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Ao meu pai, que me amou muito, que me ensinou
a amar a natureza recordo com orgulho.

«Não resisti. Regressei à minha velha casa, e ali, sob a sombra
do tamarindo, me deixei afogar em lembranças. […]
O tamarindo mais sua sombra: aquilo era feito para abraçar
saudades. Minha infância fazia ninho nessa árvore. Em minhas
tardes de menino, eu subia ao último ramo como se em ombro de
gigante e ficava cego para assuntos terrenos. Contemplava era o
que no céu se cultiva: plantação de nuvem, rabisco de pássaro. E
via os flamingos, setas rapidando-se furtivas pelos céus. Meu pai
sentava em baixo, na curva das raízes, e apontava os pássaros:
-Olha, lá vai mais outro!
O flamingo parecia retardar sua passagem.»

Mia Couto, "O ÚLTIMO VOO DO FLAMINGO"

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À minha tia Nana pela sua presença constante, pela sua preocupação e pela força dada ao longo do meu caminho, um obrigada do fundo do coração.

Aos meus pais pelo Amor, força e educação que me deram. Obrigada por tudo! Um especial obrigada à minha mãe pela força, pela atenção constante, pelo apoio inquestionável, pela opinião sempre acertada e pela sua palavra amiga e de encorajamento. Assim como pela ajuda ao tornar tudo mais fácil, suportável e possível.

Ao meu querido irmão por me ter mostrado Coimbra, por estar sempre ao meu lado, pela sua amizade, pela paciência, pelo ensinamento e pelo entusiasmo que me incute na descoberta da natureza e me guia em percursos fantásticos que deslumbram os sentidos. Obrigada, Pedro!

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Resumo

A sedimentação excessiva constituiu actualmente um dos maiores problemas para a integridade ecológica dos cursos de água. No entanto, ainda pouco se sabe sobre os efeitos deste problema no biota e processos chave para o funcionamento dos ecossistemas ribeirinhos, como a decomposição da folhada. Neste trabalho pretendemos avaliar o efeito dos sedimentos finos (< 2 mm) no processamento de folhas de carvalho por fungos (Hifomicetes Aquáticos) e invertebrados. Para isso foi avaliado em laboratório o efeito da velocidade de transporte e granulometria de sedimentos (< 0,063 mm e < 2 mm) na perda de massa da folhada e parâmetros microbianos associados. Verificou-se que as duas velocidades de deslocação de sedimentos simuladas (60 e 120 rpm) não afectaram a perda de massa, a dureza da folha ou a taxa de esporulação fúngica, independentemente da presença ou não de sedimentos. Sedimentos de granulometria mista < 2 mm estimulam as taxas de decomposição da folhada por abrasão, enquanto que sedimentos de diâmetro < 0,063 mm parecem induzir hipóxia no substrato inibindo o crescimento da biomassa fúngica e a decomposição; neste caso, ocorre um maior investimento na reprodução. O efeito dos sedimentos depositados (< 2 mm), e o efeito dos sedimentos em suspensão (< 0,063 mm) na performance dos invertebrados foram avaliados através de testes de consumo com o insecto triturador *Sericostoma vittatum*. Os resultados indicam que a deposição dos sedimentos limitam o consumo foliar uma vez que os invertebrados não são capazes de encontrar alimento enterrado a profundidades ≥ 1,5 cm; sedimentos < 0,063 mm em suspensão (105 < TDS < 110) não afectam as suas taxas de consumo.

Os testes realizados sugerem que os efeitos dos sedimentos dependem da sua deposição ou suspensão, granulometria e heterogeneidade, afectando de forma específica decompositores e detritívoros e, portanto, a reciclagem de nutrientes e fluxo de energia do ecossistema ribeirinho.

Palavras-chave: Decomposição, comportamento alimentar, fragmentação física, velocidade da corrente.

Abstract

Nowadays, the increased sedimentation due to anthropogenic activities is one of the biggest threats to the ecological integrity of watercourses. However, there are few studies about the impact of sediments on biota and on key processes for stream functioning, such as leaf decomposition. In this work we aimed to evaluate the effect of fine sediments (< 2 mm) on the breakdown process of oak leaves by fungi (Aquatic Hyphomycetes) and invertebrates. Therefore, the effect of transport velocity and sediment granulometry (< 2 mm and < 0.063 mm) on the loss of leaf mass and microbial parameters associated were assessed. The two transport velocities chosen (60 and 120 rpm) did not affect mass loss, leaf toughness or sporulation rate, regardless the presence or absence of sediments. Real mixture sediments (< 2 mm) stimulate breakdown rates due to physical abrasion. On the other and, sediments smaller than 0.063 mm seem to induce hypoxia conditions, inhibiting fungi biomass and decomposition; in this case fungi invest more on reproduction while reducing its biomass. The effect of deposited sediments (< 2 mm) and the effect of sediments in suspension (< 0.063 mm) in the performance of invertebrates were assessed through leaf consumption tests by the shredder *Sericostoma vittatum*. The results show that sediments may limit the consumption of leaves since the shredders are not able to feed on leaves buried at ≥ 1.5 cm; sediments < 0.063 mm on the water column (105 < TDS < 110) does not affect consumption rates. The experiments done suggest that the effect of sediments may depend on their deposition or suspension, granulometry and

heterogeneity, affecting decomposers and detritivores in a specific way, and therefore, nutrients recycling and energy flux of the stream ecosystem.

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CHAPTER 1

- General Introduction -

1. GENERAL INTRODUCTION

Freshwater systems are essential natural resources for life (Vörösmarty et al., 2010). Despite constituting only about 0.01% of the world's water these systems directly support 6% of all described species (Malmqvist & Rundle, 2002). Freshwaters are among the most endangered ecosystems, being threatened by anthropogenic activities such as urbanization, industrialization, water pollution, flow modification, destruction or degradation of habitat and invasion by exotic species (Dudgeon et al., 2005). Furthermore, water resources are not evenly distributed, being barely accessible in some areas (Dewson et al., 2007).

Running waters are linked to and dependent on the surrounding catchment areas, establishing strong and specific relationships with the adjacent terrestrial areas (Hynes, 1975). Streams in particular are being affected by the interaction of multiple factors including land use modification (Meyer & Wallace, 2001), deforestation (Naymik & Pan, 2005), and variations in the stream geomorphology and hydrology. In fact, recent data indicate that human activities may increase the rates of sedimentation of up to 10 times the natural inputs (Leigh & Webb, 2006).

1.1. Low order streams

Headwater streams, located at the beginning of the river continuum (Vannote et al., 1980) may constitute up to 85% of the total length of fluvial network (Allan & Castillo, 2007). Headwaters are low order streams (1-3 order; Vannote et al., 1980), usually shaded by riparian vegetation with a low

autotrophic production. Even though primary producers (*e.g.* algae) can be found attached to submerged surfaces (periphyton) like stones and wood stocks in areas where light conditions are appropriate, this autochthonous source of organic matter does not play a fundamental role in the energy flow of shaded headwaters (Allan & Castillo, 2007).

In temperate low order streams, the energy source is strongly dependent on the deciduous riparian vegetation - allochthonous inputs of organic matter - mainly composed by leaf litter (Molinero & Pozo, 2004) but also by floral fragments, bark, wood and fruits (Benfield, 1997). This material may vary in quality and quantity, depending on the composition of the riparian vegetation, and is usually supplied to the stream with a marked seasonality. The higher litter input occurs during autumn/winter (Abelho & Graca, 1996), and this leaf material is the main support of the aquatic food webs based on detritus for the following months until next fall (Sponseller & Benfield, 2001). Thus, some invertebrates have synchronized their life cycles with the litter inputs (Vannote et al., 1980; Cummins et al., 1989).

The conversion of these leaves into living biomass, *i.e.*, leaves processing, is largely promoted by the activities of decomposers (mainly fungi - Aquatic Hyphomycetes - and bacteria) and detritivores (mainly shredders; Gessner et al., 1999). Detritus decomposition constitutes a key-ecosystem level process in these ecosystems (Ferreira et al., 2013). This process is primarily determined by the chemical and physical characteristics of the leaves (Abelho, 2001) and is modulated by environmental factors (Young et al., 2008). For example, soft nutritious leaves as alder are known to decompose faster than the tough oak leaves rich in lignin, cellulose and phenolic compounds (Cortez et

al., 1996). It is also generally accepted that in warmer or nutrient enriched waters leaves decompose faster (Webster & Benfield, 1986; Menéndez et al., 2003); on the other hand, low pH usually means a slower breakdown (Young et al., 2008; Riipinen et al., 2009). Other factors such as the stream hydrological regime, water velocity (Dewson et al., 2007) and type of sediment of the stream (Chauvet, 1997), may also influence leaves degradation (Young et al., 2008).

1.2. Leaf litter breakdown in streams

Leaf decomposition starts upon leaves immersion and usually follows three main steps: leaching, conditioning and fragmentation. These steps may overlap, although a more or less defined sequence is usually observed (Gessner et al., 1999).

Leaching is a fast abiotic process that usually lasts for 48 h. During this process, soluble organic and inorganic compounds are released from leaves to the water (Abelho, 2001; Allan & Castillo, 2007), and up to 42% of leaf weigh may be lost (reviewed by Abelho, 2001). The quality and quantity of the leachates are determined *a priori* by chemical and structural differences of the leaf species (Campbell et al., 1992) and can be influenced by several abiotic factors such as pH, water temperature and turbulence (Abelho, 2001).

