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Eucalyptus plantations: can different species determine different "ecological realities" in streams?

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Resumo

Em pequenos cursos de água florestados, grande parte dos nutrientes e energia que sustentam as cadeias alimentares provêm dos ecossistemas terrestres adjacentes, nomeadamente das suas áreas ripícolas. Aqui, a produção primária é limitada pela sombra das copas das árvores e as cadeias alimentares baseiam-se em detritos, nomeadamente folhas senescentes. A composição vegetal das áreas ripícolas influencia, de forma marcante, o funcionamento destes sistemas lóticos: espécies diferentes fornecem ao biota matéria orgânica com características físico-químicas e valores nutritivos distintos.

Práticas florestais nas bacias de drenagem e, em particular, em zonas ripárias, alteram frequentemente o coberto vegetal das mesmas, afetando a quantidade, qualidade e sazonalidade do fornecimento de matéria orgânica aos cursos de água. Tais mudanças têm o potencial de impactar estes ecossistemas de forma intensa e por longos períodos de tempo.

A substituição de floresta nativa por eucaliptal é prática comum em muitos países e é um dos exemplos da intervenção humana em sistemas terrestres com consequências no estado ecológico dos cursos de água. Em Portugal, as plantações de eucalipto são maioritariamente realizadas com *Eucalyptus globulus*, em regime de monocultura; no entanto, podem encontrar-se outras espécies. As consequências destas plantações para o funcionamento dos cursos de água são largamente desconhecidas.

Tendo como objectivo comparar os efeitos de diferentes folhas de eucalipto no funcionamento dos cursos de água, foi avaliado o processo de decomposição, promovido por fungos (hifomicetes aquáticos) e invertebrados, de folhas de três espécies deste género – E*. globulus*, *E. camaldulensis*, *E. grandis*. O processo de decomposição é um processo ecossistémico chave em cursos de água florestados, que garante a reciclagem de nutrientes no sistema.

Para concretizar os objectivos, as folhas das três espécies de eucalipto foram caracterizadas, *a priori*, em termos de dureza, concentração de fenóis totais, carbono (C), azoto (N) e fósforo (P). Os resultados sugerem uma maior recalcitrância das folhas de *E. globulus* em relação às folhas de *E. camaldulensis*, que se apresentam com valores superiores

de N e P. A perda de massa, promovida por fungos, durante 4 semanas, foi distinta entre as diferentes espécies de folhas, sendo mais rápida em E. *camaldulensis* > *E. grandis* > E. *globulus.* O mesmo padrão foi observado com as taxas respiratórias. As taxas de esporulação foram diferentes apenas entre *E. camaldulensis* e *E. grandis,* mas as comunidades fúngicas foram dominadas, em todos os casos, por *Tetrachaetum marchalianum* (52% – 72% do total de esporos). As folhas de *E. grandis*, em similitude com *E. globulus*, apresentaram lixiviados (imersão 7 dias) com concentrações elevadas em fenóis o que sugere potenciais impactos destas espécies, sobretudo em períodos de menor caudal, na qualidade da água. Quando oferecidas a detritívoros – *Sericostoma vittatum* (Trichoptera) - *per se* (testes de consumo) ou em testes de preferência alimentar, não se verificam diferenças significativas nas taxas de consumo. No entanto, estes invertebrados demonstram preferência pelas folhas mais nutritivas e mais condicionadas - *E. camaldulensis* – em relação a *E. globulus* e *E. grandis*.

Este estudo sugere que os efeitos das plantações de eucaliptos em cursos de água dependem da espécie utilizada. Os resultados indicam que as folhas de *E. camaldulensis* (vs. *E. globulus* e *E. grandis*) poderão ser mais facilmente incorporadas na produção secundária que as espécies de eucalipto mais recalcitrantes. A incorporação de *E camaldulensis* em áreas de monocultura de eucaliptos com interesse económico poderá auxiliar o papel das áreas ripícolas nativas na preservação dos ribeiros ladeados por plantações destas exóticas.

Abstract

In low order and forested streams, most nutrients and energy that support food chains come from adjacent terrestrial ecosystems, namely their riparian areas. Here, primary production is limited by the shadow produced by the overhanging canopies of the trees and food chains rely on detritus, mainly senescent leaves. The vegetal composition of the riparian areas markedly influences the functioning of these lotic systems: different species give the biota organic matter with physic-chemical characteristics and distinct nutritional values.

Forestation practices in the watershed and, in particular, riparian areas, frequently change their vegetal cover affecting the quantity, quality and seasonality of the organic matter inputs to the streams. Such modifications have the potential to impact these ecosystems intensively and for long periods of time.

The substitution of native forest by eucalyptus forests is a common practice in many countries and it is one example of the human intervention in terrestrial systems with consequences to the ecological status of the streams. In Portugal, eucalyptus plantations are mainly composed by *Eucalyptus globulus*, in a monoculture regime; however, other species can also be found. The consequences of such afforestations have unknown consequences to the functioning of the watercourses. In order to evaluate the effects of different eucalyptus leaves on the streams functioning, we assessed the decomposition process, promoted by fungi (aquatic hyphomycetes) and invertebrates, of the leaves of *E. globulus*, *E. camaldulensis*, *E. grandis*. The decomposition process is a key ecossystemic process in forested watercourses that assures the nutrient cycling in the system. In order to fulfil the objectives, the leaves of the three species were characterized *a priori* in terms of toughness, total phenolic concentration, carbon (C), nitrogen (N) and phosphorous (P). The results suggest a higher recalcitrance of the *E. globulus* over *E. camaldulensis* leaves which present higher values of N and P. Mass loss, promoted by fungi, during 4 weeks, was different among leaf species, being faster in E. *camaldulensis* > *E. grandis* > E. *globulus.* The same pattern was observed regarding respiration rates. Sporulation rates were only different between E. *camaldulensis* and *E. grandis*, but the fungal communities were dominated by *Tetrachaetum marchalianum,* (52% - 72% of the spores

total) regardless of the leaf species. *E. grandis* leaves, similarly to *E. globulus*, presented high phenolic concentration in their leachates (7 days immersion), which suggests potential impacts of these species, mostly in periods of lower runoff, to the water quality. When offered to shredders - *Sericostoma vittatum* (Trichoptera) – *per se* (consumption tests) or in feeding preference tests, no significant differences were observed in the consumption rates. Nevertheless, these invertebrates showed a preference towards the most nutritious and more conditioned leaves – *E. camaldulensis* – in relation to *E. globulus* and *E. grandis*.

This study suggests that the effects of eucalyptus plantations in watercourses depend on the planted species. The results indicate that the *E. camaldulensis* leaves (vs. *E. globulus* and *E. grandis*) can be more easily incorporated into secondary production than the more recalcitrant eucalyptus species. The incorporation of *E. camaldulensis* in eucalyptus monoculture areas with economic interest could be an added value to the role of the native riparian areas in preserving the streams running through plantations of these exotic species.

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CHAPTER 1

General Introduction

1. General Introduction

Water has an undeniable importance to our planet, since about 70% of its surface is covered with it and all life forms depend on it to thrive. Freshwater ecosystems represent only 0.01% of the world's total aquatic reservoir (Schneider et al., 1996) but are utterly crucial to the worldwide biodiversity – for example, one third to a half of all vertebrates' species and 10% of the world's animal species (Balian et al., 2008; Geist, 2011; Christian et al., 2011) live in this habitat. However, this significant contribution to the planet's biodiversity is often ignored and misruled. Freshwater systems are taking their toll on the effects of human activities with important deleterious effects on the services provided to man (Vörösmarty et al., 2010). In fact, no other environment is declining as much and as severely as freshwater ecosystems (Décamps, 2011). Therefore, studies regarding these aquatic systems' functional and structural integrity are of vital importance for policy and preservation strategies.

1.1 - Low order streams and their riparian areas

Low order streams are of particular relevance, since they mark the "beginning" of the *river continuum* (Vannote et al., 1980). Any events - such as anthropogenic disturbances - taking place in these headwaters hold the potential to affect downstream areas. Small streams impairment may represent the loss of diversity in a river network (Finn et al., 2011), since they support and help to maintain biodiversity levels of the fluvial net (Meyer et al., 2007). Moreover, small streams (from first to third order) comprise the greater percentage of watercourses within the fluvial network (Allan & Castillo, 2007). These lotic systems are highly dependent and affected by external-originated factors, which influence their structure and functions. Riparian areas, in particular, are land-water interfaces that support and determine most of the headwaters undergoing ecological processes (Abelho, 2001).

In low order streams, the channel size is small, which determines a high degree of shading, produced by the overhanging canopies of the trees located near the banks. In temperate climates, debris from the riparian areas, and, in particular, leaf litter material (Abelho, 2001), constitute the major nutrient and energetic source (Vannote et al., 1980; Wallace et al., 1995; Hall et al., 2000) - up to 99% of the total energy supply (Fisher & Likens, 1973) - to the aquatic biota. Low, dense canopies and trees' proximity to the water channel increase shading, whereas taller and more open canopies allow more light to enter the system (Gregory et al., 1991). According to the degree of light limitation, temperature of the water can be influenced; it can modulate primary production, fungal and benthic invertebrate assemblages and, consequently, organic matter processing (Abelho, 2001; Franken et al., 2005; Yoshimura, 2007; Lagrue, et al., 2011).

