UNIVERSIDADE DE ÉVORA

Escola de Ciência e Tecnologia

Departamento de Biologia

Comparison of organic matter decomposition between natural and artificial ponds.

Pedro Miguel Portela Faísca

Orientação: Doutor Miguel Matias

Mestrado em Biologia da Conservação

Dissertação

Évora, 2018

University of Évora
Master in Conservation Biology

Dissertation
"Comparison of organic matter decomposition between natural and artificial ponds"

Pedro Miguel Portela Faísca

Supervisor: Doutor Miguel Matias

Index

Figure Index

Table Index

Comparison of organic matter decomposition between natural and artificial ponds

Abstract

Litter decomposition is a key ecosystem service within aquatic ecosystems and is a complex process that is sensitive to environmental factors. The role of microbial and macrofaunal decomposers, and how it changes across environmental gradients is not yet fully understood. Decomposition was assessed across 6 biogeographical regions to determine the role of macroinvertebrates in this ecosystem service. Decomposition was estimated using standardized cotton strips, which were deployed in the mesocosms of each region. The role of macroinvertebrates was tested with an exclusion experiment which allowed or prevented the access of macroinvertebrates to cotton strips, a similar experiment was also conducted in natural ponds. After 64 days the cotton strips were collected, and mass loss and tensile strength were measured. There were significant differences in the rate of decomposition across different regions and no differences were found between systems. Macroinvertebrates played an important role, with gatherers being major players.

Comparação de decomposição de matéria orgânica entre charcos naturais e artificiais

Resumo

A decomposição é um serviço de ecossistema chave e um processo complexo sensível a factores ambientais. O papel de decompositores microbianos e da macrofauna, e como este papel muda num gradiente ambiental não é completamente entendido. A decomposição foi avaliada em 6 zonas biogeográficas para determinar o papel de macroinvertebrados neste serviço de ecossistema. A decomposição foi estimada utilizando tiras de algodão, colocadas em mesocosmos nas diferentes regiões. O papel dos macroinvertebrados foi testado através de uma experiência de exclusão que permitia ou impedia o acesso de macroinvertebrados às tiras, uma experiência semelhante foi realizada em charcos naturais. Ao fim de 64 dias, as tiras de algodão foram recolhidas e a perda de massa e tensão foram quantificadas. Encontraram-se diferenças significativas na decomposição entre as diferentes regiões, mas não se observaram diferenças entre sistemas. Os macroinvertebrados têm um papel importante neste serviço de ecossistema, sendo as espécies colectoras as mais importantes.

Palavras-chave: Decomposição, Aquático, Charcos, Macroinvertebrados, Biogeografia

Introduction

Freshwater ecosystems are a major component of biodiversity and may be one of the most threatened ecosystems in the world (Szöllosi-Nagy *et al.,* 1998; Saunders *et al.,* 2002; Dudgeon *et al.,* 2005; Higgins *et al.,* 2005). Declines in biodiversity, which includes the variety of living organisms, genetic differences among them, communities and ecosystems in which they occur, and the ecological and evolutionary processes that keep them functioning (Noss & Cooperrider, 1994; Delong, 1996), are far greater in freshwater than in terrestrial ecosystems (Frissell *et al.,* 1996; Sala *et al.,* 2000). It is estimated that future extinction rates in freshwater ecosystems will be five times greater than that of terrestrial ecosystems, and three times greater than that of coastal marine ecosystems (Ricciardi *et al.,* 1999; Saunders *et al.,* 2002). The threats to global freshwater biodiversity can be grouped under five interacting categories: over exploitation; water pollution; flow modification; destruction or degradation of habitat; and, invasion by exotic species (Brönmark & Hansson, 2002; Saunders *et al.,* 2002; De Meester *et al.,* 2005; Dudgeon *et al.,* 2005; Oertli *et al.,* 2005; Declerck *et al.,* 2006; Céréghino *et al.,* 2007). The combined and interacting influences of the five major threat categories have resulted in population declines and range reduction of freshwater biodiversity worldwide (Dudgeon *et al.,* 2005).

Recent studies have explored the links between biodiversity and ecosystem function (Hooper *et al.,* 2005; McIntyre *et al.,* 2007; Vaughn *et al.,* 2007; Strayer *et al.,* 2010). Ecosystem functions depend on species richness and composition, but the size and nature of this effect depend on the species being gained or lost, the ecological process under consideration, and the characteristics of the ecosystem (Strayer *et al.,* 2010). In these key ecosystem functions there are landscape related functions (e.g., migration corridors and stepping stones; De Meester *et al.,* 2005), leaf litter decomposition that plays an essential role in controlling the carbon and nutrient cycles (e.g., Kampfraath *et al.,* 2012; Vysná *et al.,* 2014;) and primary production (e.g., Dang *et al.,* 2009).

Determining the ecological condition of ecosystems is a key challenge for effective resource management (Tiegs *et al*., 2013). Traditional assessment has focused entirely on ecosystem structure (e.g., invertebrate community composition, water quality), and neglected ecosystem processes (e.g., primary production, organic-matter decomposition; Fritz *et al.,* 2011; Niyogi *et al.,* 2013; Tiegs *et al*., 2013; Vysná *et al.,* 2014). Nevertheless, several studies have drawn attention that using only a structural organization of biota, as

indicators of ecosystem health (without considering also its functional role) contribute little to ecosystem functioning and therefore should not be used as the only indicator in assessment of the ecological status of the water bodies (Bunn and Davis, 2000). As a response to these issues, the 5th European Water Framework Directive (WFD - Directive 2000/60/CE) requires additional incorporation of the ecosystem processes in stream assessment protocols. Recently, functional measures have received considerable attention due to their sensitivity in response to environmental change (Bunn *et al.* 1999; Fellows *et al.* 2006; Young *et al.* 2008). One of the most conspicuous descriptors of the ecosystem-level processes is the measure of organic matter processing (e.g. litter breakdown, generation and export of fine-particulate organic matter, secondary production of macroinvertebrates (Gessner & Chauvet, 2002)) have been proposed as indices of ecosystem function that may add to the structural measures (Gessner & Chauvet, 2002; Young *et al.,* 2008; Silva-Junior *et al.,* 2014; Piggot *et al.,* 2015).

Ponds are small (1 m^2 to about 5 ha), man-made or natural shallow waterbodies which maintain water level permanently or temporarily (De Meester *et al.,* 2005; Céréghino *et al.,* 2007). Due to their small size and the ability to easily be manipulated experimentally, ponds represent an ideal model for controlled studies of many basic ecosystem processes (e.g., primary production, organic matter decomposition; De Meester *et al.,* 2005; Céréghino *et al.,* 2007), and might function as an early warning system for long term effects on larger aquatic systems (Céréghino *et al.,* 2007). Aquatic ecosystems have dramatically decreased during the last century (Saunders *et al.,* 2002; Oertli *et al.,* 2005; Le Viol *et al.,*2009), between 1984 and 2015 permanent surface water has disappeared from an area of almost 90.000 Km^2 (Pekel *et al.,* 2016). Ponds were found to be the most species rich aquatic habitat (Davies *et al.,* 2008), and, like natural ponds, man-made ponds support wildlife and may function as corridors and refuges for the native fauna and flora (Le Viol *et al.,* 2009). Although artificial ponds differ in abiotic conditions from surrounding ponds, they support communities of aquatic invertebrates as rich and as diverse at the family level as natural ponds and may contribute to reinforcing the pond network and consequently the abundance of such habitats on a regional scale (Le Viol *et al.*, 2009).

Temporary ponds are fluctuating waterbodies with recurrent seasonal phases of flooding and desiccation in most years. Although some macroinvertebrates persist in ephemeral habitats as resting stages in dry sediment, dispersal to more permanent waterbodies are

the main strategy through which they survive dry phases in temporary aquatic habitats (Florencio *et al.*, 2011). Temporary ponds support relatively fewer aquatic macroinvertebrates when compared to more permanent sites, being the species richness related to the length of the flooded period (Pyrovetsi & Papastergiadou, 1992; Collinson *et al.,* 1995). However, there is no evidence of temporary ponds being species-poor (Collinson *et al.,* 1995; Williams *et al.,* 2004; Oertli *et al.,* 2005), in fact shallow ponds can dry out to only mud and produce no effect on species richness, species rarity or community composition in the following year, might serve as proof (Collinson *et al.*, 1995; Céréghino *et al.,* 2007; Sayer *et al.,* 2012). Temporary ponds have very distinctive macroinvertebrate communities (De Meester *et al.,* 2005*;* Céréghino *et al.,* 2007), because periodicity of water confers specific advantages to particular species, including an absence of fish predation, nutritionally rich substrates and warm spring temperatures (Collinson *et al.,* 1995; De Meester *et al.,* 2005). The same conclusion was reached by Fairchild *et al.* (2003), in a study focused on beetle communities in temporary and permanent ponds. The author concluded that the hydroperiod together with other environmental variables has strong effect on community composition and distribution of beetles.