Conditioning is mainly performed by fungi (namely Aquatic Hyphomycetes) and also by bacteria. This prokaryotic group is usually more important in advanced stages of leaves decomposition. Aquatic hyphomycetes are usually dominant in the first phase of the colonization process and are the main responsibles for the increase in palatability and nutritional value of detritus

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to invertebrates (Gessner, 1999); this change in the detritus characteristics is known as conditioning.

Changes in leaves nutritional value include increases in Nitrogen and Phosphorous, softening, degradation of recalcitrant Carbon and enrichment with fungal enzymes (Canhoto & Graça, 2008). Fungal spores settlement (Kearns & Bärlocher, 2008), fungal biomass and sporulation also depend on leaves physic-chemical characteristics and is also modulated by environmental factors such as flow and water chemistry (Abelho, 2001). The flow allows greater turbulence and oxygenation of water, better for fungi colonization (Chauvet, 1992). According to Ferreira et al. (2006), the current velocity is able to control the fungal parameters, stimulating the spore release and the conidial production, being the presence of fungal species higher.

The last phase of decomposition is usually designated as fragmentation. It can be promoted by physical abrasion and/or invertebrates, during their feeding or "architectural" activities to build their cases (Abelho, 2001). Invertebrates activities are known to accelerate the fragmentation process (Graça et al., 2001). There are many studies that indicate invertebrates as active subjects on fragmentation (Gessner & Chauvet, 1997; Abelho, 2001; Ferreira & Graça, 2006).

Shredders usually make a great contribution to the Fine Particulate Organic Matter (FPOM) content in streams (Covich et al., 1999) by breaking leaves into smaller fragments, increasing in that way the area available for microbial activity and providing extra food for collectors downstream; 60% of the ingested material is converted into faeces (Cummins et al., 1989). Shredders, important ecological engineers in the stream (Moore, 2006), are fundamental

for the lotic system as they process fluxes of matter downstream, playing an important role on the river continuum concept (Vannote et al., 1980). Shredders prefer to feed on leaves colonized (Graça, 2001), since fungi change chemically and physically the quality of leaves (Canhoto & Graça, 2008), and might also prefer particular fungi (Lecerf et al., 2005) or even different combination of leaf/fungi species, which may provide specific and/or distinct and fundamental nutrients and carbohydrates to the consumers (Barlocher et al., 2003). On the other hand, fungi can also have preference for different leaves (Canhoto & Graça, 2008).

It is generally recognized that leaves physical abrasion promotes fragmentation (Chauvet et al., 1993); however, the relative importance of this promoter of abiotic degradation is still unknown (Heard et al., 1999).

Leaves physical fragmentation on streams is ruled by leaves characteristics and is dependent on environmental factors such as current velocity, substratum and quantity and quality of the sediment in transport (Chauvet et al., 1993). The loss of leaves integrity is facilitated by the conditioning process and is usually slower when leaves are in pools rather than in riffle areas (Casas, 1996). As stated by several authors (Benfield et al., 2001; Lepori et al., 2005) higher flows, especially during floods, increase the amount of suspended sediments, and consequently the physical abrasion of leaves. However, the opposition was also found: Matthaei et al. (2010) found an increase in decay rate at reduced stream flows, which can be explained by the retention of sediments resulting from flow reduction. In fact, when leaves are

submitted to coarse sediment, a higher breakdown rate is usually observed, when comparing to fine sediment (Young et al., 2008).

1.3. Sediments and sedimentation

1.3.1. Types of sediments

Sediments can be characterized according to their shape, size, mineralogy, colour, and orientation (Gordon et al., 2004). The type of sediments usually depends on the origin of the input: hill slopes, abrasion and/or sorting (Allan & Castillo, 2007), and their deposition will be influenced by their shape, size, density and fall velocity (Bethwell & Mutz, 2005). Nevertheless, the impacts of sediments on ecosystem will depend on the characteristics of the stream (*e.g.* topography and soil type), surrounding areas, and the volume of sediments transported (Wood & Armitage, 1997).

Sediment loads can be determined by the amount of sediments transported in a point during a specific time interval, multiplied by the water discharge (Allan & Castillo, 2007). The expression "fine sediment" usually defines elements smaller than 2 mm, including clay, silt and sand (Wood & Armitage, 1997). However, not all authors use the same designation, some considering particles smaller than 250 µm to be fine sediment (Kreutzweiser et al., 2005).

According to Gordon et al. (2004), there are three major groups of sediment loads: flotation load, dissolved load (particles carried in the solution) and sediment load. This last group is divided into two categories: washload, (< 0.063 mm), and bed-material load that can be suspended load or bedload.

Division in these categories is difficult though, because they change substantially depending on water flow (Fig. 1).
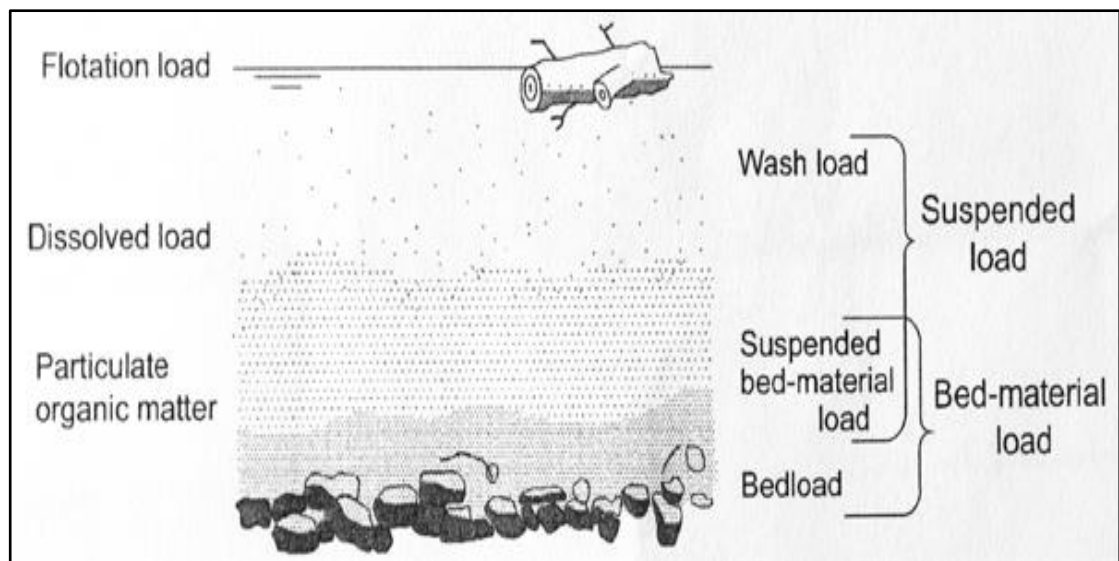
Figure 1 – Representation of the different sediment loads of the stream according to Gordon et al. (2004).

The transport of sediments is a natural feature of streams. Transportation and distance are directly related with the flow, since higher flows transport sediments further. Usually, all sediments derive from erosion of the basin slope, but urban streams have a different sediments supply, since it depends on urban infrastructure, usually presenting bed erosion, with different consequences (Allan & Castillo, 2007). Fine sediments can be transported either by rolling, sliding or salting (Wood & Armitage, 1997), or on the water column when flow is sufficient (Petts et al., 1985; Fig. 2).
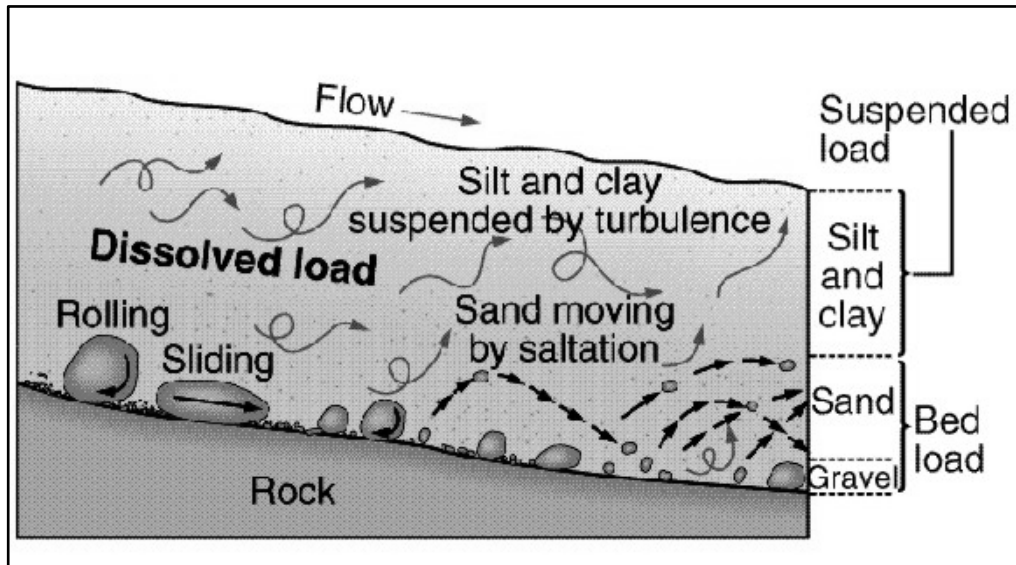
Figure 2 – Categories of sediments transportation in a stream by Petts et al., 1985.