The structural and biological composition of the forested riparian areas are important not only as determinants of the quality, quantity and seasonality of the food source to the heterotrophic aquatic food chains (Kominoski et al., 2013), but also as stabilizers of the stream banks. Vegetation also acts as filter for agricultural sediment (Cooper et al., 1987) and is a modulator of the flux of dissolved nutrient inputs from the terrestrial ecosystems. This role is related with the nutrient requirements of the species that compose the riparian area, since different biological necessities mean, in this case, different nutrient intakes (both in quality and quantity) (Gregory et al., 1991). Also, woody structures (branches, twigs, trunks, logs) provide structural and geomorphic diversity (hence habitat diversity) to the aquatic system (Allan & Castillo, 2007) and offer protection against the erosive action of floods contributing to sediment retention. Channel complexity promoted by the riparian trees also helps to decrease water and dissolved solutes flux, therefore potentially increasing biological uptake (Gregory et al., 1991).

1.2. Leaf litter decomposition

Leaf litter decomposition is a pivotal process in forested streams that allows the recycling of the organic matter and its incorporation into secondary production (Gessner et al., 1999). It proceeds through more or less distinguishable phases, which may overlap with each other in time. Leaf traits (Leroy & Marks, 2006; sections 1.2.1. – 1.2.3.) as well as environmental factors such as flow (floods: Rueda-Delgado et al., 2006; droughts: Lake, 2003; Gessner et al., 2010), temperature (González & Graça 2003; Spänhoff & Meyer, 2004; Robinson & Jolidon, 2005; Ferreira & Chauvet, 2011), nutrients concentration (Sridhar et al.,, 2000; Spänhoff & Meyer, 2004; Greenwood et al., 2007) and pH (Dangles & Chauvet, 2003; Tixier & Guérold, 2005; Löhr et al, 2006; Baudoin, et al., 2008) may influence the decomposition process.

1.2.1. Leaching

Leaf litter decomposition starts upon immersion in the stream. The leaching phase corresponds to the solubilisation of compounds such as phenols, simple sugars, soluble carbohydrates and other soluble elements (Suberkropp et al., 1976). It is an abiotic process, dependent, *a priori*, on the leaf structural characteristics such as the presence of waxes, cuticles and leaf condition (Bärlocher, 1991; Gessner, 1991; Campbell et al., 1992). Also, environmental factors such as turbulence and temperature (Chergui & Pattee 1991; Ferreira et al., 2006; Canhoto & Laranjeira, 2007) or pH of the water may affect the process (Abelho, 2001). Leaching usually lasts 48h (although it can be extended up to 7 days in some leaf species; Canhoto & Graça, 1996) and may determine an initial leaf mass loss of up to 40% (Quinn, 2000).

1.2.2. Conditioning

After the leaching period, leaves are colonized by microbial organisms – primarily aquatic fungi (aquatic hyphomycetes; Cummins, 1974; Bärlocher, et al. 1995; Abelho, 2001) but also bacteria (Baldy & Gessner, 1997). The action of fungal enzymes on the leaf promotes an increase of its palatability to invertebrates (Arsuffi & Suberkropp, 1988) mainly through nutrient immobilization and tissue softening. Several components of the leaf become gradually metabolized, depending on the leaf characteristics and the enzymatic potential of the fungal consortium (Canhoto & Graça, 2008). As the colonization progresses, fungal biomass accumulates in the leaf, reaching up to 16% of its total weight (Gessner & Chauvet, 1994).

Leaves may take months to become “fully conditioned”, which corresponds to a peak on fungal biomass and to a maximum leaf nutritional value of the leaf to its consumers (Boling et al., 1975). In a fully conditioned leaf we may find more than 20 fungal species (Bärlocher, 1991). The established assemblages may determine leaf degradability and palatability to the leaf-consuming invertebrates (Lecerf et al., 2005; see section 1.2.3.).

Leaf traits, largely determined by litter chemistry (Birouste et al., 2012; Freschet et al., 2012, 2013), are the primarily determinants of fungal-mediated decomposition. Lignin contents, the presence of secondary compounds and overall leaf toughness usually delay decomposition (Webster & Benfield, 1986; Gallardo & Merino, 1993; Gessner & Chauvet, 1994) while positive (eventually compensating; Canhoto & Graça, 1999; Gessner & Chauvet, 1994) effects have been associated with high contents of nitrogen and phosphorous. Toughness is related to the main structural compounds of the leaf (cellulose, hemicellulose, lignin, lignocellulose) and can be used as a proxy of leaf decomposition rate (Currie et al., 2010). Toughness levels can also be associated with “specific leaf area” (SLA), which can be used as an estimate of toughness (Fugère et al., 2012). Lower SLA’s are usually associated with denser and tougher leaves due to the allocation of more resources into structural components (Reich et al., 1998; Koerber et al., 2012). The presence of waxy cuticles are also known to limit conditioning as they contribute to leaf impermeability and isolation of the mesophyll from the aquatic environment (Gallardo & Merino, 1993; Canhoto & Graça, 1996; Canhoto & Graça, 1998; Canhoto & Graça, 1999). Other compounds such as polyphenols and essential oils were reported to have antimicrobial effects (Bunn, 1988; Canhoto & Graça, 1999; Canhoto et al., 2002; Andrade et al., 2011). Trichomes on the surface of the leaf might delay fungal colonization and decomposition, since the presence of phenols and essential oils and other, toxic, repellent chemicals have been identified as composition of these structures (Levin, 1973; Stipanovic, 1983; Gruner et al., 2005). Furthermore, trichomes are primarily composed of cellulose and lignin (Levin, 1973), which can also have a retardant effect on the process. They can also act as a physical barrier (Levin, 1973), eventually making it more difficult for spores to reach and attach to leaf surface (Kearns & Bärlocher, 2008).

1.2.3. Physical and Biological Fragmentation

Once conditioned, leaves become friable and easily fragmented by flow, turbulence or sediments' abrasion. Also, an improved quality status promoted by the fungal colonization makes them a preferred food source (always dependant, however, on leaf traits) for invertebrates, namely for a functional feeding group of invertebrates designated as shredders (Gessner et al., 1999). This group plays a key role in litter decomposition; their activity can be responsible for leaf decomposition by up to 64% (Cuffney et al., 1990; Wallace et al., 1995; Hieber et al., 2002). These detritivores not only prefer "fully conditioned" leaves over "unconditioned" leaves but may also show preferences for specific fungal species and assemblages (Arsuffi & Suberkropp, 1984, 1988; Chergui & Patee, 1991; Kiran, 1996; Lecerf et al., 2005; Canhoto & Graca, 2008; Gonçalves et al., 2014). The presence of a waxy cuticle is, similarly to the conditioning phase, a limiting factor for consumption (Canhoto & Graça, 1999). Nitrogen rich and softer leaves are usually preferred by shredders over harder, nutrient poorer leaves, with deterrent chemical properties (Irons et al., 1988; Canhoto & Graça, 1995; Graça & Cressa, 2010). Specific portions of leaves, such as their veins, possess refractory traits - high toughness and low nutritional values. Given these characteristics, shredders were reported to avoid the ingestion of these parts of the leaf (Martínez et al., 2016). The presence of essential oils might have negative effects on some shredder's consumption rates (Canhoto & Graça, 1999). High concentrations of phenols can also limit the decomposition rates at this stage. The presence of trichomes (both hair-like and glandular) tend to limit shredder's consumption, preventing invertebrates to access the leaf itself (Levin, 1973), negatively affecting their performance and leaf decomposition (Levin, 1973; Stipanovic, 1983; Gruner et al., 2005). High nutritional (i.e. elemental) value in the leaf can, at least partially, compensate these deterrent traits (Graça et al., 2002).

1.3. Forestation practices

Altering the structure of riparian areas - either through afforestation (Harding et al.,1998), or deforestation (England & Rosemond, 2004) – have the potential to affect stream ecosystems and to reach and affect downstream habitats (Ursic & Douglass, 1978; Abelho & Graça 1996; Harding et al.,1998; Richardson, 2008; Hladyz et al., 2009; Riipinen et al., 2010; Hladyz et al., 2011). Changes in riparian forest cover usually change the patterns and characteristics of the allochthonous detrital inputs, ultimately affecting stream communities' composition (Price et al., 2003; Reinhart & VandeVoort, 2006; Richardson & Danehy, 2007; Riipinen et al., 2010; Krenz et al., 2016). Moreover, water-related factors can also be affected due to alteration of riparian cover: among these, temperature (Johnson & Jones 2000), pH (Riipinen et al., 2010), nutrient concentrations (Wynn et al., 2000) and water velocity (Ursic & Douglass, 1978; Rezende et al., 2014) are frequently related with changes in the biota and processes such as leaf litter breakdown (section 1.2.).