Organic matter decomposition is one of the most important ecosystem processes and is essential to the trophic dynamics of freshwater ecosystems (Boyero *et al.,* 2011; Handa *et al.,* 2014; Piggott *et al.,* 2015), where the decomposition of cellulose is a central part of carbon and nutrient cycles and energy transfer within the ecosystem (Goodman et al., 2010). Decomposition processes are a complex group of physical, chemical, animal and microbial interactions (Webster & Benfield, 1986; Allan & Castillo, 2007; Piggott *et al.,* 2015; Santonja *et al.,* 2017) that are thought to be sensitive to climate warming (Boyero *et al.,* 2011; Piggott *et al.,* 2015). Also, organic matter decomposition varies locally as a function of environmental factors (e.g., temperature and nutrients) and substrate quality (Webster and Benfield, 1986; Costantini *et al.,* 2009; Goodman *et al.*, 2010; Vysná *et al.,* 2014; Martínez *et al.,* 2015; Santonja *et al.,* 2017). For example, higher temperatures and high nutrient availability can increase microbial decomposition, while an increase of fine sediments can decrease decomposition by reduction of macroinvertebrates' and microbial activity (Young *et al.,* 2008; Goodman *et al.*, 2010; Vysná *et al.,* 2014). In quantitative terms, shredder macroinvertebrates have a crucial role in decomposition (Cummins & Klug, 1979; Handa *et al.,* 2014; Garcia-Palacios *et al.,* 2016; Santonja *et al.,* 2017). They

have a direct and indirect contribution to litter decomposition by consuming and fragmenting litter material (Graça, 2001; Santonja *et al.,* 2017); which provides additional nutrients and habitats for microbes and creates new resources for other organisms (collectors and filter-feeding invertebrates) in the aquatic food web, through the production of fine particulate organic matter (Joyce & Wotton, 2008; Santonja *et al.,* 2017).

Litter-bag experiment for quantifying litter decomposition, is the most common process to assess stream-ecosystem functioning (Young *et al.,* 2008; Fritz *et al.,* 2011; Niyogi *et al.,* 2013; Tiegs *et al*., 2013; Vysná *et al.,* 2014; Ferreira & Guérold, 2017). However, standardizing litter quality is a very challenging process (Tiegs *et al*., 2013). For example, litter quality (e.g., concentrations of nutrients, secondary compounds) varies widely among species in ways that influence decomposition (Petersen & Cummins, 1974; Webster & Benfield, 1986; Newman *et al.,* 2001; Goodman et al., 2010; Tiegs *et al*., 2013; Vysná *et al.,* 2014; Piggott *et al.,* 2015; Ferreira & Guérold, 2017; Santonja *et al.,* 2017). A very simple solution would be to use a single plant species to control for among species variation in assessment procedures (Boyero *et al.,* 2011; Kampfraath *et al.,* 2012; Tiegs *et al*., 2013). However, within species variation in litter quality that exists among regions, genetic differences among individual trees and other sources of variation, for example if a tree has been exposed to herbivory, complicate this solution (Tiegs *et al*., 2013). All the shortcomings of the litter-bag experiment can be overcome with the cotton-strip experiment (Boulton & Quinn, 2000; Clapcott & Barmuta, 2010; Goodman *et al.*, 2010; Fritz *et al.,* 2011; Niyogi *et al.,* 2013; Tiegs *et al*., 2013; Vysná *et al.,* 2014; Piggott *et al.,* 2015).

The cotton-strip experiment was first developed by the textile industry as a test to evaluate the effectiveness of fungicide treatment. Eventually it became used as a standard method for decomposition studies in soil and has recently been adapted to aquatic habitats (Latter & Walton, 1988). Being 95% cellulose, cotton-strips offer numerous advantages (Boulton & Quinn, 2000; Tiegs *et al*., 2013): (1) allow a degree of standardization of the material that is not possible with plant litter; (2) cellulose is a highly ecological relevant compound because it constitutes the bulk of plant litter (Kampfraath *et al.,* 2012; Tiegs *et al*., 2013); (3) less expensive and time consuming, cotton fabric is inexpensive and loss of tensile strength typically occurs faster than litter-mass loss, requiring smaller incubation times (Boulton & Quinn, 2000; Niyogi *et al.,* 2013; Tiegs *et al.,* 2013); (4) provide a suitable

substrate for leaf-colonizing fungi and bacteria and can serve as a food source for some leaf-shredding invertebrates (Tiegs *et al.,*2007). Although, decomposition of cotton strips might offer advantages in terms of standardization, it is primarily a measure of microbial enzymes to decay cellulose and is not a perfect surrogate for leaf decomposition, because materials using natural cotton have an uncertain and variable chemical composition and is extremely simple when compared to natural plant litter. (Tiegs *et al.,* 2007; Kampfraath *et al.,* 2012; Vysná *et al.,* 2014; Piggot *et al.,* 2015). And, despite its economic and logistical ease, few studies have used the cotton strip experiment as way of assessing ecosystem function on aquatic ecosystems (Young *et al.,* 2008; Goodman *et al.*, 2010). We are using this method because we needed a material that would be economical, easy to transport and that could be deployed in all the locations in this way we would have a standard origin for the organic matter that would allow us to compare different regions without focusing on the difference in leaves.

The threats that small ponds are faced with opens the possibility to use experimental systems, like mesocosms, to test how changes in environmental variables might affect ecosystem function in a natural system. With that in mind, one of the main objectives of this study was to compare decomposition between natural ponds and mesocosms. Diversity is linked to the function of the ecosystem, therefore the decline in worldwide biodiversity might cause changes in ecosystem function (e.g. organic matter decomposition). It's crucial to understand the exact role of freshwater macroinvertebrates on one of the most important ecosystem functions (organic matter decomposition) to help predict how changes in biodiversity will affect the function of these ecosystems. For this reason, another objective of this study is to understand if and how macroinvertebrates contribute to organic matter processing. To meet this objective macroinvertebrate structure and organic matter decomposition was compared between freshwater mesocosms and natural ponds across 6 different locations. The hypotheses of this study is that there are no differences in decomposition rates between natural ponds and mesocosms, decomposition differs across different locations and macroinvertebrates play an important role in organic matter decomposition.

Methods

Study Area

The main objective of this study is to compare decomposition of organic-matter between natural ponds (Figure 1 A-E) and aquatic mesocosm (experimental enclosures that vary from one to thousands of litters and can test community and ecosystem-level responses to change (Stewart *et al.*, 2013); Figure 1 F-J). In this study we also made a comparison between six regions (Évora, Porto, Murcia, Toledo, Peñalara and Jaca; Figure 2) using mesocosms and tested the role of macroinvertebrates in this ecosystem service. Litter-bags are easy to implement, cost-effective, reliable and accurately reflect the ecosystems condition (Fritz *et al.*, 2011; Kampfraath *et al.*, 2012; Tiegs *et al.*, 2013).