1.3.2. Excessive sedimentation in watercourses: a worldwide problem

Sediments are a natural component of streams. However, the amount of sediments produced and transported are presently excessive due to human activities (Schofield et al., 2004), and sedimentation is threatening the aquatic fauna (Richter et al., 1997). The concept of sedimentation includes "the process whereby substrata are covered and interstitial spaces of the substrata are filled by deposited sediment" (Henley et al., 2000). This process may change the substrate through changes in superficial surface (Graham, 1990) and depends on the flow (Wood & Armitage, 1997). Usually, sedimentation is higher when the flow is low, since it allows the suspended sediments to settle (Wood & Armitage, 1997). However, according to Bond (2004), sedimentation can be lower when the flow is reduced, since the quantity of suspended sediments transported also decreases.

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Excessive sedimentation is a worldwide problem frequently associated with anthropogenic activities. Such activities include excavation of river sand, forest clearance (Broekhuizen et al., 2001; Larsen & Ormerod, 2010), modification of riparian vegetation and changes within the drainage basin such as agricultural activites (Townsend et al., 2008; Larsen & Ormerod, 2010), industrialization (Broekhuizen et al., 2001), construction of road structures (Extence, 1978), and/or mining (Broekhuizen et al., 2001). All these activities are known to change the dynamic of sediment transportation and settlement (Waters, 1995).

Sedimentation is presently considered one of the main threats to the ecological integrity of streams and rivers (Young et al., 2008; Benoy et al., 2012) with important consequences on the fluvial continuum. On one hand, big amounts of sediment transported can lead to problems in the estuarine areas and reefs, whereas a lack of sediment transported downstream can lead to a retreat in river deltas and coastal shorelines, causing losses of habitat and increasing vulnerability to storms (Allan & Castillo, 2007). On the other hand, overload sedimentation in potable waters requires filtration treatment, which can be avoided by reforesting some species (Lake et al., 2000).

1.3.3. Consequences of sedimentation for the biota

Sediments can be either carried by the flow, increasing turbidity, or deposited on the bottom of the stream. An excess of sediments can affect the biota (Larsen & Ormerod, 2010).

Primary producers are affected by sedimentation (Quinn et al., 1992), with macrophyte leaves and stems directly affected by scouring and abrasion

(Lewis, 1973). Thus, primary production can be reduced, especially in situations where light available is low (Wood & Armitage, 1997), producing a bottom-up effect in food webs. The diversity of benthic invertebrates can be changed indirectly, since sediments can occupy interstitial habitats, reducing the abundance and/or quality of periphyton (food source for most invertebrates; Broekhuizen et al., 2001).

Specific substrate types may determine and shape invertebrate communities present in streams. For example Oligochaeta, Sphariidae (Armitage, 1995), and Chironomidae larvae can be benefited by the abundance of fine sediments as they use these particles to construct cases and tubes (Dudgeon, 1994). Trichoptera belonging to the Families Glossossomatidae, Goeridae and Sericostomatidae use small pebbles to build cases, which provide shelter from predators (Schofield et al., 2004). But sediments can limit species survival (Jowett et al., 1991) by inhibiting, damaging or reducing their filter feeding capacity (Aldridge et al., 1987) and respiration structures (Lemly, 1982), leading to an increase in drift (Gibbins et al., 2004; Larsen & Ormerod, 2010). In fact, Suren & Jowett (2001) demonstrated that drift may be related with the size of sediments, with smaller sediments promoting higher rates of drift. Sediment inputs can increase drift directly due to particles, or indirectly because sediments may reduce the quality of food resources (Graham, 1990). Changes in habitat conditions promoted by sediments may also affect the invertebrates assemblages: Simuliidae larvae, for example, need clean rocks, not covered by sediments, to attach their silks (Management biodiversity issues, 2001). Habitats predominated by either fine or large sediments are poorer than mixture environments, suggesting that the heterogeneity of the substratum is

usually related to high diversity of invertebrates, since sediment sizes can influence the bedload as well as its mobility (Gibbins et al., 2004).

Mussels for example can also be affected by fine sediments: bivalves feed trough the filtration of water, so they are directly sensible to increases of sediments in the water column (Shin, et al., 2002). If females face limitation in food resources, the amount of nutrients needed for egg production is insufficient and reproduction might be affected (Landis et al., 2013). Furthermore, since sediments are often associated with pollutants, bivalves can bioaccumulate the pollutants present in the water (Shin, et al., 2002).

Sediment impacts on fish are well documented, due to their economic and recreational importance (Wood & Armitage, 1997). The consequences of sediment loads on fish are vast: difficulty in swimming, decrease of growth rate, lower tolerance to diseases, and clogging of the gill filaments causing asphyxiation (Bruton, 1985), which can alter the usual migration patterns of fish (Alabaster & Lloyd, 1982). They can also change the feeding behavior, since an increase of suspended sediments can obstruct the filter feeding capacity (Aldridge et al., 1987). Also, fish species that are used to clean waters will be in disadvantage, and predator-prey relation may be changed (Ranåker et al., 2012). Furthermore, deposited sediments can clog gravel-spawning grounds of several fish species affecting fish communities (Sear, 1993).

It is known that rivers suffer flow variability, from floods to low flows, and this result in variations of the suspended solids concentrations, as well as on their deposition (Wood & Armitage, 1997). Hence, benthic faunal communities is likely able to deal with these variations on sediment loads. Rapid increases of

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sediment loads may allow a rapid recovery. Nevertheless, constant sediments input due to human activities can modify the natural fauna. Furthermore, the input of sediments to streams is usually associated with nutrient enrichment as a result of watershed logging (Benfield et al., 2001), chemicals input due to agriculture intensification (Liess et al., 1999) or even heavy metals and toxic substances pollution that can be aggregated to sediments (Gordon, 2004).

Input of sediments into the streams is a natural process; it becomes a problem when higher inputs due to human activities affect and threat all natural cycles of the ecosystem (Management biodiversity issues, 2001).

1.4. Main objectives

In this study we assessed the effects of sediments, in transport or deposited, in leaves decomposition promoted by fungi and invertebrates. For that purpose, several experiments were carried out to assess the effect of water velocity (with consequent distinct intensities of bed-material loads) and sediment granulometry on microbial mediated decomposition. Tests were also performed in order to address invertebrates consumption in environments where their feeding behavior was challenged by high amounts of suspended bed-load or deposited sediment over the food items.

CHAPTER 2

- Materials and Methods -

2. MATERIALS AND METHODS

2.1. General

Oak (*Quercus robur* L.) leaves were collected after senescence, air dried in the dark at ambient temperature and stored until needed. Initial phosphorus, nitrogen and total phenols were determined (Graça et al. 2005). Before using the leaves, they were moistened with distilled water, in order to avoid breakage during handling. Leaves were assembled in groups of ~2 g in fine mesh bags (10 × 15 cm, 0.5 mm mesh) to avoid decomposition by macroinvertebrates. A total of 13 bags were incubated, for 21 (December, winter) or 10 days (March, spring), in a second order stream located at Lousã mountain, Central Portugal (Candal stream; 40º04'48.10''N, 8º12'11.16''W, 634 m a.s.l.).

After the incubation periods, bags were brought to the laboratory in a cooler and were carefully rinsed with distilled water to remove attached sediments. Stream water samples were also transported to the laboratory, filtered (fiberglass filter, Millipore APFF) and frozen at −18 °C for determination of cations and anions by ion chromatography (Dionex DX-120, Sunnyvale, California, USA) and soluble reactive phosphorus (SRP) concentration (Allan & Castillo 2007). Stream substratum was collected and sieved *in situ* in order to obtain sediment < 2 mm. Hereafter this sediment will be referred as real mixture (RM). In the laboratory, sediment was ignited (550ºC; 4 h) to remove organic matter; being separated in 2 size classes (< 0.063 mm and < 2 mm) using a sieve shaker (Fig. 3).

Figure 3 – Sieve shaker used to separate the sediment collected in Candal stream (< 2 mm) by size classes (< 2 mm and < 0.063 mm)

2.2. Microbial-mediated decomposition tests

2.2.1. Effects of sediment load velocity on leaves decomposition

Ten pairs of leaf discs were symmetrically obtained from the conditioned leaves, with a cork borer (Ø = 12 mm). One disc of each pair was used to evaluate leaf toughness expressed as the required mass (g of water in a container) to push a 1 mm diameter metal shaft through the leaf disc. These discs were then oven-dried (105ºC; 48 h) and weighed to evaluate initial dry mass (DMi). The correspondent pairs were immersed in 20 Erlenmeyers of 500 ml with 10 discs each, previously filled with 200 ml of filtered (filter paper) stream water.

A total of 20 Erlenmeyers (each one with a total of 10 discs obtained as above) were divided into two horizontal shakers rotating at 60 or 120 rotations

per minute (rpm) (Fig. 4). In each shaker, half the Erlenmeyers (n = 5) were provided with 26 g of RM while the other half only contained stream water. Incubation lasted for 4 weeks, in an acclimatized room at 15 ± 2ºC. At the end of the experiment, sets of 5 discs from each replicate were used to determine sporulation. For subsequent counting, all conidial suspensions were mixed with 100 µl of Triton X – 100 solution (0.5%), an aliquot was filtered (Millipore SMWP, 5 µm pore size) and the spores retained stained with 0.05% cotton blue in acid lactic (60%). Spores were counted under a compound microscope at 250× (Graça et al. 2005).