1.3.1. - Eucalyptus spp. forestation

Eucalyptus spp. is a widely distributed genus, covering 6 million ha and constituting 5% to 10% of land coverage in all forested plantation regimes (Mora & Garcia, 2000; Smith, 2001). Among the over 800 species of *Eucalytus* (Coppen, 2003*)*, *E. globulus* (mainly Portugal and Spain), *E. grandis (*Brazil and South Africa) and *E. camaldulensis* (widespread, mostly Mediterranean areas), are listed as three of the most planted species in the world (Mora & Garcia, 2000). In Portugal*, E. globulus* is presently the main forest occupation species. Being native to the Southeast Australia and Tasmania (Dutkowski & Potts, 1999), this species now covers 1.3 million hectares of forested area in Europe, being particularly abundant in the Iberian Peninsula (more than 80%), France and Italy (Iglesias-Trabado & Wilstermann, 2008; Cerasoli et al., 2016). In Portugal, 812000 ha of land, which represents 26% of our forested area, are occupied by this species (ICNF, 2013). *E. globulus* is mainly planted in a monoculture regime, currently constituting around 99% of the *Eucalyptus* plantations (pers. inf.). It constitutes raw material for the economically important paper mill, pharmaceutical and cosmetic industries. *Eucalyptus camaldulensis* is also one the most extensive occupation species around the Mediterranean basin (Cerasoli et al., 2016). Other species like *E. botryoides, E. dalrympleana and E. nitens* are also common species in Portuguese territory.

Portugal's original deciduous forests essentially include *Castanea sativa* Miller, *Quercus ruber* L. and *Prunus lusitanica* L. (Paiva, 1981, 2002). The unique composition gives the Portuguese forests their own specific traits and features (Carvalho, 2005, 2011), which are notoriously different from the ones we find in *Eucalyptus* dominated areas: considering riparian areas, the amount, timing, diversity and quality of the litter inputs to streams are different (Abelho & Graça, 1996). Both forests were reported to have seasonal litter inputs to streams, in different periods: around 73% of total annual litterfall in mixed-deciduous forests occurs mainly in autumn (Abelho & Graça, 1998), whereas in eucalyptus sites, an increased litterfal occurs in summer months (Abelho & Graça, 1996; Grigg & Mulligan, 1999; Graça et al., 2002). Furthermore, particularly in Mediterranean areas, eucalyptus streams' have a distinct hydrology: soil hydrophobicity increases runoff in rainy seasons (Burch, 1989) while, in summer, decreases in water levels frequently dry up entirely sections of the stream. These drought periods coincide with higher litter inputs (Scott & Lesch, 1997).

Eucalyptus forests occupy wide areas of the planet as an exotic (Ugalde et al., 2001; Iglesias-Trabado & Wilstermann, 2008; Bennett, 2010), Portugal included, constituting a main problem for the aquatic life not only in temperate (Ferreira et al., 2015) but also in tropical and subtropical systems (Raviraja, et al., 1996; Davies & Boulton, 2009).

1.4. - *Eucalyptus camaldulensis*, *E globulus* and *E. grandis* leaf traits

Eucalyptus globulus, E, camaldulensis and E. grandis, although all belonging to the same genus, are known to present distinct susceptibilities to decomposition. Breakdown rates (*k* day^{-1}) for each of the three species have been evaluated in previous studies (Table 1), which can vary from slow or medium to rapid for each species (Petersen & Cummins, 1974). This may be due to the use of distinct mesh size of the bags across the studies and to environmental contexts.

These three eucalyptus species differ in their essential oils' quantity and composition (Graça et al., 2002; Ogunwande et al., 2003; Lucia et al. 2007; Ghalem & Mohamed, 2008; Cheng et al., 2009; Akin et al., 2010): some studies indicate that *E. camaldulensis* produces considerably less amounts of these deterrent compounds than *E. globulus* (Silva et al., 2006). These chemicals, consistently located in specific semi-impervious vesicles in the leaf (Canhoto & Graça, 1999), have been reported to have significant antimicrobial effects (Canhoto & Graça, 1999; Ghalem & Mohamed, 2008; Andrade et al., 2011).

Morphological differences between the leaves of *E. camaldulensis, E. grandis* and *E. globulus* are evident (mostly size and shape) (Fig. 1). Both *E. globulus* and *E. camaldulensis* leaves present high stomatal densities and stomatal cell areas (Ali et al., 2009), which can facilitate fungal mycelium invasion (Canhoto & Graça, 1999).

According to previous reports, *E. camaldulensis* leaves are softer (Lignin:N ratio = 9.01; Tarafdar et al., 2003; Rezende et al., 2012) than *E. globulus* (Lignin:N ratio = 38; Ribeiro et al., 2002;) and slightly tougher than *E. grandis* (Lignin:N ratio = 4.4; Tonin et al., 2014). *E. globulus* leaves have low nutrient contents (Pozo et al., 1998; Sampaio et al., 2001; Pérez, et al. 2014) whereas *E. camaldulensis* leaves can produce high levels of phenols (Rezende et al., 2012). Such trait is in line with what has been reported for *E. grandis* - high phenolic complexity and quantity (Hepp et al., 2009; Tonin et al., 2014). However, *E. grandis* thin leaf cuticle was found to facilitate a more rapid solubilisation of these compounds (Tonin et al., 2014).

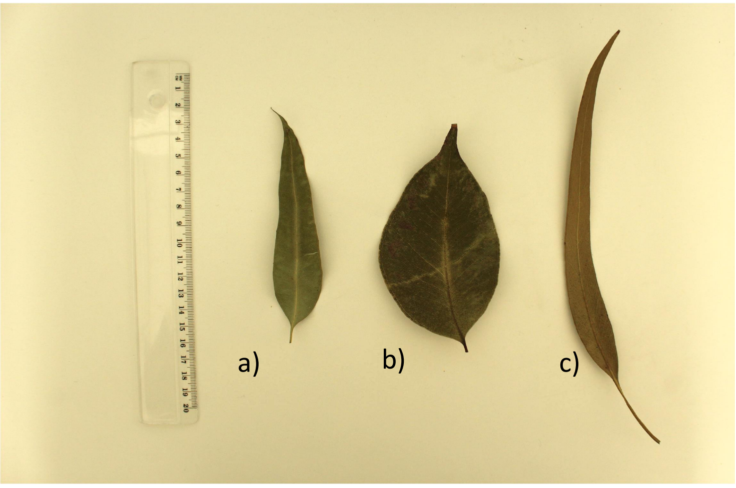

Figure 1. a) *Eucalyptus camaldulensis,* b) *E. globulus* and c) *E. grandis* leaves.

Table 1. Breakdown rates (*k* day^{-1}) of *E. camaldulensis, E. globulus* and *E. grandis* leaves contained in bags with specific mesh sizes (mm).

Species	Mesh Size (mm)	K day^{-1}	Reference
E. camaldulensis	0,3	0.0149 – 0.0163	Schulze & Walker, 1997
E. camaldulensis	2	0.0747	Parnrong et al., 2002
E. camaldulensis	5	0.005 – 0.008	Arroita et al., 2012
E. camaldulensis	5	0.0131	Galizzi et al., 2009
E. camaldulensis	10	0.0138 – 0.0147	Schulze & Walker, 1997
E. camaldulensis	10	0.017	Gonçalves et al., 2017
E. camaldulensis	10	0.0445	Rezende et al., 2012
E. camaldulensis	10	0.1192	Abelho et al., 2010
E. camaldulensis	25	0.0176 – 0.0253	Schulze & Walker, 1997
E. globulus	0.5	0.0068	Canhoto & Graça, 1996
E. globulus	0.5	0.009 – 0.019	Mesquita et al., 2007
E. globulus	0.5	0.026	Gonçalves & Canhoto, 2009
E. globulus	1	0.0071 – 0.0074	Bärlocher, 1998
E. globulus	5	0.0027 – 0.0158	Molinero et al., 1996
E. globulus	5	0.0066 – 0.0169	Pozo et al., 1998
E. globulus	5	0.0083	Sampaio et al., 2001
E. globulus	10	0.011 – 0.029	Mesquita et al., 2007
E. globulus	10	0.035	Gonçalves & Canhoto, 2009
E. globulus	-	0.0092	Campbell & Fuchshuber, 1995
E. globulus	-	0.0092	Schulze & Walker, 1997
E. grandis	1	0.001	Costa et al., 2005
E. grandis	2	0.003	Bachega, et al., 2016
E. grandis	2	0.005	Trevisan & Hepp 2007
E. grandis	2	0.005	Hepp et al., 2009
E. grandis	5	0.007 – 0.015	Rezende et al., 2010
E. grandis	6	0.005	Gonçalves et al., 2012
E. grandis	10	0.012	Tonello et al., 2014
E. grandis	10	0.074	Tonin et al., 2014

1.5 Objectives and hypothesis

This study aims to compare leaf decomposition of leaves belonging to three different species of *Eucalyptus - Eucalyptus globulus, E, camaldulensis and E. grandis* – present in the forests of Portugal. The importance of the leaf traits on leaf litter mass loss and associated microbial (aquatic hyphomycetes) descriptors will be assessed using a microcosm approach. The importance of leaf litter characteristics and quality to shredders (and invertebrate (*Sericostoma vittatum* (Trichoptera)) will also be evaluated through consumption and food preference trials. Results will help to gain insights on the relative impact of these species in low order streams not only in Portugal but also in areas where these species are dominant.