Figure 1- Aquatic systems used in the experiment. A-E) natural ponds; F-J) mesocosms
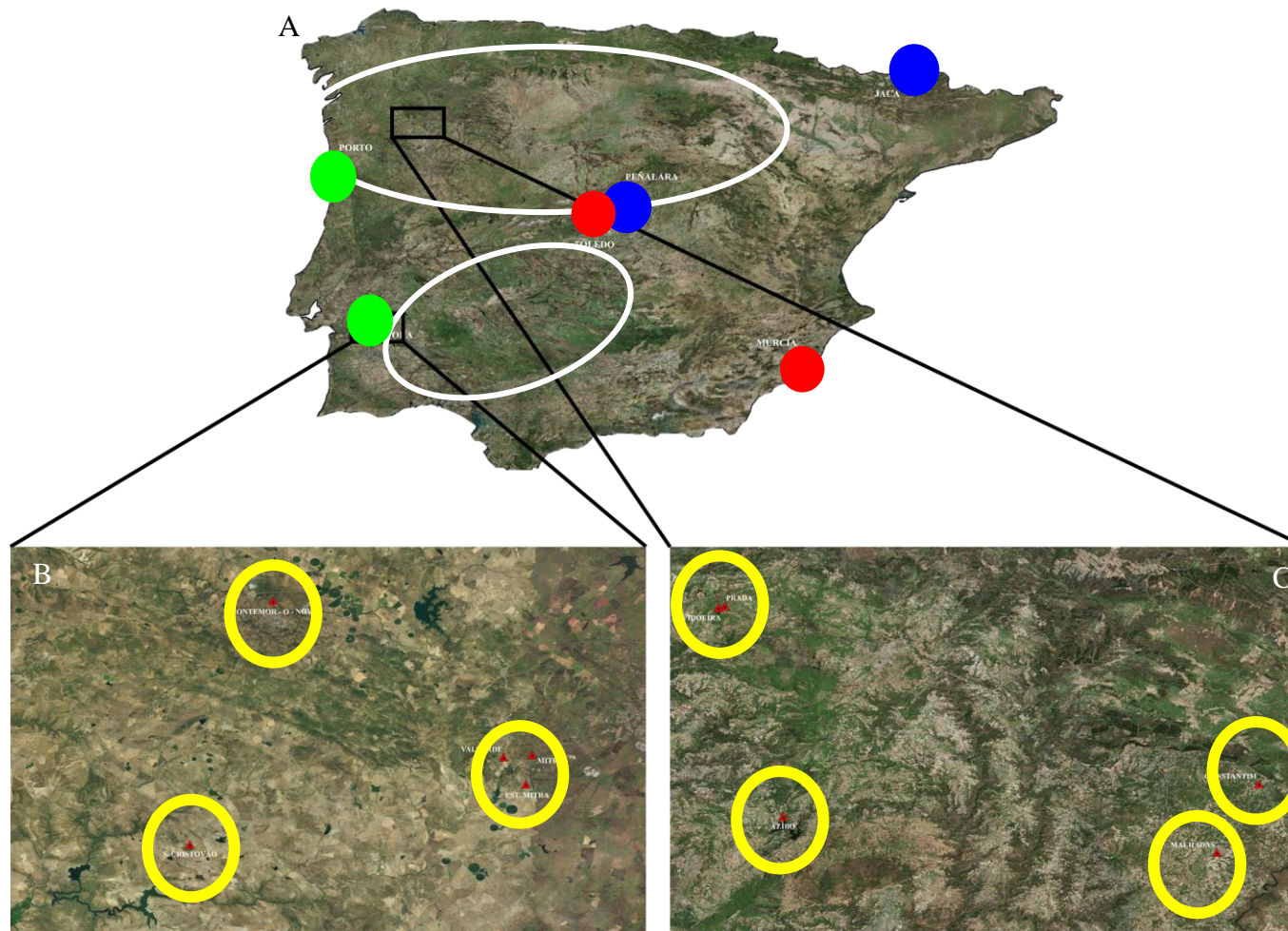
Figure 2- Map of the study area. A) Locations of the mesocosms in the Iberian Peninsula; red circles represent arid locations (Murcia and Toledo), green circles represent temperate locations (Évora and Porto) and blue circles represent mountain locations (Jaca and Peñalara); white circles represent Douro and Guadiana basins. B) Locations of the natural ponds in the Guadiana basin; red triangles and yellow circles represent the location of the natural ponds. C) Locations of the natural ponds in Douro basin; red triangles and yellow circles represent the location of the natural ponds.

These locations have very distinct climates (Figure 3). The climate in Murcia is Mediterranean with semi-arid features, with an average annual temperature between 15.0 ℃ and 19.0 ℃ and short winters and long and hot summers. The annual rainfall is less than 350 mm, except for some areas in the upper northwestern lands where it exceeds 600 mm, rainfall distribution is irregular with long dry periods combined with short and intense rainfall events (Alonso-Sarría *et al.,* 2016). Toledo has a continental semiarid climate with an annual rainfall of 487 mm and an average annual temperature of 14.0 ℃ (Hernández *et al.,* 2007). The climate in Évora is typically Mediterranean, with a hot and dry summer. More than 80% of annual precipitation occurs between October and April. The long-term mean annual temperature is 15.0-16.0 ℃ and average annual precipitation of 669 mm (Pereira *et al.*, 2007). The most significant feature of the Porto climate is the annual rainfall level (1236 mm) and its irregular distribution throughout the year, mainly concentrated in winter and spring. Due to the maritime influence Porto has mild temperatures with an annual mean of 14.4 ℃. No cold season can be found in Porto, being January the coldest month, with an average of 9.3 ℃. The mean summer temperature is about 18.1 ℃, although between May and September, very high temperatures can be reached (Abreu *et al.,* 2003). In Jaca, climate conditions are typically alpine, with cold mean annual temperatures that ranged between -0.7 ℃ and 5.0 ℃ and high-mean annual precipitation values well distributed along the year (Garcia-Pausas *et al.,* 2007). In Peñalara, the mean annual temperature is 6.3 ℃, with the coldest months being between December and April and hottest months being July and August. Annual precipitation is 1357 mm, the wettest months are between October and May and the driest being between July and August, with higher precipitation being in late autumn and early winter (Palacios *et al.,* 2003).

The Douro basin, which is located across the northern-central Iberian Peninsula, is characterized by having a temperate climate with some continental and Mediterranean influences, this is evident by the annual precipitation values that vary within the region from 3000 mm in the upper Minho mountain tops, to 400 mm in the upper Douro valley, and the severe summer drought that are felt in the region (Asensi *et al.,* 2011; Reis *et al.,* 2014). The Guadiana river basin is enclosed in the Mediterranean region with semi-arid and sub-humid conditions (Valverde *et al.,* 2015), it has a typical Mediterranean hydrological regime in which more than 80% of rainfall occurs between October and March (Collares-Pereira *et al.,* 2000).

The mesocosms consist of small circular plastic tanks (160x60cm; 1000 L) that mimic small ponds. Each location has 32 mesocosms that were installed in 2014 and were initiated by adding 100 kg of topsoil collected locally and filled with local water. After the addition of water, the mesocosms were left to settle without further manipulation for a month, to allow the establishment of primary producers and insects. Following this initial settling period, mesocosms were inoculated with water collected from local natural and artificial ponds within a few kilometers from the experimental site. Finally, the mesocosms were inoculated with macroinvertebrates, macrophytes and sediment samples, adding a range of larger organisms. This sequential inoculation minimized potential differences among the sites associated with starting date, but also allowed to simulate a natural process of colonization in natural ponds. The natural ponds (Figure 1) used in this study ranged from farmland ponds to dams. All the habitats used were naturalized.
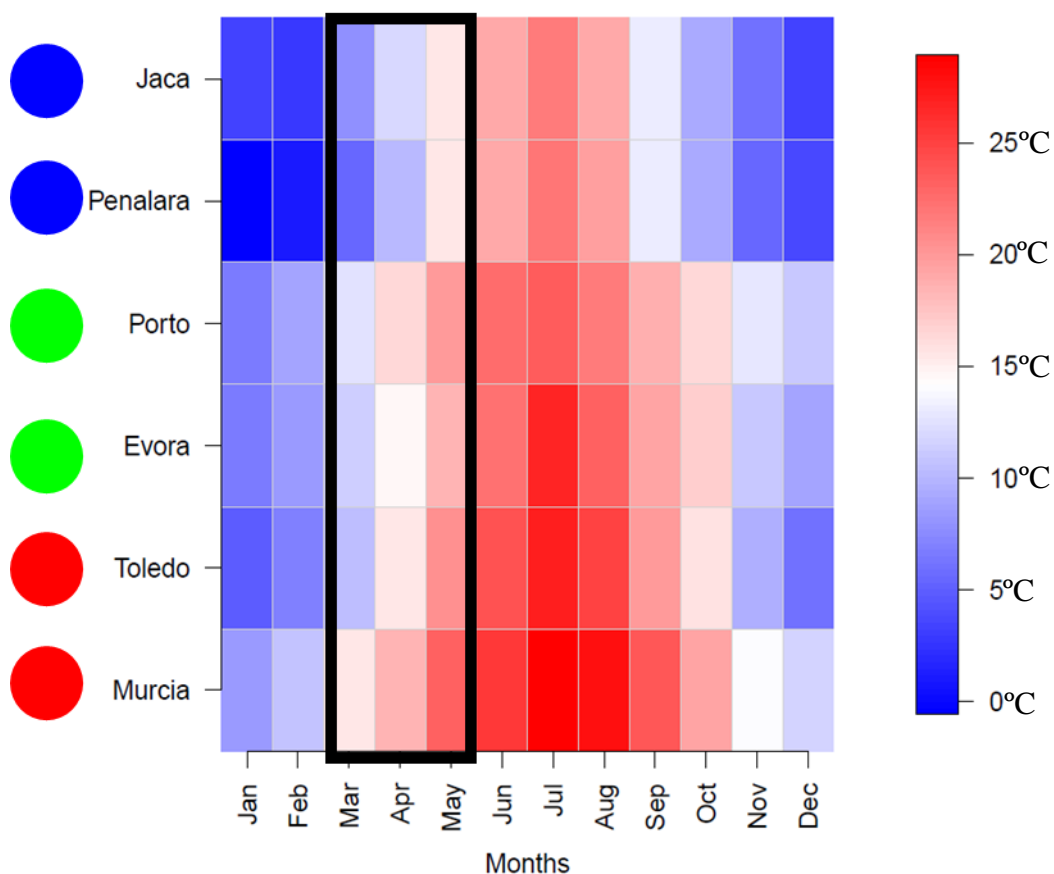
Figure 3- Monthly mean water temperature of the mesocosm through the year of 2015.

Decomposition experiment

The decomposition experiment was done using cotton-strips (8x 2,5 cm) as surrogates for leaf-litter, following Tiegs *et al.* (2013). To test the role of macroinvertebrates in the decomposition of organic-matter, net bags with two different mesh-sizes were used, following previous studies (e.g., Tiegs *et al.,* 2007; Raposeiro *et al*., 2014. Coarse net bags (CR; with a mesh size of 5 mm) were used to allow macroinvertebrates to access the cotton-strips, which gives total decomposition (TD). Fine net bags (FN; with a mesh size of 0.1 mm) were used to prevent macroinvertebrates to access the cotton-strips, which gives microbial decomposition (MD).