All incubated leaf discs were oven dried (105ºC; 48h) and weighted. Remaining dry mass was evaluated as the difference between the initial and the final dry mass of the leaf circles in each microscosm.

2.2.2. Effects of sediment size on leaf litter decomposition

Oak leaves conditioned in the stream for ten days (in Spring) were used to obtain pairs of leaf discs as above: half the discs were oven-dried (105ºC; 48h) and weighted to determine initial dry mass. The correspondent pairs were equitatively distributed by Erlenmeyers, in groups of ten: groups of five Erlenmeyers were provided with 26 g of RM, 26 g of extra fine sediment (Ø < 0.063 mm; EFS treatment) or No sediment was added. After this period, as above, sets of five discs from each microcosm were used to induce sporulation. For counting and identification, all conidial suspensions were mixed with 100 µl of Triton X – 100 solution (0.5%), an aliquot was filtered (Millipore SMWP, 5 µm pore size) and the spores retained were stained with 0.05% cotton blue in acid lactic (60%). Spores were identified and counted under a compound

microscope at 250× (Graça et al. 2005). Sporulation rates were expressed as number of conidia/mg DM/day.

Three out of the ten leaf discs from each microcosm were used to evaluate ergosterol content as a proxy of fungal biomass (Gessner & Chauvet 1993; Young, 1995). Leaf discs from all replicates and treatments were freeze dried, lyophilized and weighted. Ergosterol extraction was performed according to Gessner et al. (2003): ergosterol concentration was quantified by high performance liquid chromatography (HPLC) using a Merck LiChroCART 250-4 (LiChrospher 100) RP-18 column, by measuring absorbance at 282 nm (Young 1995). Ergosterol was converted into fungal biomass using a conversion factor of 5.5 μg ergosterol/mg fungal DM (Gessner & Chauvet, 1993). Results were expressed as mg fungal biomass/g DM. Dry Mass remaining (DMr) was evaluated and expressed as percentage of initial dry mass.

2.3. Invertebrates feeding tests

Larvae of *Sericostoma vittatum* Rambur (Trichoptera; Sericostomatidae) were collected in a low-order stream of Central Portugal (Ribeira de São João, Lousã mountain, Central Portugal; 40º05'59"N, 8º14'02"W) and transported to the laboratory in cooled plastic boxes. In the laboratory, animals were kept in plastic containers filled with stream water with stream sediment in the bottom (15ºC; 12 h light: 12 h dark photoperiod). They were fed *ad libitum* with stream conditioned oak leaves.

Consumption tests were run with individuals of medium size (8.8 mg ± 3.3) evaluated using the diameter of their case opening; relationship between

the case opening and dry mass was achieved by the formula: DM = 0.0136 x CO − 0.0162 (R^2 = 0.83) where DM is dry mass (mg) and CO is case opening (mm) (Ferreira et al., 2010).

In all tests, measured individuals were starved for 24 h prior to the experiments and then randomly allocated in the microcosms (5.5 cm diameter; 15.5 cm height) provided with ignited (550ºC; 4 h) stream sediment (EFS or RM) at the bottom, and filled with 300 ml of filtered stream water (filter paper) continuously aerated. Experiments were stopped when half of the discs were eaten in 50% of the microcosms.

2.3.1. Effects of sediment in suspension on invertebrates consumption

A total of 60 microcosms were provided with 3 g of sediment (< 0.063 mm). In half of the containers a longer oxygenation tube (Suspended sediments treatment; SS) ensured a higher turbulence of the water, allowing the sediments to be continuously suspended in the water column instead of deposited in bottom (Control treatment; C). Three small stones were placed in each container in order to provide the possibility of refuge from turbulence to the invertebrates. At the beginning of the experiments, two symmetrical discs were cut from the conditioned oak leaves conditioned in the stream; leaf discs were obtained avoiding the central vein. Discs from each pair were assumed to have identical initial mass. Each microscosm was provided with one oak disc placed on the top of the sediments and another enclosed in a fine mesh bag (0.5 mm mesh size; 3 x 4 cm) attached with a clip to the top of the microscosm. This bag was kept inside the water, but no contact was allowed between the leaf disc and the animal (control disc). The discs were stuck to the bottom of the cup with

pins to ensure food availability in spite of the turbulence. Oxygenation and total dissolved solids (TDS) were daily monitored. Samples of water were taken from each microscosm at three water depths (top, middle and bottom) in order to check for sediment suspension uniformity. In this case, 2 ml of water were collected with a plastic pipette, placed into pre-weighed eppendorf tubes, centrifuged and dried (60ºC; 48 hours). Eppendorf tubes were then re-weighted and sediment quantity trapped in the water column assessed as the difference between the eppendorfs weight. After 2 days, the experiment was stopped and discs were dried (60° C; 48 h). Consumption in each microcosm was estimated as the difference between the control and the correspondent disc offered to the animal. Consumption was expressed as mg leaf consumed/g animal/day.

2.3.2 Effects of sediment deposition on food consumption

A total of 45 microcosms were provided with 90 g of ignited RM originating a total sediment depth in the microcosm of 3 cm. Pre-measured *S. vittatum* larvae were randomly distributed by the microscosms and carefully placed on the top of the sediment. For each microscosm, two symmetrical discs were cut as above from the conditioned oak leaves avoiding the central vein; one was used to feed the animal and the other placed inside a fine mesh bag (0.5 mm mesh size; 3 x 4 cm) in order to prevent invertebrate's consumption – control disc. On the first treatment (n = 15) a single disc hold by a pin were placed on the top of sediment (hereafter called Top); on second treatment (n=15) the leaf disc was placed in mid-depth of the sediment ($\sim$1.5 cm depth; hereafter called Middle) while the last fifteen replicates had the disc placed in the bottom of the sediment (3 cm depth; hereafter called Bottom). The

experiment lasted for 6 days when 50% of the discs were half eaten by half the invertebrates in one of the treatments.

2.4. Data analysis

Leaves mass loss and toughness promoted by sediment abrasion at two water velocities, and sporulation rates observed in each treatment were compared by 2-way analyses of variance (ANOVA).

The effect of sediment granulometry on leaf mass loss, fungal biomass sporulation rates and total number of aquatic hyphomycete species were compared by 1-way ANOVA. Whenever necessary, a Tukey test was used. Leaf species specific fungal assemblages associated to all sediment treatment were analyzed by non-metric multidimensional scaling (NMDS) ordination, based on Bray Curtis similarity index of log (x+1) transformed conidial abundance data (PRIMER v6; Clarke & Gorley, 2001). An analysis of similarity (1-way ANOSIM) was also performed to test the similarity between treatments, with sediment granulometry as factor.

Leaves consumption by the invertebrates under distinct sediment suspension intensity was compared by a t-test. Invertebrate consumption of leaves covered by sediment was compared among treatments by 1-way ANOVA, followed by Tukey test whenever necessary.

Data were log (x+1) transformed when necessary to achieve the assumptions, normality and homogeneity of variances. All statistical analyses were performed to a significance level of $P = 0.05$ and carried out using STATISTICA v7 for Windows.

CHAPTER 3

- Results -

3. RESULTS

In Candal stream, the water was well oxygenated (>100%), circumneutral (pH = 6.8) and nutrient poor (NO^{3-} = 537 µg/L; NH^{4+} = 10 µg/L; soluble reactive phosphorus = 45.33 µg/L).

The physico-chemical characteristics of the pre-conditioned oak leaves (mean ± SE; n = 3) incubated in the stream for 28 (Winter) and 10 days (Spring) are shown in table I. Differences in incubation time were decided in order to provide similar leaf conditioning status in the leaves used in both microbial mediated experiments due to differences in mean water temperature (Winter, 6.3 ± 0.2; Spring, 11.9 ± 0.3).

Table I – Physico-chemical characteristics of the pre-conditioned oak leaves (mean ± SE; n=3).