We expect *E. camaldulensis* to decompose faster than *E. grandis* due to its expected higher quality. *E. globulus* the most recalcitrant, giving its poor nutritional value and higher toughness, will likely present the lowest decomposition rates. Leaf physico-chemical defences will be more effective towards invertebrates rather than fungi considering the potential ability of the mycelium to overpass *Eucalyptus* leaf defences.

CHAPTER 2

Materials and Methods

2. Materials and Methods

2.1. Initial litter physic-chemical composition

Senescent leaves of *Eucalyptus globulus, E. grandis* and *E. camaldulensis* were collected just after abscission in October 2016. Leaves were air dried and stored until needed in the dark. In order to characterize initial litter physic-chemical characteristics, air-dried leaf samples were oven dried (105ºC, 24h), milled (0.5mm powder size) and subsamples analysed for nitrogen (N) (Kjeldahl method; Kjeldahl, 1883; Hach et al., 1985; Automated Wet Chemistry Analyzer, SanPlus System - Skalar), phosphorus (P) (Hach method), carbon (C) (dry combustion method; TOC, multi EA 4000 - Analytik Jena AG) and total phenolic (Graça et al., 2005) concentrations. Results were expressed as percentage of dry mass (%DM). Additionally, toughness of the leaves (initial and after the total conditioning period) was measured using a penetrometer (Graça et al., 2005). A leaf disc was placed in between plates, aligned with a needle-entry hole. A water recipient was placed on top of the puncturing device. Water was carefully added (i.e. drop by drop) to the recipient, until the needle pierced the disc. Results were expressed as penetrance, i.e., the required mass (g of water in a container) to push a 1 mm diameter metal shaft through the leaf disc.

The objectives of the study were achieved by two sets of experiments: (1) microbial-mediated decomposition and associated fungal parameters in microcosms and (2) consumption and feeding preference tests.

2.2. Leaf litter decomposition

2.2.1. General

Leaf discs (13 mm diameter) were punched out from leaves of each species with a cork borer. Discs were oven dried (105 °C, 24 h) and individually pre-weighed (0.0504g ± 0.0007). Groups of twenty leaf discs of each species were placed in 100 ml Erlenmeyer flasks - 8 replicates / species - with 40 ml of distilled water. In each Erlenmeyer, 5 (out of 20) randomly chosen discs were weighed and tied together for further evaluation of mass loss (section 2.2). Leaching was allowed for 7 days; after this period, 3 microcosms of each species were sacrificed to evaluate mass loss (section 2.2.2.) and phenolic concentration of the leachates; The remaining 6 microcosms were re-filled with 40 ml of sterile (autoclaved: 121ºC, 20 min.) nutrient solution (75.5 mg CaCl2, 10 mg MgSO4.7H2O, 0.5 g 3-morpholino propanesulfonic acid (MOPS), 5.5 mg K2HPO4 and 100 mg KNO3 per litter of sterile distilled water; Dang et al., 2005). Cultures of 6 hyphomycetes species (previously grown on malt extract agar) commonly found in eucalyptus streams (*Articulospora aquatica; Flagellospora curta; Heliscus lugdunensis; Lemoniera aquatica; Tetrachaetum elegans; Tetracladium marchalianum*) were used to inoculate microcosms (with agar plugs) which were then closed with cotton bungs. The erlenmeyers were continuously aerated on orbital shakers (100 rpm; 18ºC) under a 12 hour light/12 hour dark photoperiod for 4 weeks. The medium was renewed every 2 days. Three replicates/leaf species were used to evaluate **mass loss** (5 discs), **fungal respiration** (5 discs), **sporulation rates** (5 discs) and **leaf toughness** (2 random measurements in a set of each of 5 discs) (sections 2.2 – 2.4). An additional Erlenmeyer was prepared for consumption and feeding preference test (section 2.3.)

2.2.2. Leaf mass loss

The five pre-weighed leaf discs incubated in each replicate from each treatment were used for the evaluation of mass loss. After the incubation period (4 weeks) each set of 5 leaf discs were oven-dried for 48h at 105ºC and weighed to determine dry mass remaining (%DMr). A similar procedure for abiotic-derived mass loss was applied in the discs after the leaching period, i.e., 7 days. Assuming a linear mass loss, decay rates were also determined using an exponential decay model:

$$M_t = M_0 \text{ x } e^{-kt}$$

where M_t is the mass at time t, M_0 the initial mass and k the decay coefficient.

In order to characterize litter physic-chemical composition after leaching and conditioning, the sets used in mass loss evaluation were analysed for toughness and milled for total phenolic concentration evaluations (section 2.1).

2.2.3. Fungal respiration

Five discs from each replicate were used to evaluate microbial, i.e. fungal, respiration. The discs were immersed in an O_2 saturated nutrient solution (Dang et al., 2005), inside 50 ml falcon tubes, filled up to the top. After 22 h in the dark, O_2 concentrations in the falcon tubes were measured. Oxygen consumption was assessed by calculating the difference between initial and final concentrations. Respiration rates were expressed as mg O_2/g leaf DM/h.

2.2.4. Sporulation rates

The conidia suspensions were removed from the microcosms 1 week before the end of the conditioning phase (i.e. 3 weeks) and fixed with formalin for later counting and identification. Aliquots of the suspension were then filtered and filters stained with cotton blue in lactic acid; spores were identified (Graça et al., 2005) and counted under a compound microscope at 200x. Sporulation rates were expressed as number of conidia released/mg leaf DM/day

2.3. Consumption and feeding preferences

Consumption and feeding preference tests were performed with *Sericostoma vittatum* (Trichoptera) larvae (Fig. 4). This species is an endemic and abundant shredder in low order streams of Centre Portugal. Larvae were collected from Ribeira de S. João (Lousã, Central Portugal). Individuals were kept in the laboratory for 7 days for acclimation in plastic containers filled with filtered aerated stream water covered with a fine layer of ashed (500ºC, 6h) stream substratum. The invertebrates were fed *ad libitum* with detritus collected in the stream of origin.

Leaf discs of each of the three species of *Eucalyptus* were punched out in pairs, one from each side of the main vein of the same leaf and tied together with a thin wire. Discs from each pair are assumed to have identical initial mass. A total of 20 pairs of *E. globulus*, *E. grandis* and *E. camaldulensis* each were conditioned for 3 weeks as in section 2.1.

One invertebrate (0.22 mg ± 0.0004 SE) was individually allocated into individual containers filled with 200ml of filtered stream water**.** Each container was aerated and provided with a similar amount (30 g ± 0.0001g SE) of ashed stream substratum (500ºC, 6h). Invertebrates were submitted to a 48h-starving period before the beginning of the tests. **Consumption tests** (10 replicates/species) consisted on placing one disc of each pair of conditioned eucalyptus leaves inside a 0.5 mm mesh bag (4.0 × 3.5 cm) attached to the edge of each container to be used as control for leaf mass losses other than by consumption. The pair was offered to the larvae. Tests run until half the leaf disc was consumed in half the containers i.e. 24 hours. For the **feeding preference tests** we used a similar procedure, but each container was provided with three discs one of each species of eucalyptus (Figs. 2 and 3). Tests ran until half the leaf discs in one of the species was consumed in half the containers. In both cases, leaf consumption or each specimen (C) was estimated as the difference between leaf discs dry mass in the control (DM_c) and offered (DM_o) and expressed per mg of dry mass of individual larva (DM_l), per day (d) according to the equation $C = (DM_c – DM_o) / (DM_l \times d)$ (Graça et al., 2005). The dry mass of each larvae (Dw) was calculated by measuring the diameter of the anterior opening of the invertebrate's case (Lc), according to the equation (Canhoto, 1994):

$$\ln Dw = (Lc – 1.664)/0.599$$

$$(R^2 = 0.83, p < 0.001, n = 35)$$

Statistical analysis

In order to analyse initial litter and fungal parameters and the consumption rates, a 1-way ANOVA, followed by a post-hoc Tukey's-test, whenever necessary, were performed. The assumption of homogeneity of variance was confirmed regarding each ANOVA performed, using Levene's test. Regarding respiration rate, the data obtained was transformed (Log_{10}) before the statistical test was performed in order to fulfil the ANOVA assumptions (Zar, 1999). As for the invertebrates' preference tests, a permutation test was applied (Graça et al., 2005).