In each site, five mesocosms were selected for this experiment, the selection was done to have all dominant states represented (e.g., dominated by macrophytes, microalgae or animals), due to the high diversity of habitats that can be found between mesocosms within a location. In each mesocosm three treatments were implemented (Figure 4: A-FN; B- CR; C- Controls) with three replicates (Figure 5) each placed near the center of the pond (Figure 4 D).

Five natural ponds with longer flooded period were selected in each river basin. Here, cotton strips were placed in different types of habitat. In each pond the same three treatments were implemented (Figure 4: A- FN; B- CR; C- Controls) with seven replicates each (Figure 4 E) to ensure that at least three were retrieved.

The cotton strips were wet weighted before the experiment (T1), it was found that dry weight and wet weight were correlated (Appendix 1 Figure 1), so it was possible to estimate the dry weight of the cotton strips before the experiment using the formula, $Y = 0,982*X+(-0,00778)$, and retrieved after 64 days. The cotton strips were cleaned using 80% ethanol to avoid the growth of fungi and microorganisms, and to wash sediment and algae build up. In the laboratory, they were dried at 38°C for 24 hours (Tiegs et al., 2013), and weighed to obtain the total mass loss (T2). Tensile strength was measured using a tensiometer (Hounsfield Test Equipment model H5KT-0088). Before the cotton strips were placed in the grips they were measured to see if they had the minimum length (4 cm) required to conduct the test and were marked at approximately 1 cm from the edges, they were placed in the grips in a way that they didn't slipped or ripped in the points of contact. The tensiometer pulled at a rate of 2 cm/min. After the test the cotton strips were stored again.
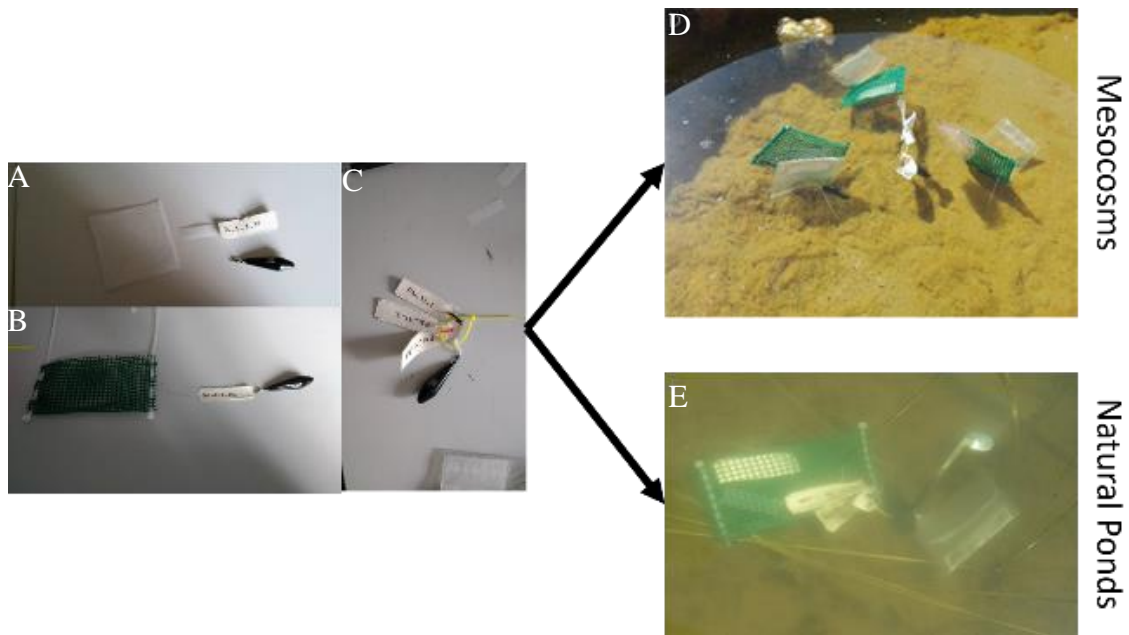
Figure 4- Net bags used in the decomposition experiment. A) Fine mesh bag (FN), which gives microbial decomposition (MD); B) Coarse mesh bag (CR), which gives total decomposition (TD); C) Controls; D) Experiment placed in mesocosms; and, E) Experiment placed in natural ponds.
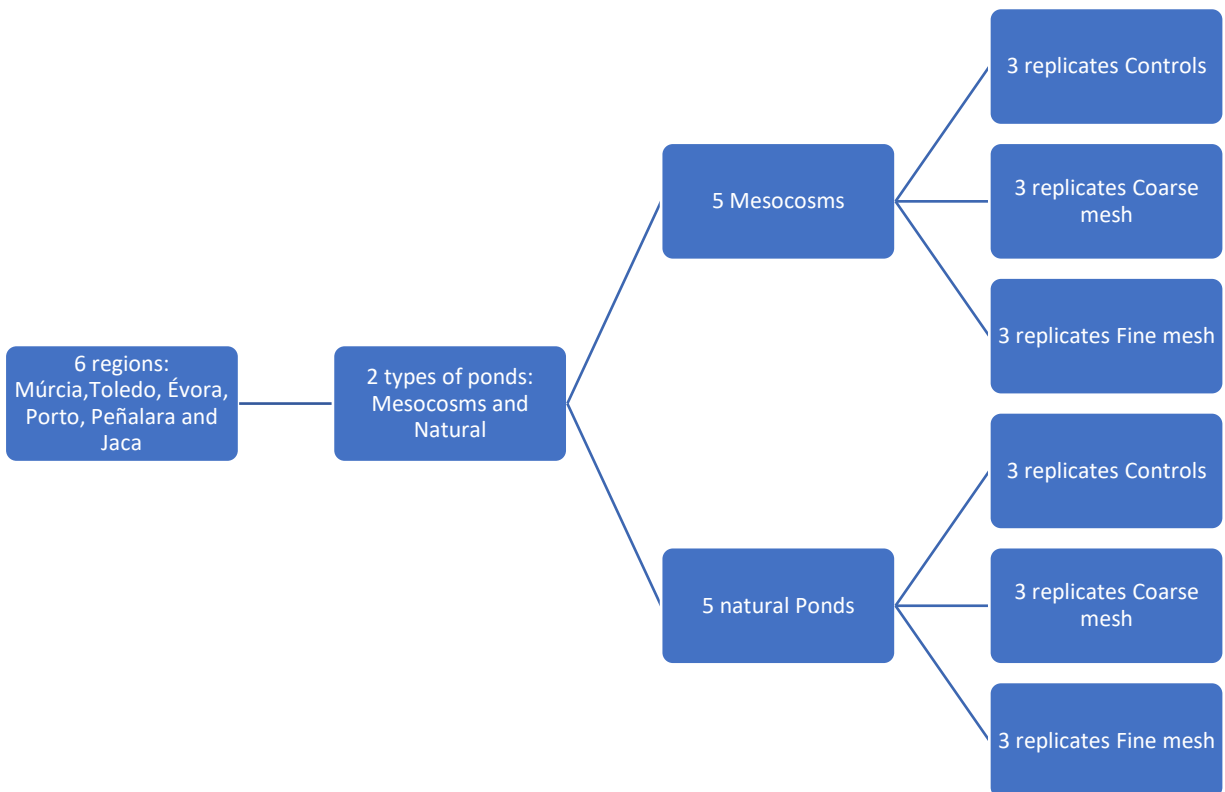
Figure 5- Experimental design of the decomposition experiment.

Environmental and biological sampling

The abiotic variables were measured using a HQ40D probe (Hach; Figure 6). The variables measured were pH, water temperature (ºC), conductivity (µS/cm) and dissolved oxygen (mg/L). In the case of the mesocosms, the oxygen measurements were done over two days at sunrise, midday and sunset to get an idea of the gross primary productivity and net primary productivity. Water temperature was measured continually in intervals of 30 minutes in 5 different mesocosms in each site using TidbitV2 HOBO data loggers (Onset). Detailed description of the physical and chemical characteristics of the mesocosms used in this study (Table 1).

Figure 6- Probe used to measure abiotic variables.