	Oak	
	Winter	**Spring**
N (% DM)	2.250 ± 0.631	1.42 ± 0.064
C (% DM)	53.440 ± 2.271	48.04 ± 0.196
P (% DM)	0.050 ± 0.008	0.070 ± 0.010
Phenolics (% DM)	11.702 ± 0.755	10.09 ± 0.375
Toughness (g)	89.163 ± 3.173	146.13 ± 14.807
Lignin (% DM)	41.338 ± 0.288	41.513 ± 0.300

3.1. Microbial-mediated decomposition tests

3.1.1. Effects of sediment load velocity on leaves decomposition

Dry mass remaining ($P > 0.16$; Fig.4), leaf toughness ($P > 0.14$; Fig. 5) and sporulation rates ($P > 0.39$; Fig. 6) of the pre conditioned oak leaves were not different among treatments (presence of sediments and sediment loads velocity). Dry mass remaining varied between 35% (at 120 rpm with sediment) and 39% (at 60 rpm with sediment).
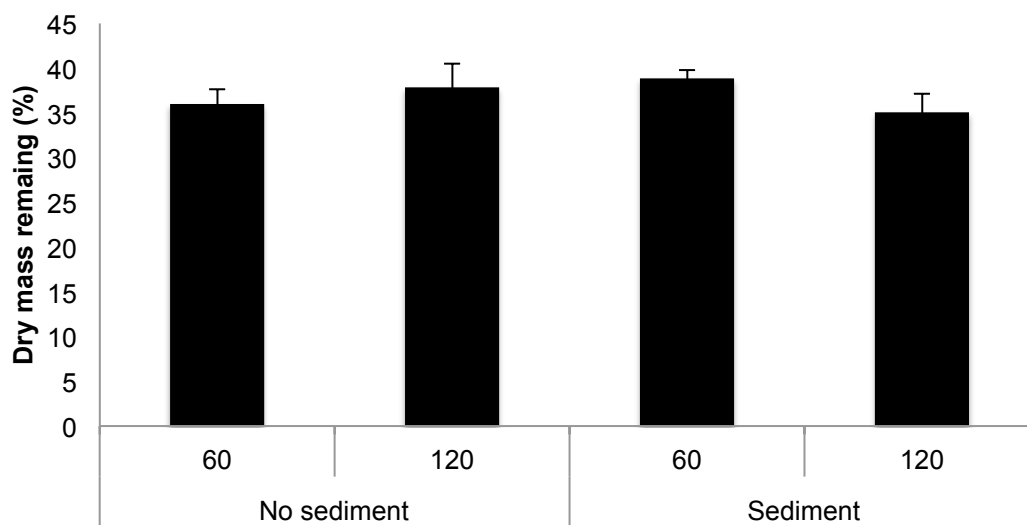
Figure 4 – Dry mass remaining (percentage ± SE) of oak leaves, after 28 days of incubation in the lab. Leaves were conditioned in microcosms with or without sediments (< 2 mm) and were maintained at 60 or 120 rpm. No letters indicate absence of differences within the two main factors (presence of sediments and sediment load velocity).
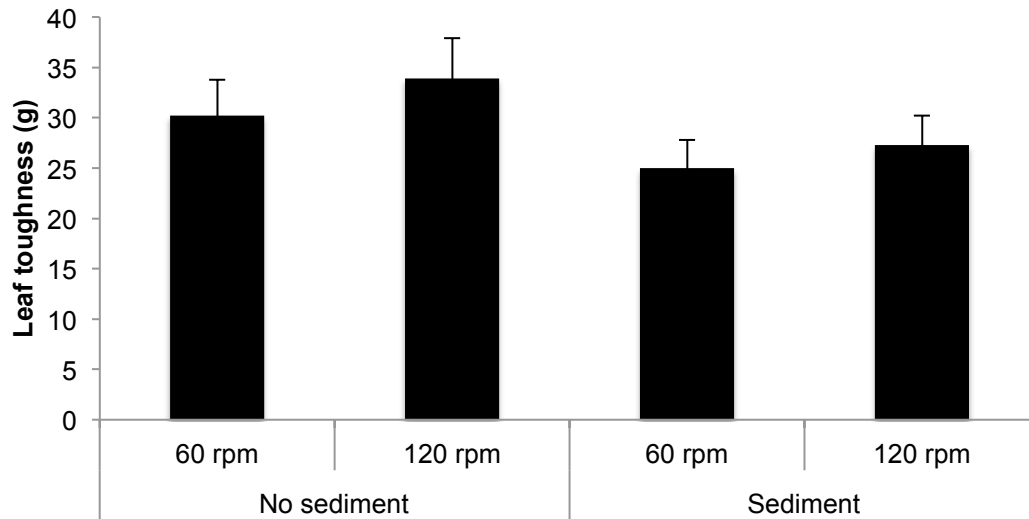
Figure 5 – Toughness of preconditioned oak leaves (mean ± SE), after 28 days of incubation in the lab. Leaves were conditioned in microcosms with or without sediments (< 2 mm) and were maintained at 60 or 120 rpm. No letters indicate absence of differences within the two main factors (presence of sediments and sediment load velocity).
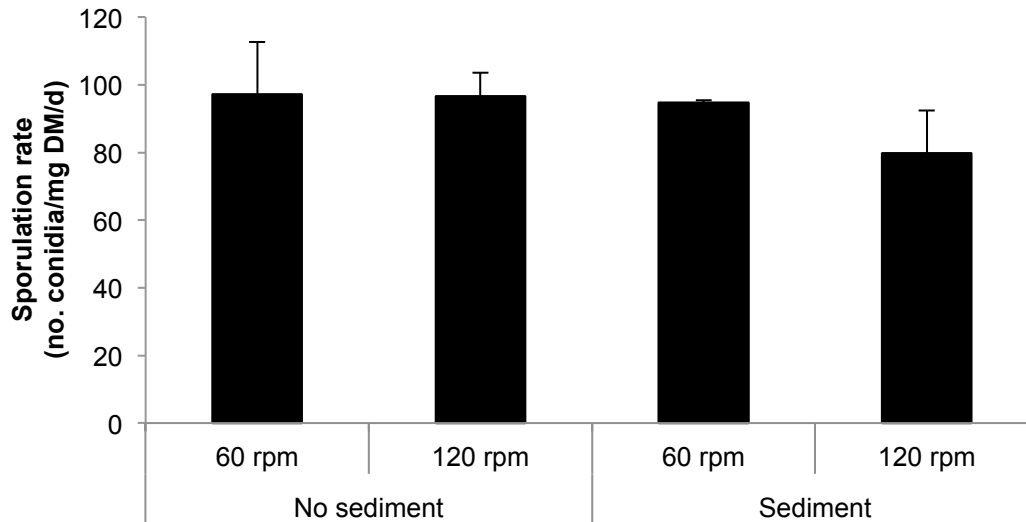
Figure 6 – Mean (± SE) soprulation rates (per mg DM) of aquatic hyphomycetes associated with oak leaves conditioned in the lab for 28 days. Incubation was made in the presence and absence of sediment (< 2 mm) at two shaking conditions – 60 and 120 rpm. No letters indicate absence of differences within the two main factors (presence of sediments and sediment load velocity).

3.1.2. Effects of sediment size on leaf litter decomposition

Sediment granulometry affected leaves mass loss (P = 0.02; Fig. 7). Significant differences were found in leaves mass loss between the real mixture and particles smaller than 0.063 mm (Tukey test, P = 0.02). Dry mass remaining was highest in leaves conditioned in the presence of the finest sediment. No significant differences were found between dry mass remaining of the control leaves (No sediment) and the other treatments (RM or EFS; Tukey test, P > 0.07).

The presence of sediment did not affect fungal biomass associated with the oak leaves (1-way ANOVA; P = 0.43; Fig. 8). However, an obvious decrease in the amount of biomass associated with the leaves was observed:

fungal biomass of leaves incubated with no sediment attained values twice as high as the leaves incubated in microcosms with EFS (439.60 mg/g in No sediment vs. 239.30 mg/g in the EFS).