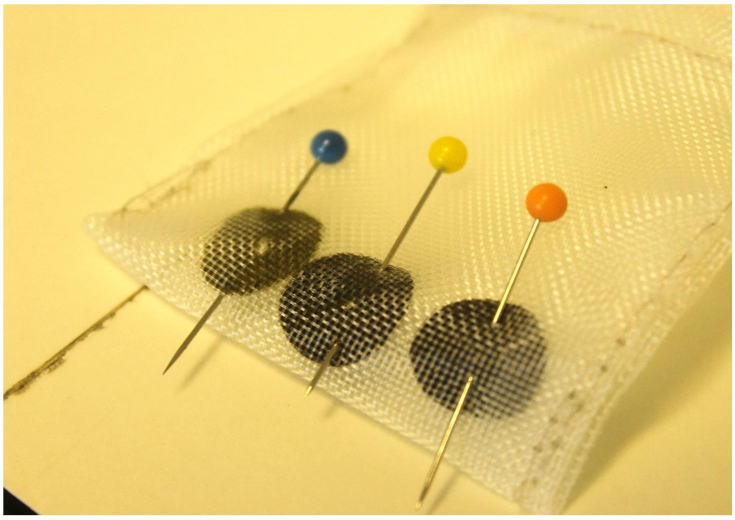

Figure 2. Preference tests: control discs inside a bag (Ø = 0.5mm). Blue – *E. camaldulensis*; Yellow – *E. grandis*; Orange – *E. globulus*

Figure 3. Feeding preference tests: each replicate contains 3 leaf discs offered to the invertebrate and respective pairs (i.e. controls) placed inside the mesh bag. Each invertebrate was allocated in the cup bottom that was covered with stream sand, and filled with arerated and filtered stream water. Blue and green – *E. camaldulensis*; Yellow and purple – *E. grandis*; Orange – *E. globulus*.

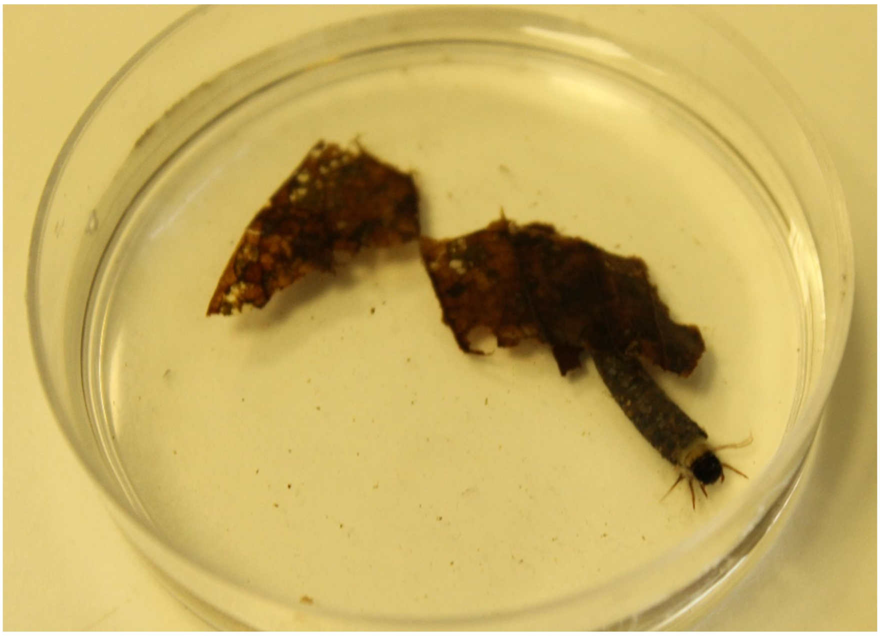

Figure 4. *Sericostoma vittatum* (Trichoptera; Sericostomatidae) larvae used in consumption and feeding preference tests.

CHAPTER 3

Results

3. Results

3.1. Litter physic-chemical composition

Carbon percentages were statistically different between species (1-way ANOVA, $F_{(1,2)}$= 25.43; p = 0.001). *E. camaldulensis* presented lower values than *E. grandis* (Tuckey's test; p = 0.024) and *E. globulus* (Tuckey's test; $p < 0.001$) (Table 2). Also, C percentages of *E. globulus* were higher than the values found for *E. grandis* (Tuckey's test; p = 0.024). As for N percentages, the opposite tendency was observed (1-way ANOVA, $F_{(1,2)}$ = 41.58; $p < 0.001$). *E. camaldulensis* presented higher values than *E. grandis* (Tuckey's test; $p < 0.001$) and *E. globulus* (Tuckey's test; p = 0.001). No differences were recorded between *E. grandis* and *E. globulus* (Tuckey's test; $p < 0.270$) (Table 2). *E. camaldulensis* P concentration was higher than *E. grandis* and *E. globulus* (Table 2).

Table 2. Carbon, nitrogen, phosphorus, initial toughness and phenolic content of *E. camaldulensis, E. grandis* and *E. globulus* expressed as percentages. Values are means (n = 3) ± SE for C, N and phenolic content, except for toughness (n = 6) ± SE. Different letters indicate statistical differences ($p < 0.05$).

Species	Carbon (%)	Nitrogen (%)	Phosphorus (%)	Initial Toughness (g)	Initial Phenols (%)
E. camaldulensis	38.00 ± 0.94^a	1.74 ± 0.08^a	0.18	71.38 ± 7.14	3.95 ± 1.01
E. grandis	42.33 ± 0.64^b	1.04 ± 0.01^b	0.11	82.90 ± 8.27	5.31 ± 0.75
E. globulus	44.91 ± 0.39^c	1.18 ± 0.06^b	0.12	95.37 ± 3.51	6.98 ± 0.67

No differences were recorded among initial leaf species toughness (1-way ANOVA, $F_{(1,2)}$ = 1,77; p = 0,206) although a gradient of toughness could be observed in the order *E. globulus* > *E. grandis* > *E. camaldulensis* (Fig. 5). After the conditioning period, toughness followed the same pattern and significant differences could be found among species (1-way

ANOVA, $F_{(1,2)}$ = 9,49; p = 0,002). Toughness was higher for *Eucalyptus grandis* and *Eucalyptus globulus* and lower for *Eucalyptus camaldulensis* (Tuckey's test; $p < 0.014$).

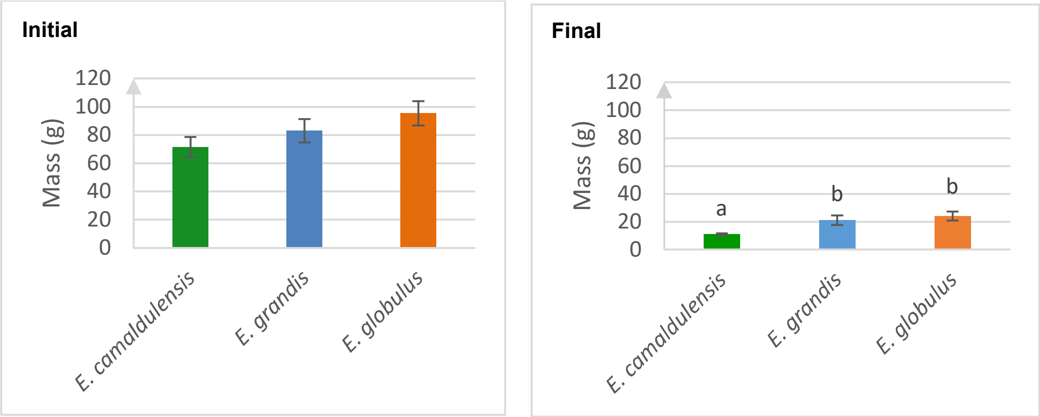

Figure 5. Initial and final toughness (g), after a conditioning period of 4 weeks, of *E. camaldulensis, E. grandis* and *E. globulus* leaves. Values are means (n = 6) ± SE. Different letters indicate statistical differences ($p < 0.05$).

Initial phenolic concentrations were not different among leaf species (1-way ANOVA; $F_{(1,2)}$ = 3,39; p = 0,103) (Table 2; Fig. 6). But after the leaching period, values of phenolics found in *E. camaldulensis* leachates were lower than *E. grandis* (Tuckey's test; p = 0.003) and *E. globulus* (Tuckey's test; p = 0.002). *E. grandis* and *E. globulus* recorded no differences between them (Tuckey's test; p = 0.80). Final concentrations (1-way ANOVA; F = 13.56; p = 0,006), were different among species, being lower for *E. camaldulensis* and *E.* globulus (Tuckey's test; $p < 0.011$), with no differences between them (Tuckey's test; p = 0.980) (Fig. 6).

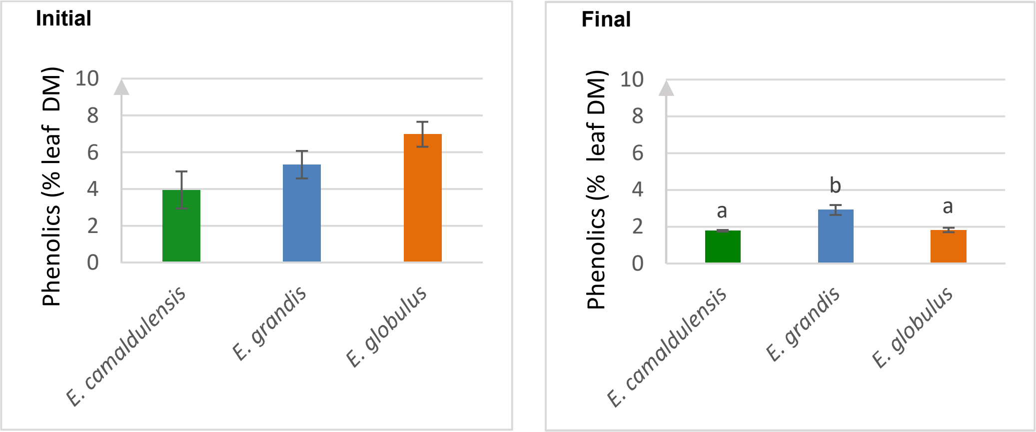

Figure 6. Initial and final percentages (after a leaching period of 7 days) of phenols/mg leaf DM on *E. camaldulensis, E. grandis* and *E. globulus* leaves. Values are means (n = 3) ± SE. Different letters indicate a statistical difference ($p < 0.05$).