Table 1- Physical and chemical characteristics of the studied mesocosms (mean values and standard deviation from the year 2017)

	Location					
Variables	Múrcia	Toledo	Évora	Porto	Peñalara	Jaca
Temperature (°C)	19.04 ± 2.68	18.92 ± 3.38	17.64 ± 3.03	18.2 ± 1.95	17.48 ± 2.62	17.11 ± 3.08
pH	9.97 ± 0.20	10.57 ± 0.15	9.74 ± 0.40	8.13 ± 0.76	9.13 ± 0.76	9.55 ± 0.85
Conductivity (µS/cm)	7775.63 ± 1389.45	1760.00 ± 259.23	598.50 ± 94.56	90.40 ± 17.29	102.26 ± 16.59	111.63 ± 29.61
chlorophyll a (µg/L)	34.21 ± 30.43	154.44 ± 168.42	45.45 ± 30.26	70.52 ± 97.87	71.40 ± 79.60	21.08 ± 1.76
Turbidity (NTU)	0.006 ± 0.004	0.02 ± 0.03	2.60 ± 2.91	7.19 ± 4.29	0.01 ± 0.02	0.003 ± 0.002
Dissolved oxigen(mg/L)	4.97 ± 3.34	5.32 ± 2.12	5.09 ± 3.48	8.43 ± 2.74	11.99 ± 3.92	10.17 ± 1.33

The macroinvertebrates sampling in mesocosms consisted in a quantitative. For the quantitative sample, a core (50 L) was used (Figure 7), which represents 5% of the volume of the mesocosm. Ten swipes were done with aquarium net (mesh size of 500 µm) along the water column and sediment surface. All the matter that was scooped (e.g., organisms, organic-matter, sediment) was sieved through a 500 µm sieve and conserved in 96% ethanol.

Figure 7- Core used for quantitative sampling in the mesocosms.

In the natural ponds a D-frame net (mesh size of 250 µm) was passed 15 times considering the proportion of different micro-habitats found in the pond. The samples were conserved in ethanol at 96%.

In the laboratory, all samples were washed, and macroinvertebrates were sorted and then identified and counted under a dissecting microscope (SZX7 Olympus). In some cases, samples were subsampled using an 8x8 grid, and then sorted as many squares as possible in 2 hours. All macroinvertebrates were identified to the lowest taxonomic level possible using Tachet *et. al* 2010 (see Apendix 4 Table 1 and Figure 8 for data on species composition in all of the locations). In other cases, we used group specific ID keys to reach the species level (Figure 9 A and C) Odonata: Cham, 2012; Askew, 2004; Brooks & Cham, 2014. F) Ephemeroptera: Sowa, 1975; Alba-Tercedor, 1997. E) Chironomids: Wilson & Ruse, 2005; Langton, 1984; Brooks *at al.,* 2008; Andersen *et al.,* 2013). After identifying all the species, a biological trait table was created (Appendix 2, Table 1) with the functional feeding group (FFG) of each species using the Freshwater Ecology database (https://www.freshwaterecology.info).
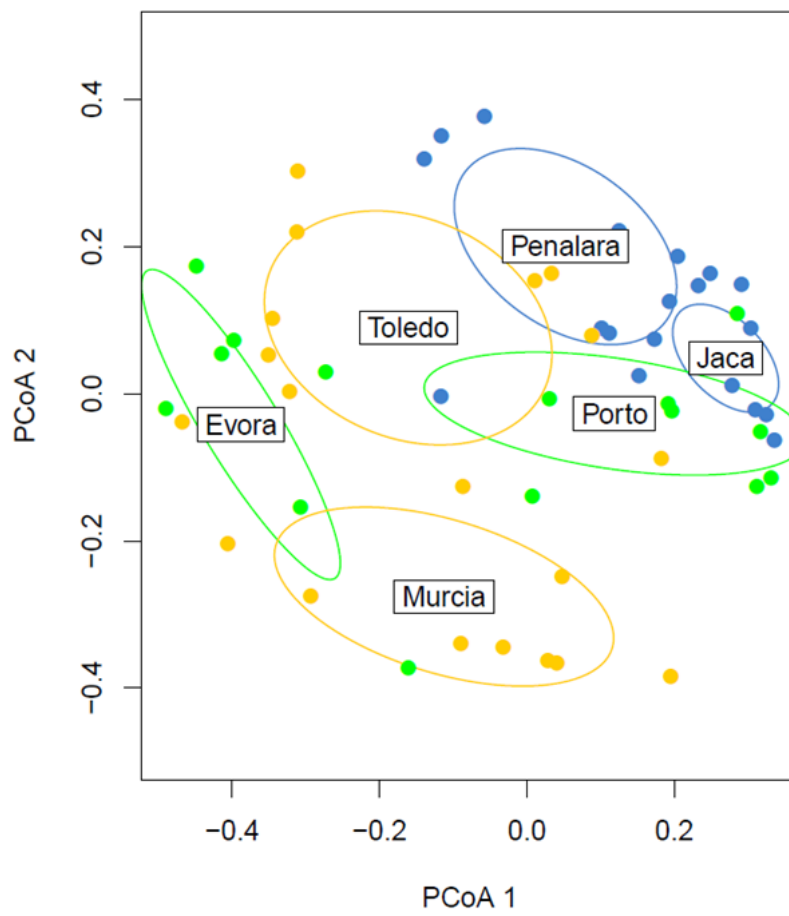
Figure 8-Principal coordinate analysis (PCoA) of the macroinvertebrate community of the mesocosms of each location
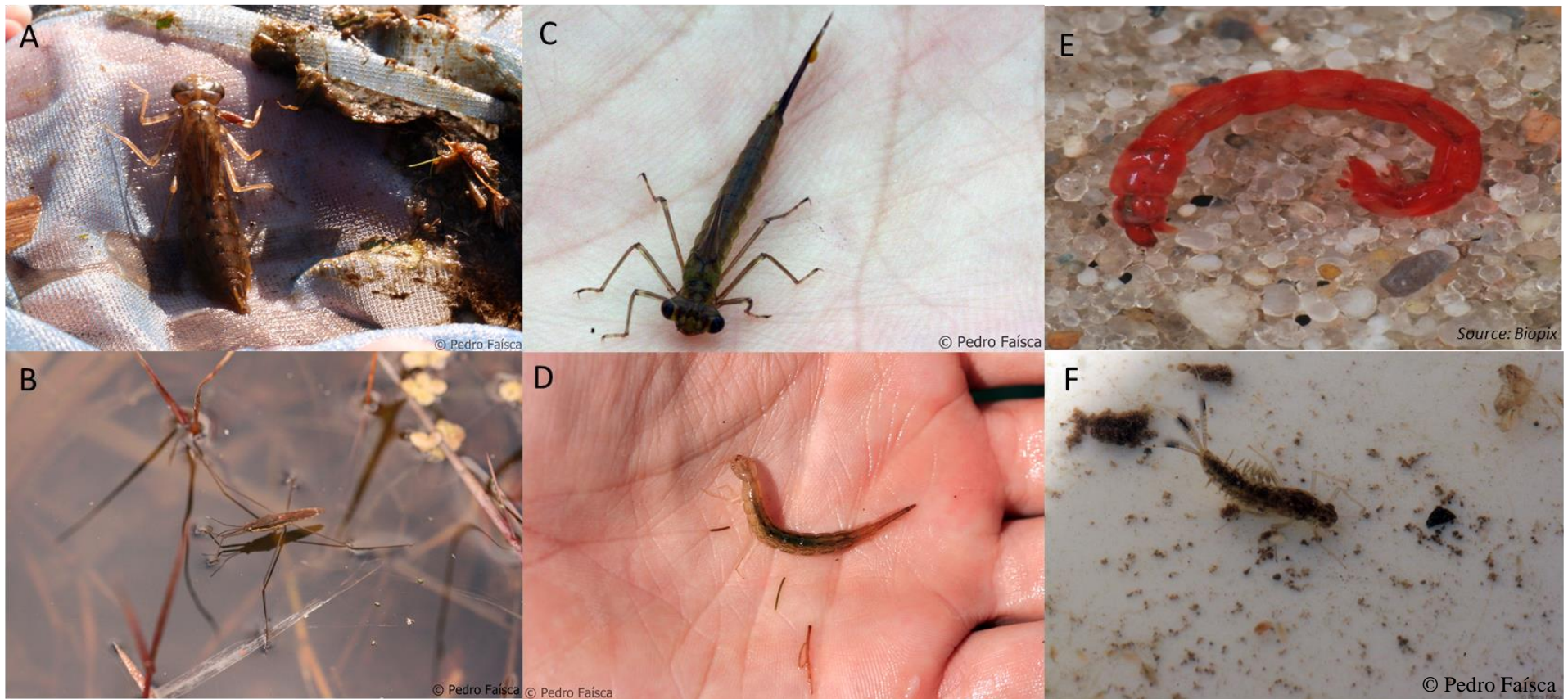
Figure 9- Major groups of macroinvertebrates present in the samples. A –Dragonfly (*Anax* sp.); B –Water strider (*Gerris* sp.); C –Damselfly (Zygoptera); D – Beetle larvae (Dytiscidae); E- Chironomid larvae (*Chironomus* sp.); F- Mayfly Larvae (Baetidae).

