated with *Al. glutinosa* leaf litter decomposition in the present study. High variation among *Al. glutinosa* samples was surprising in this microcosm experiment, which highlights the need to consider more replication, even in controlled laboratory experiments.

The three other litter types (*Ac. dealbata* leaflets and flowers and *Q. robur* leaves) generally showed values for microbial variables and decomposition between *Ac. dealbata* pods and *Al. glutinosa* leaves (although differences were not always significant), which likely resulted from a trade-off between nutrient concentrations (and C:nutrient molar ratios) and lignin concentrations. *Acacia dealbata* leaflets had high nutrient concentrations (low C:nutrient molar ratios) but they also had high lignin concentration. High lignin concentration generally contributes to litter recalcitrance and it is generally detrimental to microbial colonization and litter decomposition (Lecerf & Chauvet, 2008; Schindler & Gessner, 2009; Jabiol et al., 2019). Interestingly, however, is the fact that *Ac. dealbata* leaflets accumulated the highest fungal biomass compared with all other litter types, in agreement with a previous study (Pereira & Ferreira, 2021). This may result from the large surface area:volume ratio of the leaflets facilitating microbial colonization, their high lignin concentration allowing for increased structural stability, and their high nutrients concentrations (N and P) decreasing fungal nutrient limitation, which may have favored fungal biomass buildup.

Acacia dealbata flowers had moderate nutrient concentrations, but they also had high lignin and polyphenols concentrations. High concentrations of structural and secondary compounds generally make litter less appealing for decomposers. Polyphenol compounds are secondary metabolites, widely known for having antimicrobial activity and preventing spore germination and fungal colonization (Ostrofsky, 1997; Constabel et al., 2014; Osswald et al., 2014; Zhang et al., 2019), which combined with lignin (see above), form a physical-chemical barrier that

prevents microbial activity. Also, the configuration of *Ac. dealbata* flowers, which are gathered in small globose flower heads of 1 – 4 dozens of individual flowers (~ 0.6 mm diameter), may challenge microbial colonization. Although flowers differ from leaves in their characteristics (e.g., nutrients concentration, concentration of secondary compounds) and can make relevant contributions to litter inputs to streams (up to 11 % of annual litterfall) (Larned, 2000; Molinero & Pozo, 2004; Lee et al., 2010; Medina-Villar et al., 2015; Garthwaite et al., 2021), their role on stream energetic budgets has been neglected. Few studies have addressed instream flower effects on stream biota and processes, with varying results (Custer et al., 2017; Garthwaite et al., 2021). Garthwaite et al. (2021) found similar overall decomposition rates (over 37 days) between *Salix sitchensis* Sanson ex. Bong (willow) catkins and leaves, while decomposition rates during the leaching phase (first 7 days of incubation) were slower for catkins. Also, abundance, evenness and diversity of invertebrates were higher in catkins than in leaves, likely due to higher structural complexity of catkins (Garthwaite et al., 2021). In contrast, Custer et al. (2017) found increasing mortality in Hawaiian stream macroinvertebrates with exposure to increasing biomass of invasive *L. maackii* flowers.

Quercus robur leaves had low lignin concentration but also had low nutrient concentrations and high C:nutrient ratios, which may have limited microbial activity in the oligotrophic incubation conditions. *Quercus robur* leaves generally decompose slower than *Al. glutinosa* leaves owing to its poor nutritional quality (Gulis et al., 2006; Ferreira et al., 2012; Pereira et al., 2016; Pereira & Ferreira, 2021). However, differences between *Q. robur* and *Al. glutinosa* leaves were not significant here, despite the 2.2-fold faster decomposition of the latter leaves.

Litter characteristics influenced the colonization by aquatic hyphomycetes

The composition of aquatic hyphomycetes communities varied among litter types, likely due to differences in their physical-chemical characteristics (Canhoto & Graça, 1996; Gulis,

2001; Ferreira et al., 2006b; Ferreira & Graça, 2016). Differences in litter chemical composition select aquatic hyphomycetes species based on their enzymatic capabilities and nutrient requirements. Aquatic fungal species have specific nutritional needs (Bisht, 2013; Brosed et al., 2017), varied ecological stoichiometry (Danger et al., 2016; Brosed et al., 2017), and different enzymatic performance (Zemek et al., 1985; Abdel-Raheem & Ali, 2004; Bisht, 2013). Therefore, fungal species have preferences for litter types with chemical composition similar to their own chemical composition in terms of C:nutrients, and that they can process, which may explain the differences in community composition among litter types that have unique chemical characteristics. Although physical characteristics were not quantified, litter types likely differed in toughness and surface roughness, which have been shown to distinctly affect conidial attachment among aquatic hyphomycetes species (Canhoto & Graça, 1999; Dang et al., 2007; Kearns & Bärlocher, 2008). Especially evident was the difference in aquatic hyphomycetes communities between *Ac. dealbata* pods and all other litter types. Strong differences in aquatic hyphomycetes communities between leaf litter and distinct litter types (e.g., wood, bark, and grass) have been shown before (Gulis, 2001; Ferreira et al., 2006a; Gonçalves et al., 2007).

It is interesting to note that, while species richness of aquatic hyphomycetes in *Ac. dealbata* pods per sampling date was the lowest among litter types, total number of aquatic hyphomycetes species over the incubation period was relatively high, indicating a high species turnover among sampling dates. Different fungal species have specific complementary enzymes to degrade plant polymers (Gulis, 2001; Osono, 2007; Bisht, 2013) and are thus capable of acting at different stages of decomposition. Also, since microbial inoculation of litter was done at the beginning of the experiment and all species detected in litter were present there since the beginning, the latter sporulation of some species suggests that they may need more time to accumulate enough biomass to invest in reproduction (Gessner & Chauvet, 1994; Maharning & Bärlocher 1996; Ferreira et al., 2016b).

Invasion of native riparian forests by *Acacia dealbata* may alter stream functioning by contributing recalcitrant litter types

The invasion of native forests by exotic species can lead to strong changes in litter inputs into streams. Changes in litter quality resulting from changes in riparian species composition alter the patterns and rates of litter use by aquatic communities (Hladyz et al., 2011; Ferreira et al., 2016a; Stewart et al., 2018). The invasion of native deciduous forests by *Ac. dealbata* implies the entry of recalcitrant litter (pods and flowers), which supports lower microbial activities and slower decomposition, potentially affecting the functioning and structure of streams in invaded forests. This was a laboratory study that focused on the role of litter characteristics on its colonization and decomposition by microbes, and did not consider variation in the amount and phenology of litter inputs, which likely vary between litter types, neither it considered the role of invertebrate detritivores. Field studies are therefore needed to fully address the contribution of pods and flowers to litter inputs to streams in invaded forests, and to determine how these litter types are exploited by aquatic communities (including both trophic and non-trophic relationships). Still, this was the first study addressing the colonization and decomposition of *Ac. dealbata* pods and flowers by aquatic decomposers, contributing to highlight the potential relevance of these litters as a long lasting, stable substrate.

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