3.2. Mass Loss

Species identity affected leaching (1-way ANOVA; $F_{(1,2)} = 47{,}95$; $p < 0{,}001$; Fig. 7) and mass loss promoted by fungi (1-way ANOVA; $F_{(1,2)} = 22.76$; $p = 0{,}001$; Fig 8). Leaching determined a stronger decrease in dry mass in *E. camaldulensis* followed by *E. grandis* and *E. globulus* (Tuckey's test; $p < 0.001$).

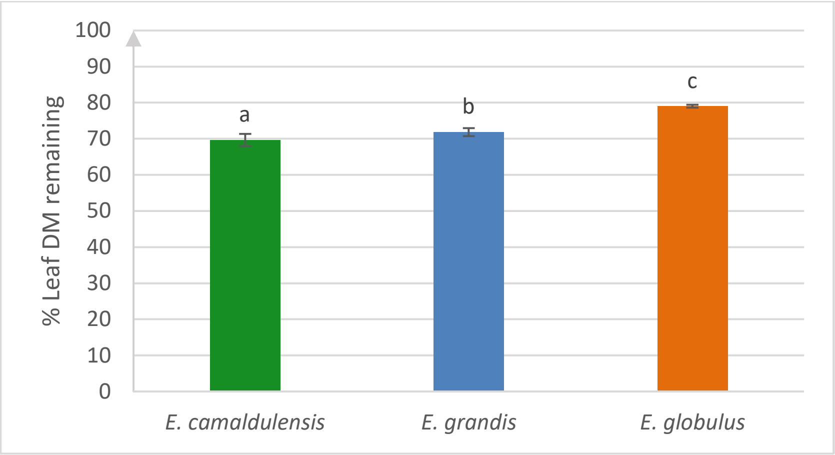

Figure 7. Leaf dry mass (DM) remaining expressed as % of *E. camaldulensis, E. grandis* and *E. globulus* after leaching. Values are means (n = 3) ± SE. Different letters indicate statistical differences ($p < 0.05$).

Regarding fungal-mediated degradation, *E. globulus* presented higher dry mass remaining than the other two leaf species. No statistical differences were recorded between *E. camaldulensis* and *E. grandis* (Tuckey's test; $p < 0.40$) (Fig. 8).

Decay rates (*k* day^{-1}) were, in all cases, fast (according to Petersen & Cummins, 1974) and in the order *E. camaldulensis* (*k* = 0.0224 day^{-1} ± 0.0037; $R^2 = 0.838$) > *E. grandis* (*k* = 0.0201 day^{-1} ± 0.0032; $R^2 = 0.847$) > *E. globulus* (*k* = 0.0134 day^{-1} ± 0.0019; $R^2 = 0.872$).

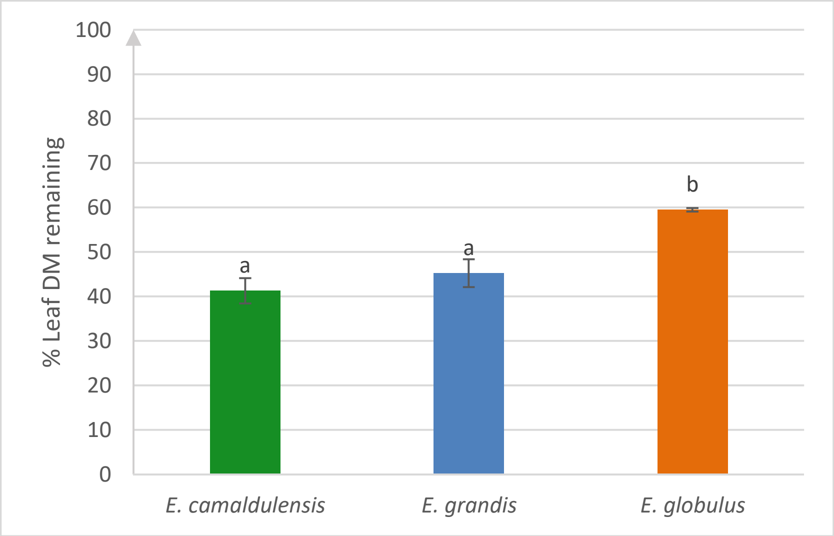

Figure 8. % leaf DM remaining on *E. camaldulensis, E. grandis* and *E. globulus* after conditioning for 4 weeks. Values are means (n = 3) ± SE. Different letters indicate statistical differences ($p < 0.05$).

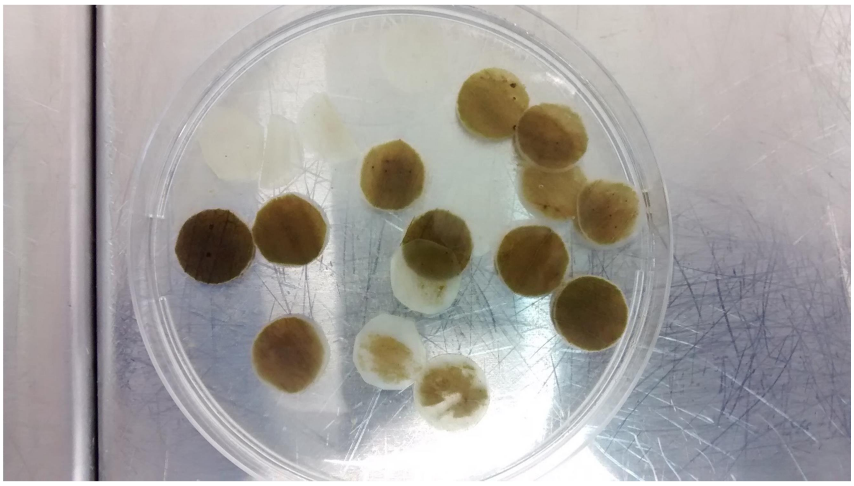

Figure 9. *E. camaldulensis* discs after a conditioning period of 7 days.

Figure 10. *E. globulus* discs after a conditioning period of 7 days

Figure 11. *E. grandis* discs after a conditioning period of 7 days.

3.3. Fungal respiration

Fungal respiration was dependent on the substrate, (1-way ANOVA; $F_{(1,2)} = 202.73$; $p < 0.001$). Higher fungal respiration values were associated with *E. camaldulensis*, followed by *E. grandis* (Tuckey's test; $p < 0.001$) and *E. globulus* (Tuckey's test; $p < 0.001$) (Fig. 12). Respiration rates of the fungal assemblages associated with *E grandis* represented about 34% of the O_2 consumption rates observed in *E. camaldulensis* and about 20% of the rates evaluated for *E. globulus*.

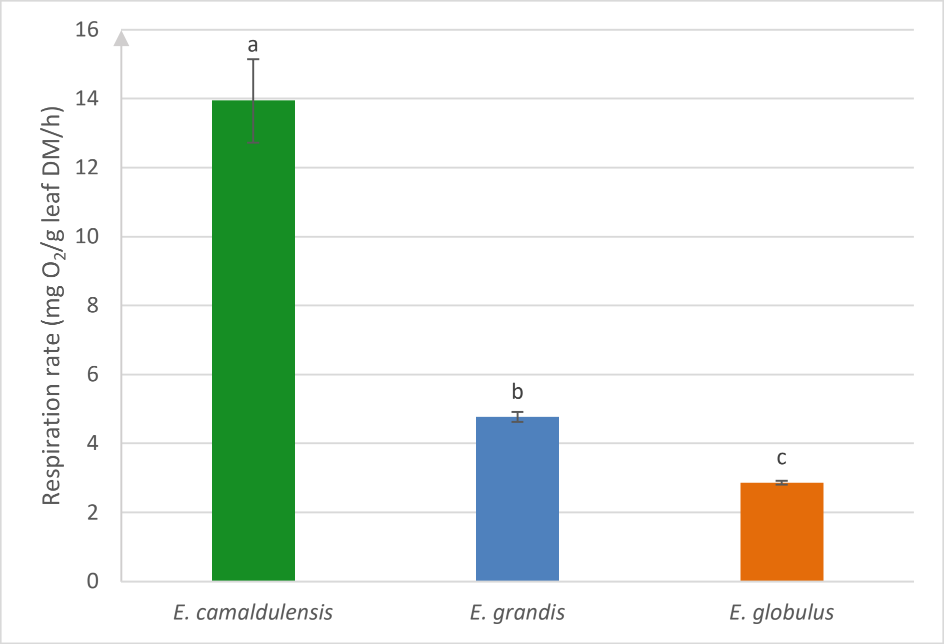

Figure 12. Respiration rates (mg O_2/g leaf DM/h) of the fungal assemblages colonizing *E. camaldulensis, E. grandis* and *E. globulus* discs. Values are means (n = 3) ± SE. Different letters indicate statistical differences ($p < 0.05$).