Statistical analysis

For the analysis tensile strength and mass loss data was used, despite showing some correlation (Figure 10). However, tensile strength loss appears to be more sensitive to small differences than mass loss (Tiegs *et al.,* 2007). The analysis was done using R version 3.4.2, to test the differences between locations an analysis of variance (one-way ANOVA) was used with 'location' and 'tension loss' or 'mass loss' as main factors. To test the difference between treatments we used linear mixed-effects models using the 'lmer' function in R package lme4 (Bates *et al.,* 2015), with mesocosm number as a random factor, and two-way ANOVA with 'location', 'treatment' and 'tension loss' or mass loss' as main factors. To test the differences between systems a one-way ANOVA was used with 'system' and 'tension loss' or 'mass loss' as main factors. Differences between treatments was tested using linear mixed-effects models using the function 'lmer' in R package lme4 (Bates *et al.,* 2015), with pond number as a random factor, and two-way ANOVA with 'system', 'treatment' and 'tension loss' or 'mass loss' as main factors.
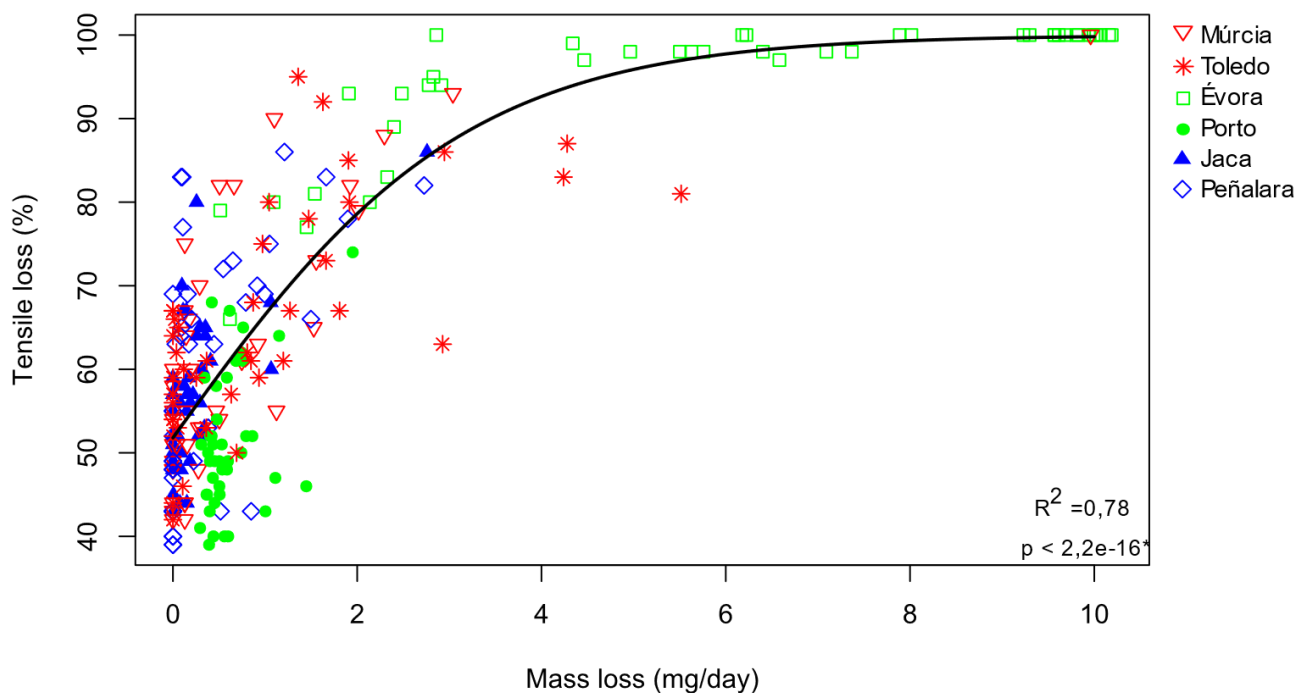
Figure 10- Relation between Mass loss (mg/day) and Tension loss (%).

To assess the effect of macroinvertebrates in the tensile strength of the cotton strips a ratio was done using the formula: ln (TD/MD), where TD represents total decomposition, which is related with the coarse mesh bags, and MD represents microbial decomposition, which is related with the fine mesh bags. This ratio is similar to what other studies have done (Cardinale *et al.*, 2006, Mayer-Pinto *et al.*, 2016). If the result was positive there is a positive effect of macroinvertebrates and if its negative it will mean a positive effect of microbial decomposition (Figure 11).
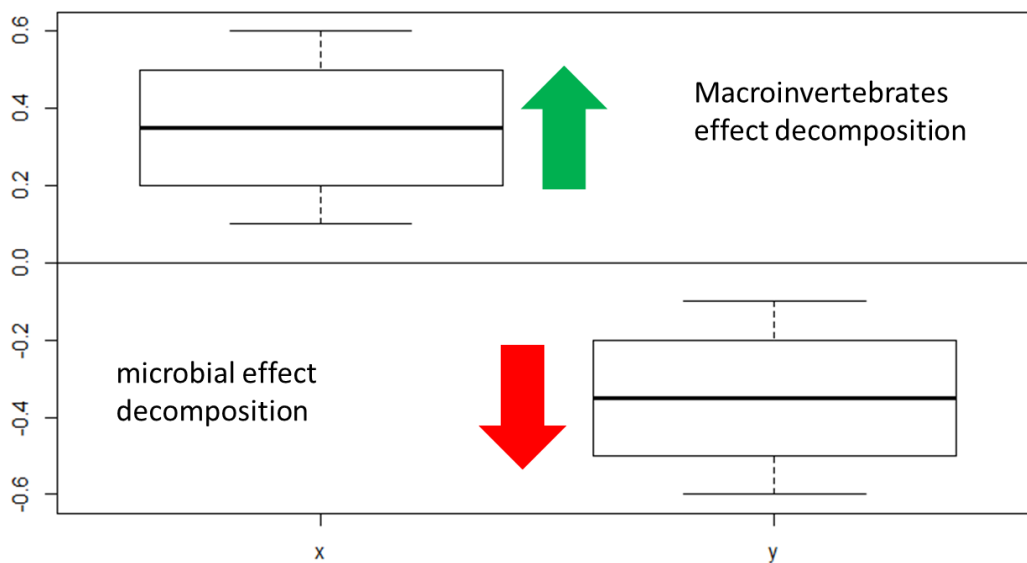
Figure 11- Example of the ratio used in this study. If results were positive there is an effect of macroinvertebrates and if the results were negative, there is a positive effect of microorganisms.

To check for differences in the macroinvertebrate community between natural ponds and mesocosms a principal coordinate analysis (PCoA) was done using the function 'betadisper' in R package vegan (Oksanen *et al.,* 2018). To determine which environmental variables and macroinvertebrate's feeding type best explained the decomposition in the mesocosms, a variable selection by Akaike information criterion (AIC) was used. Relevant variables were then plotted and tested to determine if there was a correlation using linear models

.

Results

Decomposition in mesocosms and natural ponds

There were no apparent differences between both systems in mass loss (A) and tension loss (B) between mesocosm (Meso) and natural ponds (Natural) using controls (Figure 12). Table 2 shows that there are no significant differences between systems for mass loss (A) and tension loss (B) (p-value > 0.05).
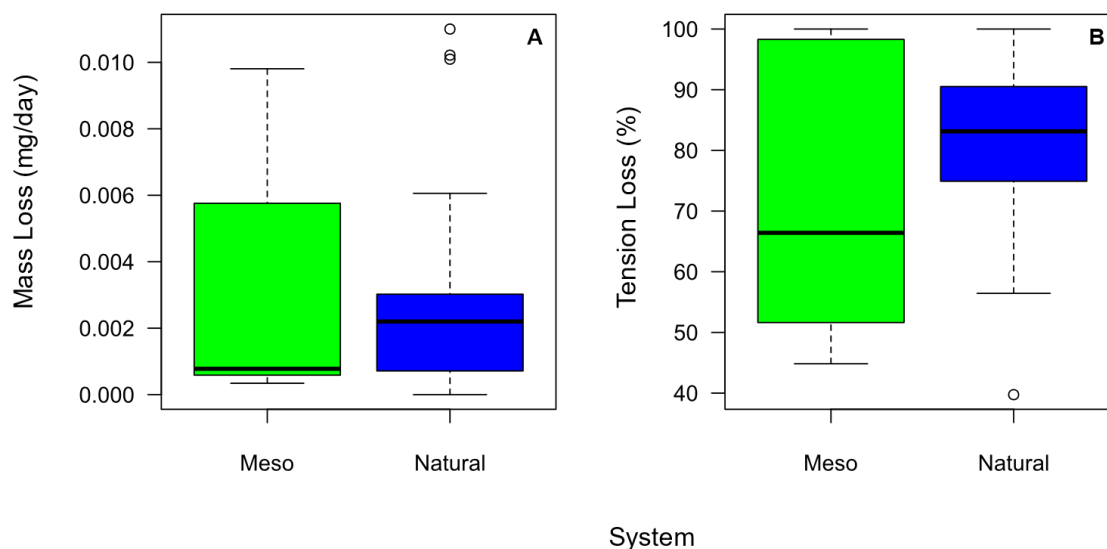
Figure 12- Decomposition in the different systems. These plots are "box-plots" which are used to show variation in the samples. The bar represents the median and the two lines (or whiskers) indicate the spread of the data. In this plot we can see the mass loss (A) and tensile loss (B) of the cotton strips. These plots used the data from the controls of each of the systems. There are no differences between the systems.