The presence of sediment seems to stimulate sporulation rates, which was highest in leaves conditioned in microcosms with sediment < 0.063 mm. Significant differences were found among treatments (1-way ANOVA; $P < 0.05$; Fig. 9). Accordingly, the lowest number of fungal species was found in the No sediment treatment and the highest number in the EFS treatment; significant differences were found between these two treatments (Tukey's test; $P = 0.02$). The total number of species increased in the order No sediment < RM < EFS (Fig. 10). The structure of oak fungal communities was consistently affected by the sediment granulometry (1-way ANOSIM, $R = 0.77$, $P = 0.004$). In fact, the major communities' differences were promoted by the presence of sediments, but the effects tended to be attenuated when we consider the sediment sizes (Fig. 11). *Articulospora tetracladia* dominated in the No sediment treatment while *T. elegans* was only dominant in the presence of sediments. Results suggest that the sporulating capacity of *Flagellospora curta* may be affected by the presence of sediment independently of the granulometry (Fig. 12).
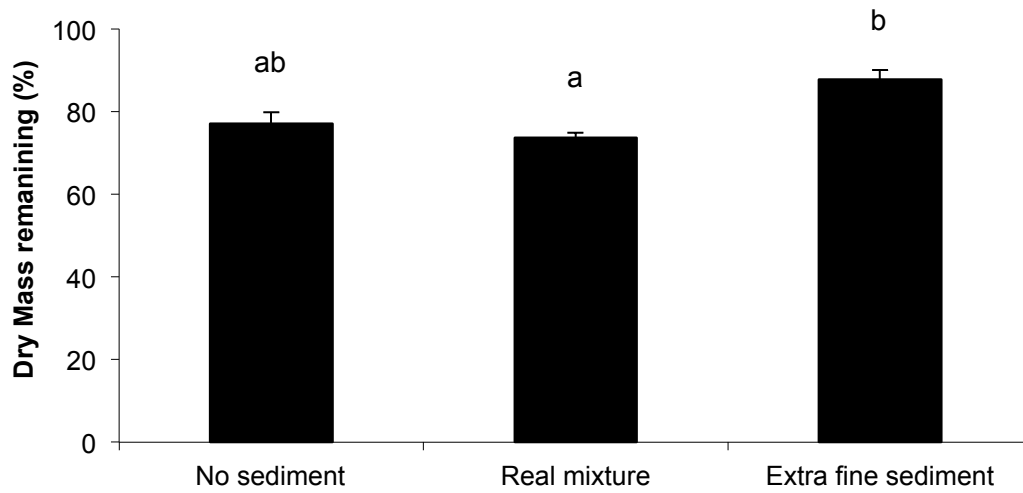
Figure 7 – Dry mass remaining (percentage ± SE) of oak leaves after 28 days of incubation in the lab. Incubation was made in the absence (No sediment) and presence of sediment (Real mixture and Extra fine sediment) and maintained at 120 rpm. Different letters indicate significant differences among treatments ($P < 0.05$).
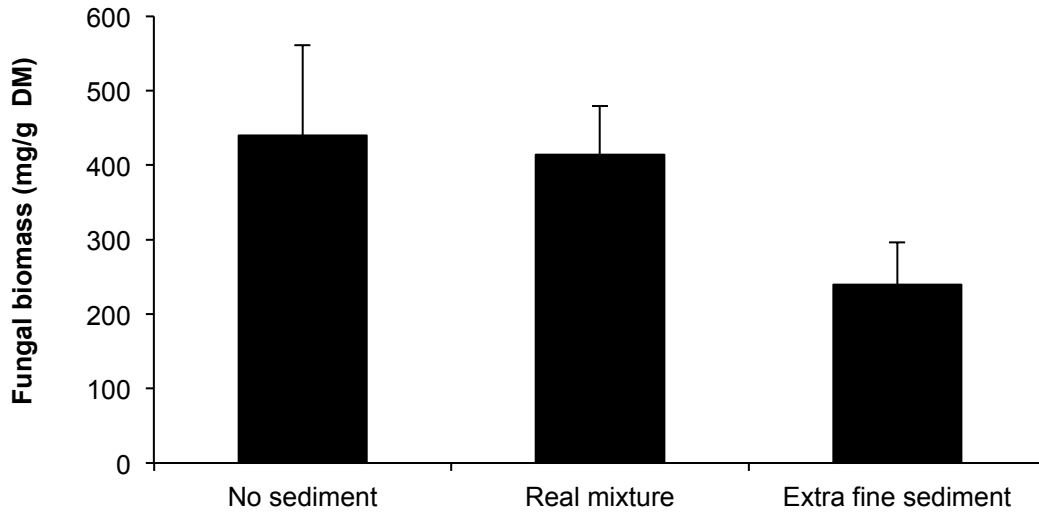
Figure 8 – Mean (± SE) fungal biomass of aquatic hyphomycetes associated with oak leaves after 28 days of incubation in the lab. Leaves were conditioned in microcosms in the absence (No sediment) and presence of sediment (Real mixture and Extra fine sediment) and maintained at 120 rpm. No letters indicate absence of differences within the three treatments.
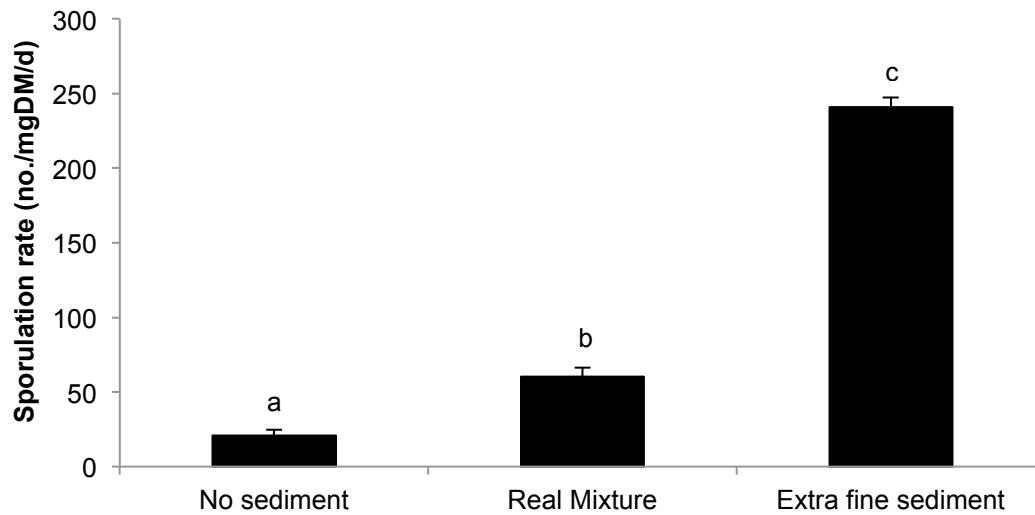
Figure 9 – Sporulation rates (conidia/mg DM/d) ± SE) of oak leaves conditioned for 28 days of incubation in the lab. Incubation was made in the absence (No sediment) and presence of sediment (Real mixture and Extra fine sediment) and maintained at 120 rpm. Different letters indicate significant differences among treatments ($P < 0.05$).
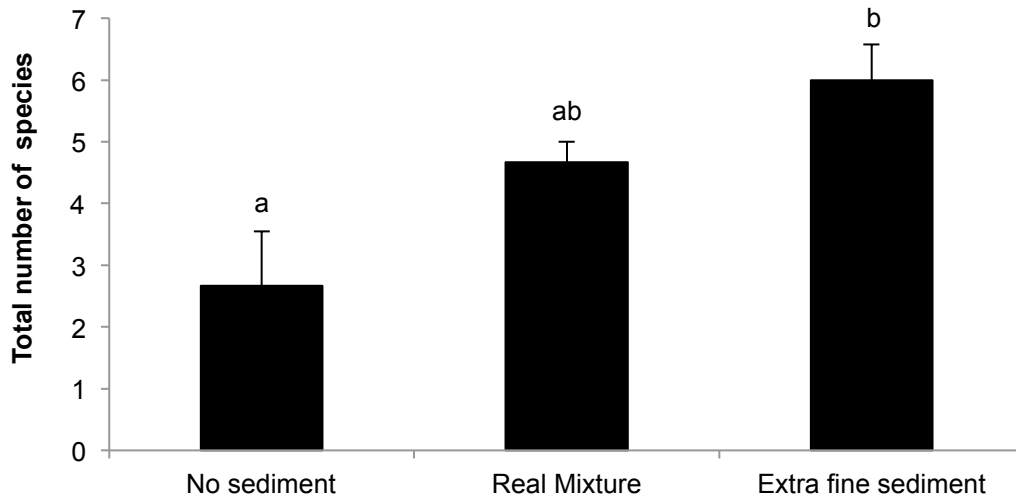
Figure 10 – Total number of species (± SE) of fungi (Aquatic Hyphomycetes) produced during sporulation of oak leaves conditioned in the lab for 28 days. Incubation was made in the absence (control) and presence of sediment (real mixture and extra fine sediment) and maintained at 120 rpm. Different letters indicate significant differences among treatments ($P < 0.05$).
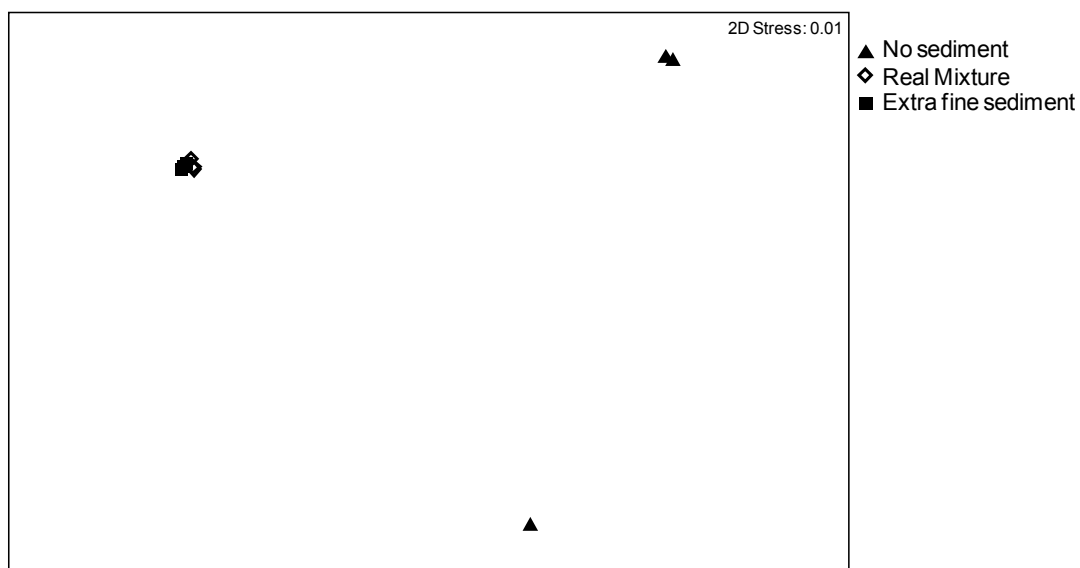
Figure 11 – Ordination (NMDS) of oak fungal communities after 28 days of incubation in the laboratory, based on Bray-Curtis similarity matrix of relative abundances of aquatic hyphomycete conidia. Incubation was made in the absence (No sediment) and presence of sediment (Real mixture and Extra fine sediment) and maintained at 120 rpm.
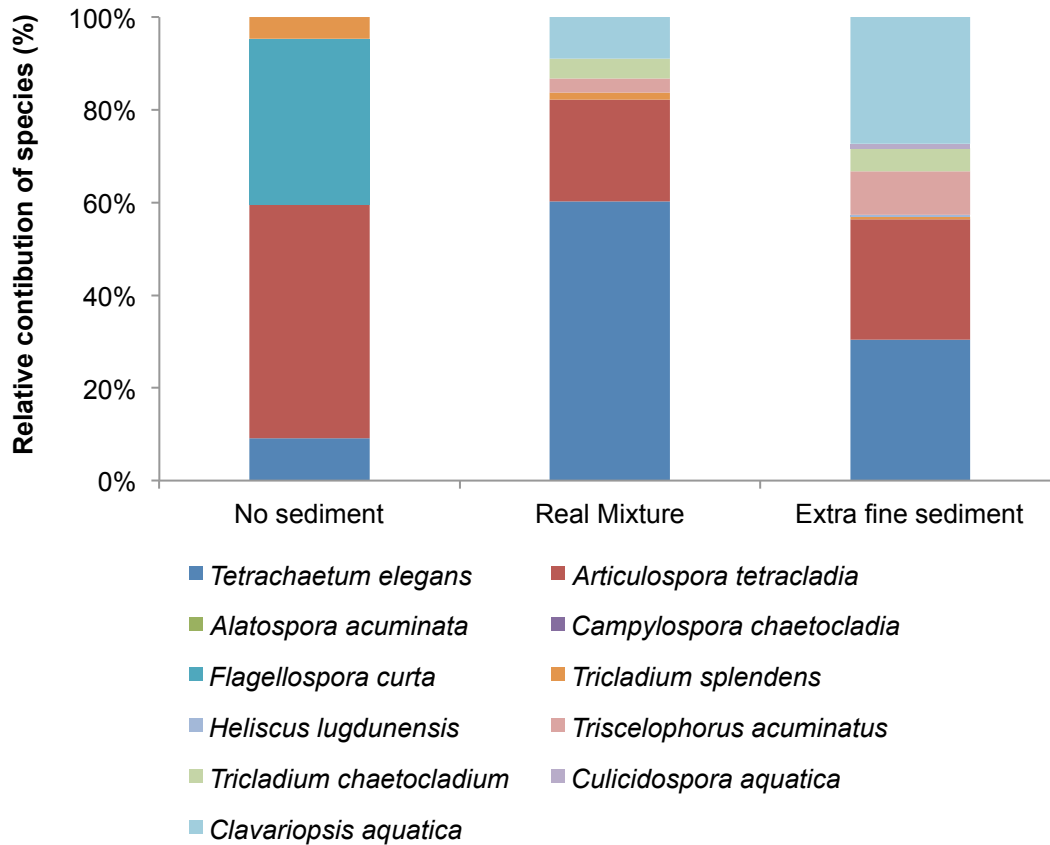
Figure 12 – Mean relative abundances (%) of aquatic hyphomycetes conidia from pre-conditioned oak leaves incubated for 28 days in the lab. Incubation was made in the absence (No sediment) and presence of sediment (Real mixture and Extra fine sediment) and maintained at 120 rpm.