3.4. Sporulation Rate

Sporulation rates were different between eucalyptus species (1-way ANOVA; $F_{(1,2)}$ = 6,21; $p = 0.036$). Sporulation rates of *E. camaldulensis* were significantly higher than of E*. grandis* (Tuckey's test; $p = 0.029$). *E. globulus* was not statically different from the other two species (Tuckey's test; $p > 0.05$) (Fig. 13).

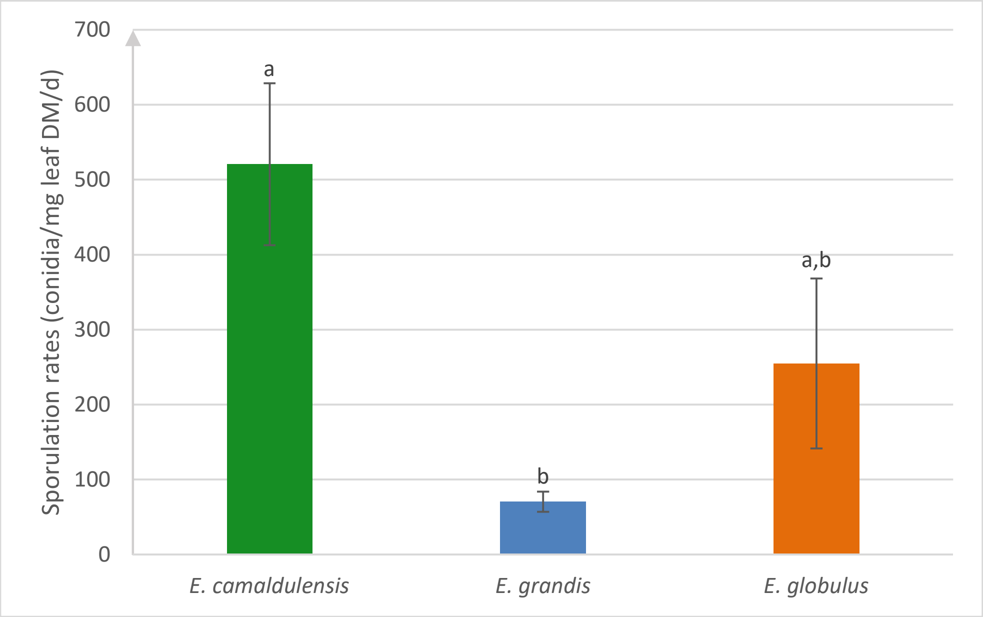

Figure 13. Sporulation rates (conidia/mg leaf DM/d) of aquatic hyphomycetes colonizing *E. camaldulensis, E. grandis* and *E. globulus* discs**.** Values are means (n = 3) ± SE. Different letters indicate statistical differences ($p < 0.05$).

All 6 species of hyphomycetes produced spores in *E. camaldulensis* substrate, in contrast with the other two species (Fig. 14). The species responsible for the highest sporulation rates in all eucalyptus species was *Tetracladium marchalianum* comprising 52%, 61% and 72% of the total conidial production of *E. globulus, E. camaldulensis* and *E. grandis,* respectively. *Tetrachaetum elegans* only produced spores on *E. camaldulensis.*

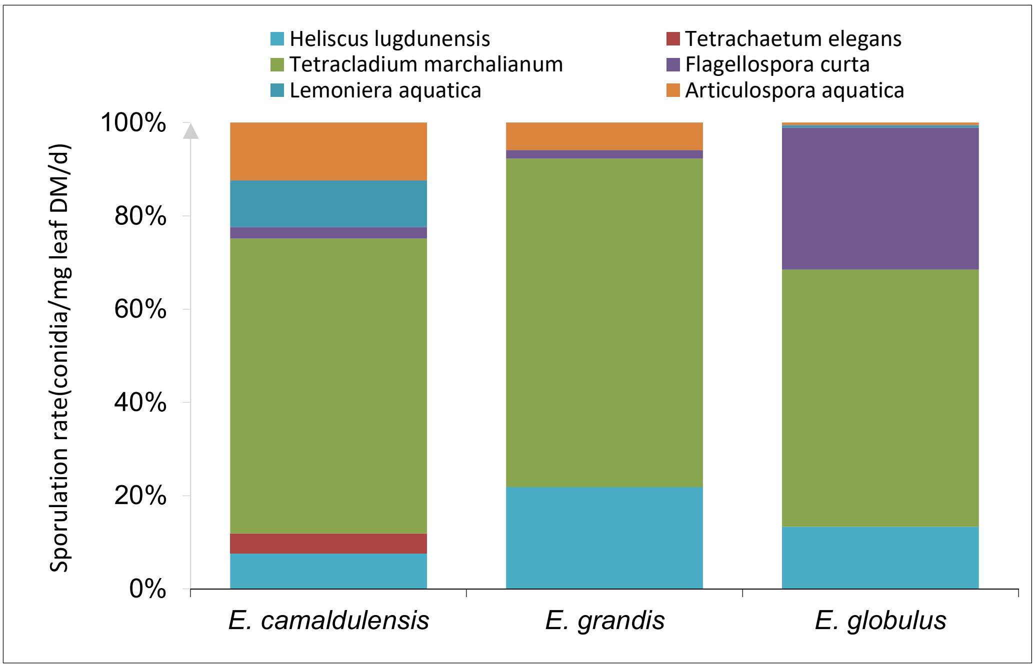

Figure 14. Mean relative abundances of aquatic hyphomycetes conidia produced in *E. camaldulensis, E. grandis* and *E. globulus* microcosms after for 4 weeks.

3.5. Consumption and feeding preferences

Invertebrates' consumption was not affected by leaf species (1-way ANOVA; $F_{(1,2)}$ = 2.44; $p < 0.12$) (Fig. 15). *E. globulus* was, nevertheless, less consumed (50% less than *E. grandis*) than the other two eucalyptus species.

Specimens discriminated *E. camaldulensis* over *E. grandis* and *E. globulus* in the feeding preference test (Permutation test; $p = 0.034$) (Fig. 16). This species was consumed about 5 times more than *E. grandis* and about 3.3 times more than *E. globulus*.

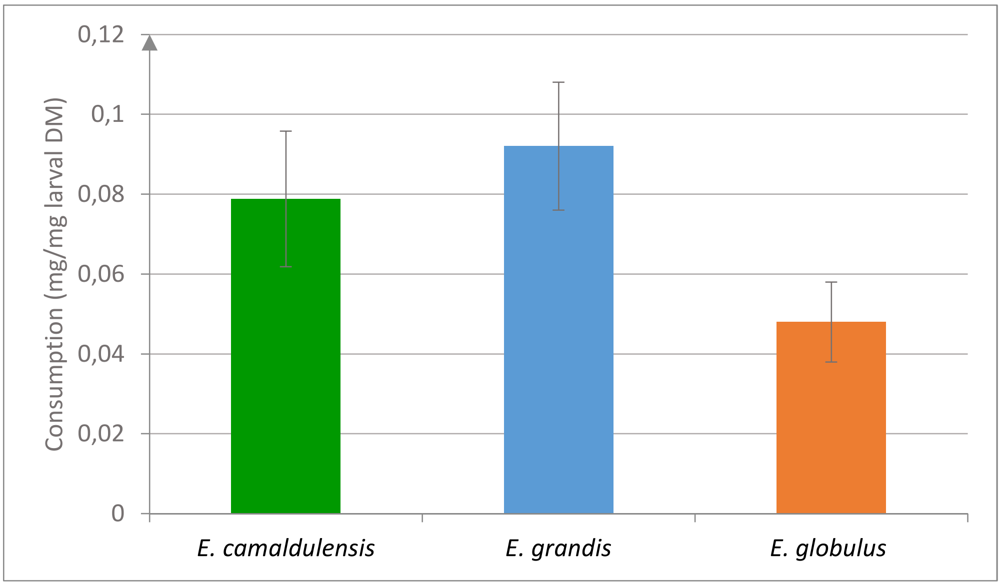

Figure 15. Consumption of 4-weeks conditioned *E. camaldulensis, E. grandis* and *E. globulus* by *S. vittatum* larvae during a period of 24h. Items were singly offered to the invertebrates. Values are means (n = 10) ± SE.