Table 2- One-way ANOVA between systems and mass loss (mg/day) (A) and tension loss (%) (B). The differences between systems were not significant in both cases (p-value > 0.05).

A) Mass Loss					
	Df	Sum Sq	Mean Sq	F value	Pr(>F)
System	1	7e-7	6.51E-07	0.064	0.801
Residuals	55	5.61e-4	1.02E-05		

B) Tension Loss					
	Df	Sum Sq	Mean Sq	F value	Pr(>F)
System	1	703	703.4	1.983	0.165
Residuals	55	19512	354.8		

The ratio between treatments in Mesocosm (Meso) and Natural Ponds (Natural) for Mass loss (A) and Tension loss (B) showed that both systems showed a positive effect of macroinvertebrates for mass loss, while in tension loss natural ponds have a positive effect of microbial decomposition (Figure 13).
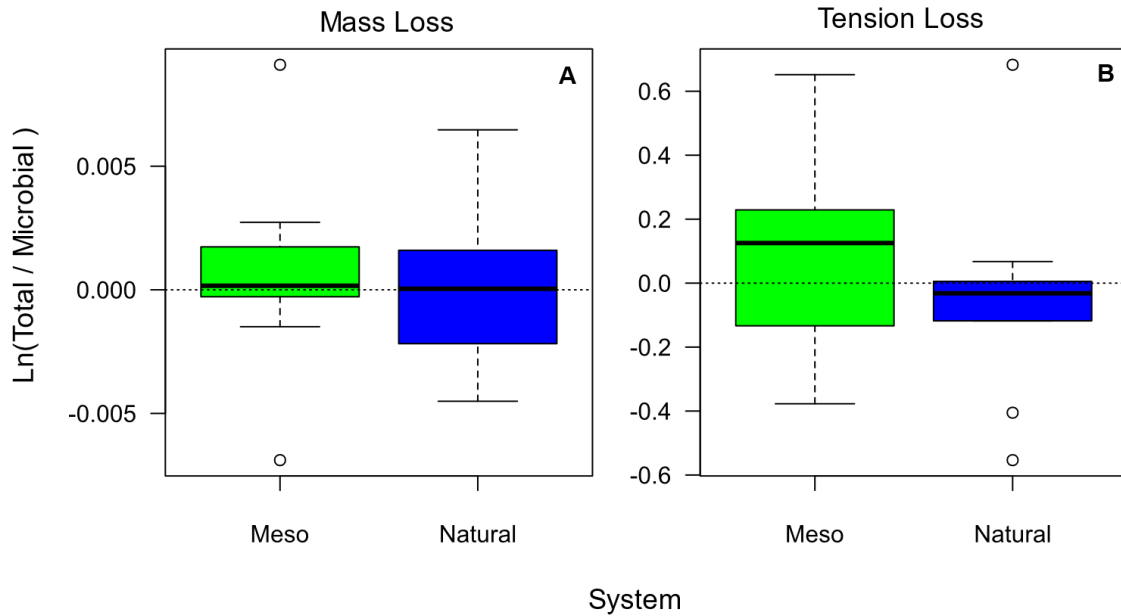
Figure 13- Ratio between treatments in Mesocosms (Meso) and Natural Ponds (Natural) for Mass loss (A) and Tension loss (B). Both systems show a positive effect of macroinvertebrates for mass loss, while in tension loss natural ponds had a positive effect of microbial decomposition. See Methods section Statistical analysis for further details on the calculation of the ratio.

Mass loss (A) and tension loss (B) between the two treatments in Mesocosms (Meso) and Natural ponds (Natural) showed that TD had the highest mass loss and tension loss in both systems (Figure 14). The differences between system were not significant in both mass loss and tension loss (ANOVA: p-value > 0.05), while the differences in treatment where only significant for tension loss (ANOVA: p-value < 0.05) (Table 3). PCoA showed that in Natural Ponds there are no significant differences between both locations, while in the mesocosm there are differences between locations (Appendix 3, Figure 1; Table 1, p-value > 0.05).
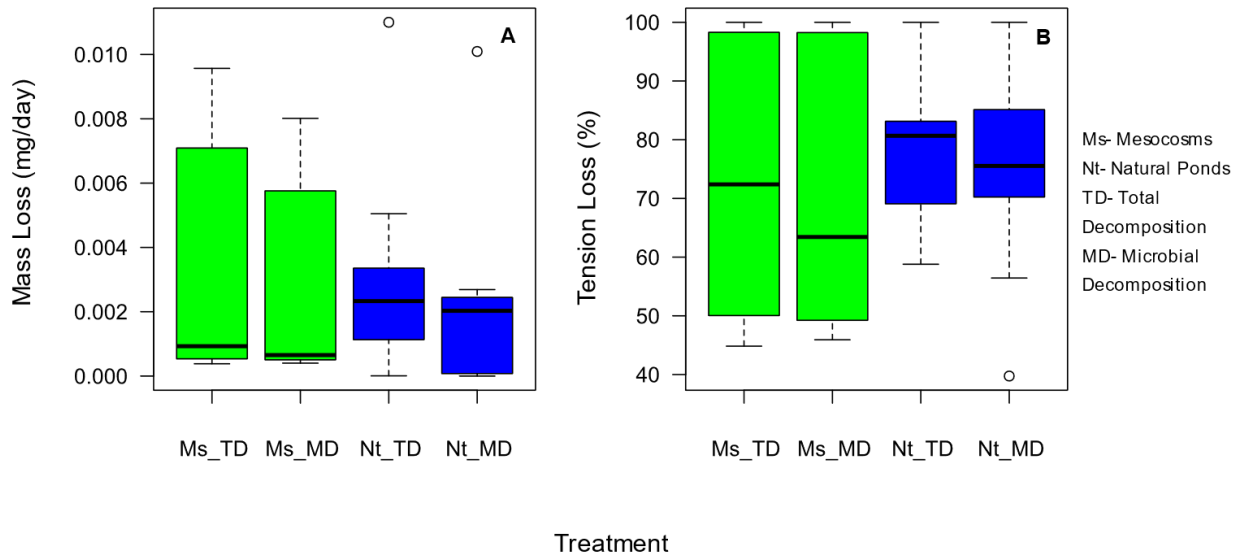
Figure 14- Mass Loss (A) and tension loss (B) of the cotton strips between treatment. TD had the highest mass loss and tension loss in both systems.

Table 3- ANOVA with pond number as a random factor, between system and treatment for mass loss (mg/day) (A) and tension loss (%) (B). There were no significant differences for mass loss (p-value > 0.05), while in tension loss the differences between treatments was significant. * indicates significative differences at p-value < 0.05.

A) Mass Loss						
	Sum Sq	Mean Sq	NumDF	DenDF	F value	Pr(>F)
System	2.56e-07	2.56e-07	1	14.878	0.0999	0.7564
Treatment	3.50e-06	1.75e-06	2	36.025	0.6825	0.5118
System:treatment	2.98e-06	1.49e-06	2	36.025	0.5816	0.5641

B) Tension Loss						
	Sum Sq	Mean Sq	NumDF	DenDF	F value	Pr(>F)
System	80.89	80.89	1	14.739	0.6173	0.4445
Treatment	1031.07	515.54	2	36.005	3.9344	0.0285*
System:treatment	443.63	221.81	2	36.005	1.6928	0.19832

Decomposition in the mesocosms among different locations and treatments

Decomposition was different across all locations (Figure 15). Évora was the location with the highest mass loss and tension loss (mean mass loss = 6.01 mg/day; mean tension loss = 95%), while Jaca had the lowest mass loss (mean mass loss = 0.018 mg/day) and Porto was the location with the lowest tension loss (mean tension loss= 51%). The differences between locations were significant (One-way ANOVA: p-value < 0.05) in both mass loss and tension loss (Table 4).
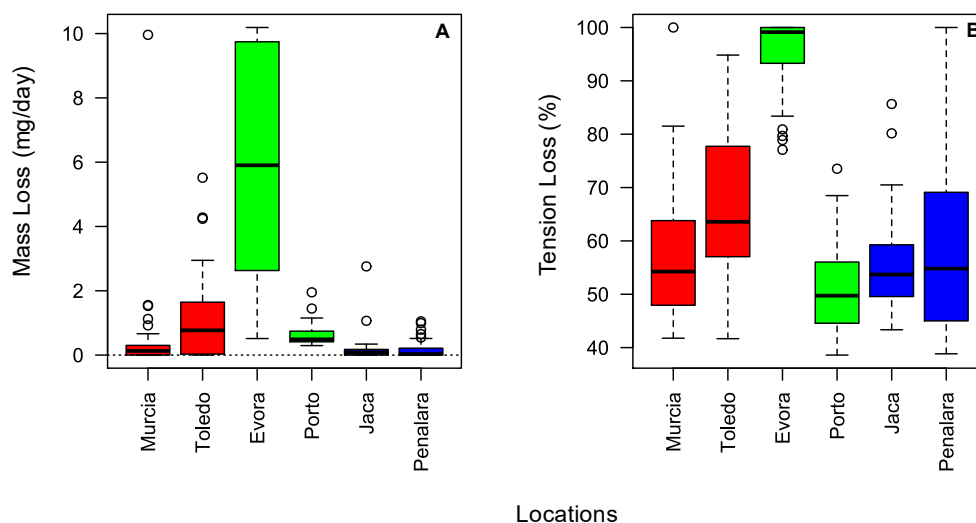
Figure 15- Decomposition across different location. These plots showed the mass loss (A) and tensile loss (B) of the cotton strips. These plots used the data from the controls of each of the locations separately. Évora was the location with the highest decomposition and Jaca was the location with less mass loss, while Porto was the location with less tension loss.