3.2. Invertebrates feeding tests

3.2.1. Effects of sediment in suspension on invertebrates consumption

Suspended solids and oxygen were measured daily in all microcosms during the experimental time. The amount of suspended solids was significantly higher (181 mg/L vs. 163 mg/L) when a long tube was used to promote suspension of the (t-test; $P < 0.05$). Also, sediments present in the water column were significantly different between treatments (t-test; $P < 0.05$). In both

treatments a 100% oxygenation was maintained. The presence of the sediments in suspension did not affect the invertebrates consumption of oak leaves (t-test; P = 0.49; Fig. 13).
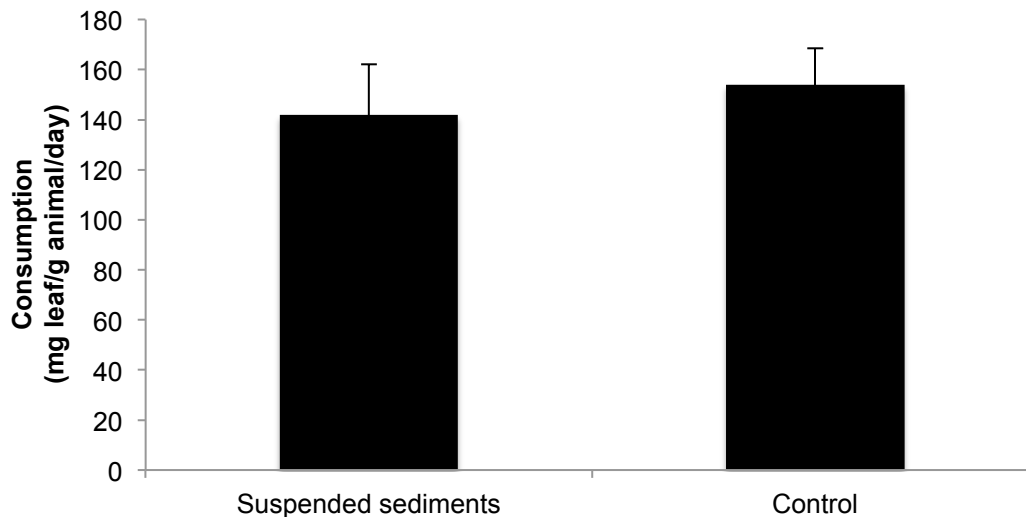
Figure 13 – Consumption (mean ± SE) of *Sericostoma vittatum* larvae kept in microcosms with suspended sediments (< 0.063 mm) and without sediments in suspension. No significant differences were observed between treatments.

3.2.2. Effects of sediment deposition on food consumption

The location of the food item affected the feeding behavior of *Sericostoma vitattum*. Consumption of conditioned oak leaves was significantly higher when the disc was at the top of the sediment (1-way ANOVA; P < 0.01; Fig.14). Although no significant differences were found between the other two treatments (middle and bottom), leaves that were less buried in the sediment (1.5 cm) were 58% more consumed than the leaves maintained at 3 cm depth. In this case consumption was negligible (7.85 mg leaf/g animal/day).
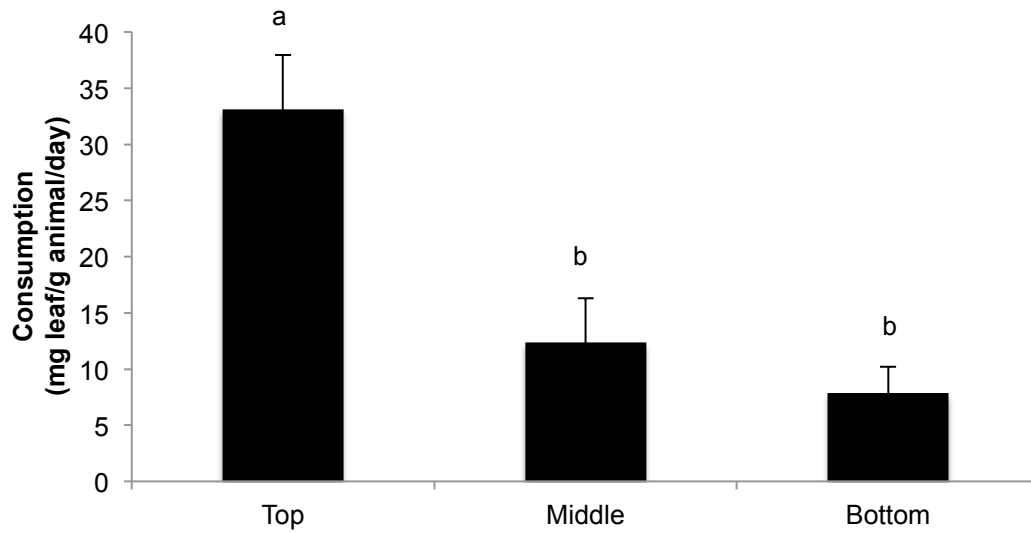
Figure 14 – Consumption (mean ± SE) of conditioned oak leaves by
the *Sericostoma vittatum* larvae when the food items were maintained
at the surface of the microcosm substratum or covered by 1.5 or 3 cm
of sediment (< 2 mm). Different letters indicate significant differences
among treatments (P < 0.05).

CHAPTER 4

- Discussion -

4. DISCUSSION

The number of studies on the importance of sediment loads or sediment deposition on leaves decomposition and shredder ecology are still few considering the spatial dimension and relevance of this problem to streams ecological integrity (Cornut et al., 2010; Matthaei et al., 2010), and the results gathered are frequently contradictory. Furthermore, it is difficult to isolate the impacts of sedimentation on the biota or stream processes, because sedimentation is most of the times associated to other factors like nutrient enrichment or canopy removal (Schofield et al., 2004). To avoid this problem, a large number of studies (*e.g.* Rosenberg & Wiens, 1978; Sanpera-Calbet et al., 2011) were performed in artificial streams or in laboratory (this one included); although such approaches are accurate and scientifically sound they make difficult to assess biotic interactions (Schofield et al., 2004) and to extrapolate results for real ecosystems. Nonetheless they certainly help to explain the effects of sedimentation observed in stream communities, in particular stream macroinvertebrates (Piggott et al., 2012) and processes as leaf decomposition (Cornut et al., 2010; Danger et al., 2012). In this study the effects of sediment loads were assessed at two distinct levels: microbial-mediated decomposition and invertebrate-mediated decomposition of leaf litter, evaluating the effects of sediments on the main protagonists of leaves processing in heterotrophic systems based on detritus - microbes (mainly Aquatic Hyphomycetes) and shredders - and their function. Results pointed to specific impacts at both basic levels of the stream detrital-based food chains, which may suggest potential bottom-up consequences in the stream biota.