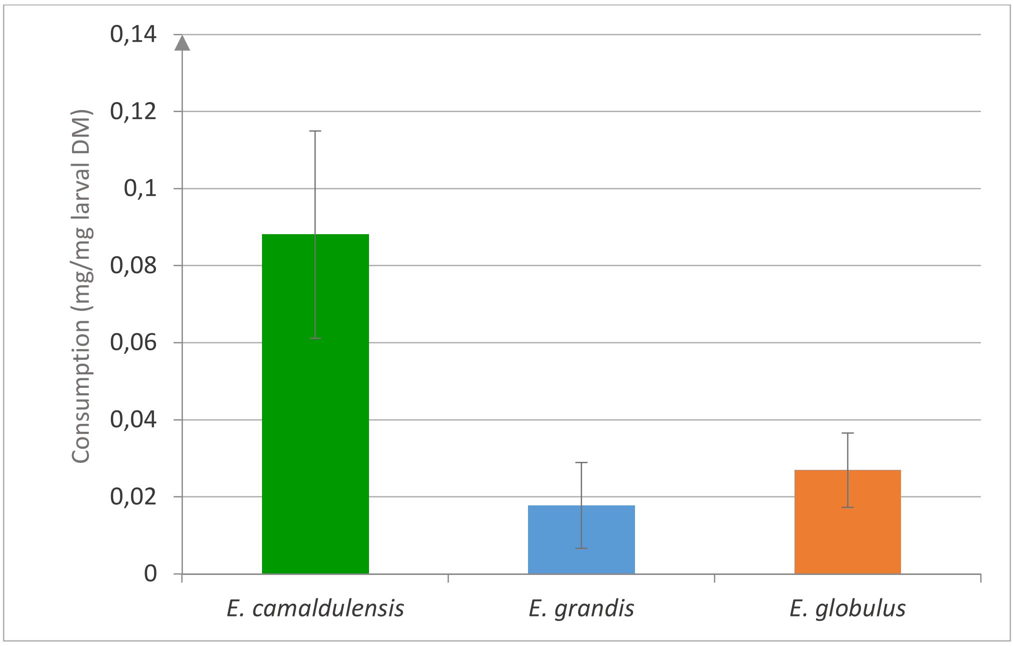

Figure 16. Consumption, by invertebrates, of 4-weeks conditioned *E. camaldulensis, E. grandis* and *E. globulus*, offered simultaneously to *S. vittatum* larvae, for a period of 16h. Values are means (n = 10) ± SE.

CHAPTER 4

Discussion

4. Discussion

According to the results, *E. camaldulensis* seems to be the less recalcitrant of the three tested eucalyptus leaves: leaf litter decomposition mediated by fungi presented the fastest decomposition rate (k = 0.0224 day^{-1}), the highest microbial respiration and the highest sporulation rates. This may be related with a higher nutritional value of this eucalyptus species in relation to the others, as *E. camaldulensis* was softer and presented higher N, P and lower initial phenolic concentrations (~4 %). Several studies indicate that fungi (and invertebrates) prefer N rich leaves with low amounts of secondary compounds. In fact, *S. vittatum* larvae preferred this species in the simultaneous presence of the others, which is in agreement with the general belief that invertebrates may detect leaves of higher nutritional quality (Irons et al., 1988; Danger et al., 2012). This discriminative behaviour may occur due to a higher colonization (expressed by increased respiration) and to an inherent comparatively higher quality. Several studies indicate strong relationships between microbial respiration, nutritional quality of substrates and decay rates (Gulis & Suberkropp, 2003; Stelzer et al., 2003; Fanin et al., 2011). Although no statistical differences were found in consumption rates, values were 50% lower in *E. globulus* and, despite no differences between *E. grandis*, preference tests show a clear tendency towards *E. camaldulensis*, which corroborates the idea that this species is more easily degradable by invertebrates.

Toughness is strongly correlated with low decomposition rates (Gessner and Chauvet 1994; Hutchens and Benfield 2000; Royer and Minshall, 2001; Ardón et al., 2009; Tonin et al., 2014). While initial toughness values of *E. camaldulensis* were close to the ones found in recalcitrant native species like oak (e.g. Gonçalves et al. 2013), these values decreased 84.6 % after the conditioning period (more than *E. grandis* - 74.4% - and *E. globulus* - 74.6%) This was likely related with a conditioning effect – fungal enzymatic enzymes such as cellulases breakdown the structure of the cell walls, degrading their refractory structural compounds (Chamier, 1985) – and with a richer fungal colonizing assemblage (Danger et al., 2012), but also with a detachment of the cuticle (Fig. 9) – as opposed to the other species (Figs. 10 and 11), which facilitated mycelium progress in the mesophyll (and sporulation), overall decomposition and consumption, similarly to previous studies (Tonin et al., 2014). *A priori*, a higher stomatal cell area observed in the leaves of this species might also have facilitated an earlier and faster access of the fungal mycelium to the mesophyll contributing to the conditioning effect (Canhoto & Graça, 1999; Ali et al., 2009). Furthermore, these differences in toughness loss could also be explained by different percentages of structural compounds of these species (Ribeiro et al., 2002; Tarafdar et al., 2003; Tonin et al., 2014). The relationship between low contents of structural compounds (and though toughness) and fast decomposition rates in streams was also referred in a previous study using *E. camaldulensis* (Gonçalves et al., 2017).

E. grandis presented higher breakdown rates (k = 0.0201 day^{-1}) than *E. globulus (k* = 0.0134 day^{-}1) which may be related with a thicker waxy cuticle of *E. globulus*, more difficult to degrade (*pers. obs*), along with, eventually, a distinct quality and quantity of essential oils and phenolic compounds that may delay and/or limit degradation. Higher respiration rates (and though fungal activity; Canhoto et al., 2017) could, in fact, be observed in *E. grandis* (vs. *E. globulus*). It seems that, in this case, mycelial activity occurs at the expenses of sporulation, which presented the lowest rates (and spore diversity). An investment in biomass production and/or degradative activity of the mycelia (Maharning & Bärlocher, 1996) may occur in the fungal communities colonizing *E. grandis,* which may stimulate degradation. Nonetheless, no differences could be detected between both leaves consumption in either feeding trials. Based on the composition of the fungal communities after the conditioning period, and assuming that the proportion of the spores corresponds to a trend in each species relative biomass (Maharning & Bärlocher, 1996), this may result from a less diverse community or to the presence of a less palatable assemblage in relation to *E. camaldulensis*. It is generally recognised that fungal richness may stimulate consumption and that invertebrates prefer some fungal species to others (Canhoto & Graça, 2008; Gonçalves et al., 2014). In all cases (particularly in *E. grandis*), *T. marchalianum* dominates the assemblages after 4 weeks conditioning; this species is frequently considered unpalatable (Gonçalves et al., 2014) and dominant (Dang et al., 2005; Pérez & Galán, 2014). The absence or reduction of other fungal species enhancing such negative effect seems likely, but needs to be clarified. Furthermore, fungal consortium is also said to influence decomposition; both in *E. grandis* and *E. globulus*, fungal assemblages are poor, which may be related with a higher degradative capacity of the hyphomycetes species, allowing them to cope with more recalcitrant leaves. On the other hand, *E. camaldulensis* held a more complex fungal assemblage. This could determine an easier degradation of structural compounds and overall decomposition, since different species detain not only different enzymatic potentials, but also different chemical compositions and nutritional values (Canhoto & Graça, 2008). As leaf litter quality also determines fungal interactions, these different fungal assemblages can be related with strong antagonistic relationships between species, inhibiting sporulation on some of them (Treton et al., 2004), although synergistic effects can also occur between species (Treton et al., 2004; Duarte et al., 2006).

The feeding trials results are consistent with previous studies (Canhoto et al., 2013; Martins et al., 2013) and particularly support a difficult incorporation of *E. globulus* leaves into secondary production. *E. grandis* was also proved to have a similar tendency (e.g. Tonello et al., 2014) in comparison with *E. camaldulensis*.

In Portugal, exotic eucalyptus streams are usually intermittent (Canhoto & Laranjeira, 2007). Drought periods usually occur in summer - which can isolate small patches of water filled with leaves (Abelho & Graça, 1996; Grigg & Mulligan, 1999; Graça et al., 2002) that release into a warmed water toxic compounds, which contribute to a decrease in pH and dissolved oxygen (Canhoto & Laranjeira, 2007; Canhoto et al., 2013). Only a limited number of invertebrates (and aquatic hyphomycetes) can cope with such conditions (Canhoto & Graça, 1999; Otermin et al., 2002; Medeiros et al., 2009; Canhoto et al., 2013). *E. globulus* and *E. grandis* do not differ in the amount of phenolic compounds released during leaching. Considering the potential of these compounds to change water quality (Canhoto & Laranjeira, 2007; Canhoto et al., 2013) and their direct toxic and indirect (i.e. through leaf litter quality) deleterious effects on stream biota (Hepp et al., 2008; Canhoto et al., 2013; Martins et al., 2013; Tonello et al., 2014), it seems likely that, specially under the expected increase in global temperature, both species might have a stronger nefarious effect on streams structure and function than *E. camaldulensis*.

Nowadays, *Eucalyptus spp.* is usually planted in a monoculture regime to increase economic incomes, which often constrains the allochthonus food supply source to one species. According to our results, *E. camaldulensis* (and *E. grandis*) might have less ecological impacts than the already observed negative effects that *E. globulus* plantations have to low order streams (Larrañaga, 2009, Ferreira et al., 2015). However, this study has the limitations of laboratory studies and only considered short-term tests with invertebrates. Whether the tolerance shown towards *E. camaldulensis* is real under long-term scenarios (e.g. growth tests) is yet to be defined. Several studies in Portugal and Spain advise the maintenance or reforestation of riparian areas with native trees (Abelho & Graça 1996; Graça et al., 2002; Molinero & Pozo, 2004) in areas forested with eucalyptus monocultures. Our results suggest that, in areas were eucalyptus are being explored for economical purposes, the inclusion of *E camaldulensis* could support the role of the native riparian areas in preserving the streams bordered by plantations of these exotic species.

CHAPTER 5

References

5. References

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