Bedload of fine sediments (simulated by microcosms shaking) did not affect leaves degradation promoted by fungi; a higher water velocity (120 rpm) did not affect leaves mass loss, toughness or sporulation rates. This was partially surprising, as higher water velocities are known to stimulate sporulation rates and the number of conidia produced, independently of the colonization rate (Ferreira et al. 2006). Furthermore, the presence of sediments may increase friction by rolling and sliding on the leaves surface potentially stimulating mycelial growth, which could determine an increase in the number of spores produced. Such effects did not seem to have occurred since mass loss or toughness decrease (also a proxy of leaves mass loss; Medeiros et al., 2009) promoted by fungal activities did not differ between water velocities. The present results are in agreement with Sanpera-Calbet et al. (2011). These authors associated the lack of sediments effect with the initial colonization of the leaves. Since all leaves were colonized with no sediments, the following contact with this material made no difference (once fixed on the leaf substrate, spores germinate quickly and mycelia adhere to the leaves; Dang et al., 2007). On the other hand, significant differences between treatments were expected since higher water velocity, like the one observed in riffles, is known to promote higher physical fragmentation in comparison with pools or dam zones, where the flow is reduced and leaves decompose slowly (Casas, 1996; Young et al., 2008). In fact, several studies argue in favour of significant effect of sediment loads on leaves decomposition (Benfield et al., 2001; Matthaei et al., 2010). It seems possible that the incubation period of the leaves in the stream was too long (in spite of low winter temperatures) or that heavy precipitation during the conditioning period in the stream promoted an excessive degradation of the

leaves used in the microcosms, masking the potential differences between treatments. Furthermore, we cannot rule out the possibility that the amount of sediments added or even the water velocity was not sufficient to accelerate the process of breakdown by physical abrasion (Schofield et al., 2004).

Sediment granulometry affected leaves decomposition. A heterogeneous fine sediment ($\varnothing$ < 2 mm; RM) seem to maintain/stimulate leaves mass loss while the EFS sediment tend to depress leaves degradation. Although no significant differences could be observed in fungal biomass (p < 0.43) the concentration of ergosterol evaluated in leaves incubated in microcosms with EFS showed about half the values of the leaves incubated in No sediment or RM microcosms, which suggests that such small particle sizes may depress fungal growth, and mass loss. Leaves in the microcosms could have been buried and protected from turbulence and collisions of the sediment during rotation. In this treatment, and in spite of the water movement (120 rpm), the contact between the water and the leaves may be limited by an eventual sediment compaction inhibiting O_2 access to the mycelium. Such decrease in fungal biomass may be in agreement with Kreutzweiser and co-workers (2005) results that indicate that fine sediments can decrease the palatability to shredders due to a lower microbial activity. In opposition, a heterogeneous substratum as the one present in the real mixture may favor oxygenation that, along with the sediments abrasion effect (in particular of the larger particles; Heard et al., 1999; Young et al., 2008), may facilitate leaves mycelial growth and breakdown. Although this could not be confirmed by our results, it seems clear that the smaller the particle size, the higher the sporulation rates and total

number of fungal species in the media. A clear reproductive investment occurs in the presence of sediments, in particular of EFS. This was unexpected as the presence of sediments is usually associated with a decrease in number of species (Sanpera-Calbet et al., 2011) and not the opposite. Nonetheless, we could detect a clear effect of the presence of sediments (both types) on fungal assemblages structure in relation to the media with no sediment. The higher contribution was done by *Tetrachaetum elegan*s (30%), followed by *Clavariopsis chaetocladium* (27%). *Articulospora tetracladia* is abundantly present in all treatments (50% in no sediment; 22% in real mixture; 26% in EFS), showing that it is a specie well adapted for different sediment conditions (Medeiros et al., 2009; Cornut et al., 2010; Sanpera-Calbet et al., 2011). Surprisingly, *T. elegans* was found in all treatments and well represented; other studies showed that this specie is usually very sensitive to sediments and inhibited in their presence (Cornut et al., 2010; Sanpera-Calbet et al., 2011). These differences in the composition and structure of fungal assemblages between treatments indicate that different species present different answers to the stress promoted by the sediments. It seems possible that the reduced O_2 levels nearby the leaves (particularly in EFS) might have reduced the mycelial growth of dominant species, reducing competition and allowing other species to grow and sporulate. It is generally accepted that sporulation may occur in hypoxic/anoxic media as the hyporheic areas (*e.g.* Bärlocher et al., 2008; Cornut et al., 2011), and that species differ in their tolerance to low levels of O_2 (Medeiros et al., 2009). In fact, it is not completely clear why we registered increasing sporulation rates and diversity from no sediment < real sediment <

extra fine sediment; a "survival" strategy may eventually help to understand the results.

Sediment cumulating on leaves affected invertebrates consumption. Shredders consumption decreased between 63% (middle) and 76% (bottom) when leaves were buried in the substratum. Although the depth effect of sediment on leaves decomposition promoted by shredders was previously stated (Cornut et al., 2010; Sanpera-Calbet et al., 2011; Danger et al., 2012) this is, to my knowledge, the first study where the effect of depth was considered independently of the leaf litter quality as all leaf circles had a similar conditioning period. *S. vittatum* larvae seem unable to locate the food source if sediments are buried. The mechanisms of food location by shredders are still not clear, but it seems that the chemical clues possibly guiding the invertebrates to their food sources (*e.g.* Rong et al, 1995) may be limited by the presence of few centimetres of sediment. It seems possible that a limited burying capacity of the invertebrates could constitute an impediment to gain access to buried leaves; however, according to the *Sericostoma* larvae distribution in the stream, the capacity of digging the sediment does not seem to be an issue. Although a finer scale of depths should be further used to assess the effects of sediment deposition on invertebrates feeding behaviour (leaves coated by the sediment seem to be consumed by the invertebrates), this study indicates that sediments deposition of over 1.5 cm may inhibit foraging. This may suggest that sediment loads may clearly affect not only the quality but also the quantity of available resources to the invertebrates, with potential consequences on population dynamics. In fact, considering our results, the proclaimed idea that the adverse

effects of sediments are reduced or negligible when they are present in low to moderate concentration (Fairchild et al., 1987) might be challenged.

Contrary to my expectations, suspended sediments in the microcosms did not affect shredders consumption. This is in accordance with Schofield et al., (2004) and Sanpera-Calbet et al. (2011), but in contradiction with most studies (Bunn, 1988; Benfield et al., 2001; Sponseller & Benfield, 2001 and Matthaei et al., 2010). In fact it seems that this species, like other invertebrates (*e.g. Gammarus*; Sanpera-Calbet et al., 2011), may be resistant to short term stress promoted by sediments, in particular when refuge (stone) and food are available. It seems likely that the presence of the case in *Sericostoma* allows an additional protection to the impact of the suspended bed material load. It is still not known which is the limit of sediment load tolerated by this species, or what would be the impact if this sedimentation lasted for longer periods. Sediment increase can have a major influence on several aspects such as biomass, diversity and even change in drift behaviour of macroinvertebrates (Quinn et al., 1992; Wood & Armitage, 1997), which tend to support the idea of a threshold of tolerance for each species, likely modulated by other environmental factors (*e.g.* water temperature, pH or current velocity).

Briefly, our laboratorial results suggest that the granulometry of the sediments may affect the decomposition dynamics through physical fragmentation, direct and indirect (O_2 availability) effects on microbial assemblages, colonization status and shredders performance. It is generally recognized that invertebrates prefer colonized detritus and may present specific feeding behaviour towards particular fungal species or assemblages (Canhoto & Graça 2008). Although it was already stated that leaves burial may affect its

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quality due to lower concentration of oxygen, influencing the fungal biomass (Cornut et al., 2010; Danger et al., 2012), this study contributes to the recognition of the sediment impairment on the foraging behaviour promoted by very thin layers of sediment. Most likely, sediments accumulation inhibits the diffusion of signals needed for food detection. *S. vittatum* larvae were able to maintain their feeding behaviour in the presence of high amounts of suspended sediments in a turbulent aquatic environment most likely due to its protective case. It remains unclear if such behaviour will be maintained for longer periods and in what extent.

The effect of sediments transport should be assessed in the future, by controlling flow in natural streams, and during a longer exposure, in order to understand the behaviour of the invertebrates. Also, tests with leaves buried at different depths and with different shredders should be done.

4.1. Final Remarks

There is no doubt that sediment loads are a natural feature of the streams; the problem is the unnatural increase of sediment inputs due to anthropogenic activities. It is therefore fundamental to understand how these changes will affect aquatic habitats as well as the whole ecosystem. Activities like agriculture and deforestation are becoming a threat to stream ecosystem functions. It is important to take in consideration the recovery of riparian forests, since they give the necessary resources for streams and act as a natural stopper and filter for sediments inputs (Cavalcanti & Lockaby, 2005), also avoiding margins erosion and an intensification on sedimentation and turbidity (Swank et al., 2001).

CHAPTER 5

- References -

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