Table 4. One-way ANOVA between locations and mass loss (mg/day) (A) and tension loss (%) (B). In both cases the differences between location were significant (p-value < 0.05). *- significative variable.

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
A) Mass Loss					
Locations	5	1024.3	204.9	75.8	<2e-16 *
Residuals	245	662.2	2.7		
B) Tension Loss					
Locations	5	49205	9841	67.1	<2e-16 *
Residuals	246	36079	147		

30

The ratio between both treatments, Total decomposition (TD) and Microbial decomposition (MD), using mass loss and tension loss showed there was a very clear positive effect of macroinvertebrates in all locations when we use either mass loss or tension loss (Figure 16).
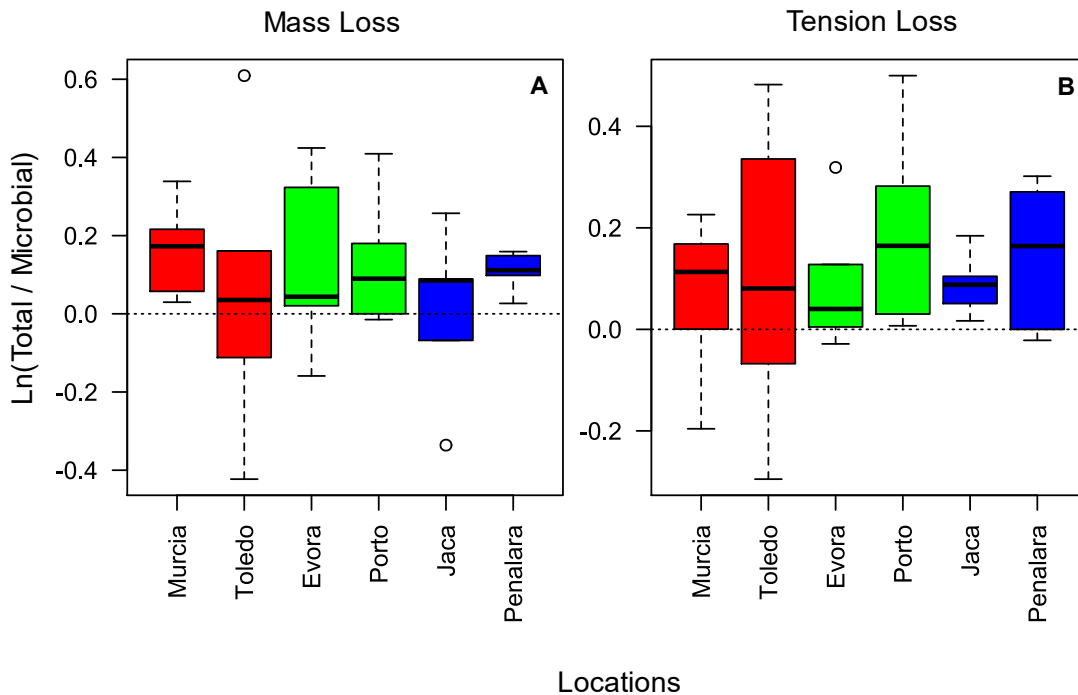
Figure 16- Ratio of the mass (A) and tensile loss (B) of the cotton strips between the two treatments. If the result was positive there is a positive effect of macroinvertebrates and if its negative it will mean a positive effect of microbial decomposition.

Mass loss (A) and tension loss (B) between the two different treatments in all locations showed that TD had the highest mass loss in all the locations (mean mass loss = 0.17 mg/day), while tension loss was higher for MD in Évora and Jaca (mean tension loss = 77 %) (Figure 17). For mass loss, only the differences between locations were significant (ANOVA: p-value < 0.05) while the differences between treatments were not significant (ANOVA: p-value > 0.05) with no interaction between both (ANOVA: p-value > 0.05; Table 4). For tension loss the differences between locations and treatments were significant with interaction between both (ANOVA: p-value < 0.05) (Table 4), but there were two exceptions to this interaction in Évora and Jaca with higher decomposition in MD.
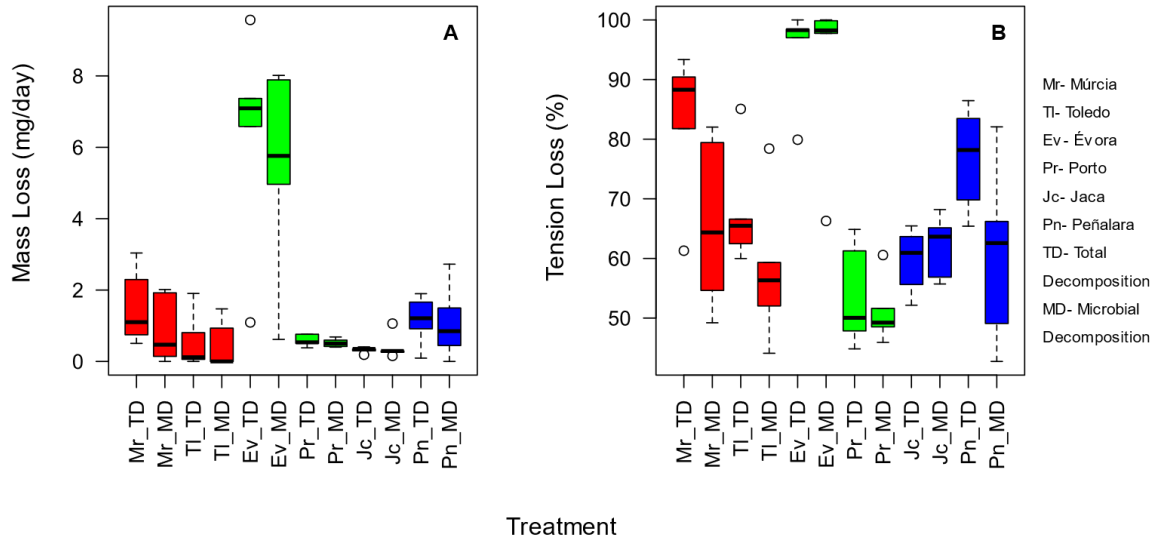
Figure 17- Mass Loss (A) and tension loss (B) of the cotton strips between treatment. These plots had data from the different treatments from each of the locations separately. TD had the highest mass loss and MD had the highest tension loss in Évora and Jaca.

Table 5- ANOVA with mesocosm number as a random factor, between location and treatment for mass loss (mg/day) (A) and tension loss (%) (B). Mass loss only had significant differences in locations, while tension loss had significant differences in location, treatments and both interact (p-value < 0.05). *-significative variable.

A) Mass Loss						
	Sum Sq	Mean Sq	NumDF	DenDF	F value	Pr(>F)
Locations	170.129	34.026	5	40.071	26.2151	1.177e-11 *
Treatment	1.124	1.124	1	30.051	0.8662	0.3594
Location:treatment	1.914	0.383	5	30.051	0.2949	0.912

B) Tension Loss						
	Sum Sq	Mean Sq	NumDF	DenDF	F value	Pr(>F)
Locations	6081.4	1216.28	5	39.955	22.1228	1.468e-10 *
Treatment	868.8	868.83	1	28.038	15.8032	4.481e-4 *
Location:treatment	800.6	160.12	5	28.038	2.9124	0.03064 *

Decomposition and environmental variables in mesocosms

The variable selection procedure done using Akaike information criterion (AIC) showed that the variables that explained decomposition in both treatments were accumulative daily temperature (Tacc) and conductivity (Cond) for both mass loss and tension loss. The relation between Tacc and mass loss between TD (A) and MD (B) , the model showed a pattern for TD with higher mass loss in the intermediate values of Tacc (Figure 18A) TD: $R^2 = 0.16$, p-value < 0.05), while in MD the model did not show a clear pattern (Figure 18B) MD: $R^2 = 0.12$, p-value > 0.05). The relation between Tacc and tension loss

32

between TD (A) and MD (B), in both treatments, the model did not show any clear pattern (Figure 19A) TD: $R^2 = 0.02$ p-value > 0.05; Figure 19B) MD: $R^2 = -4.1e^{-4}$, p-value < 0.05).
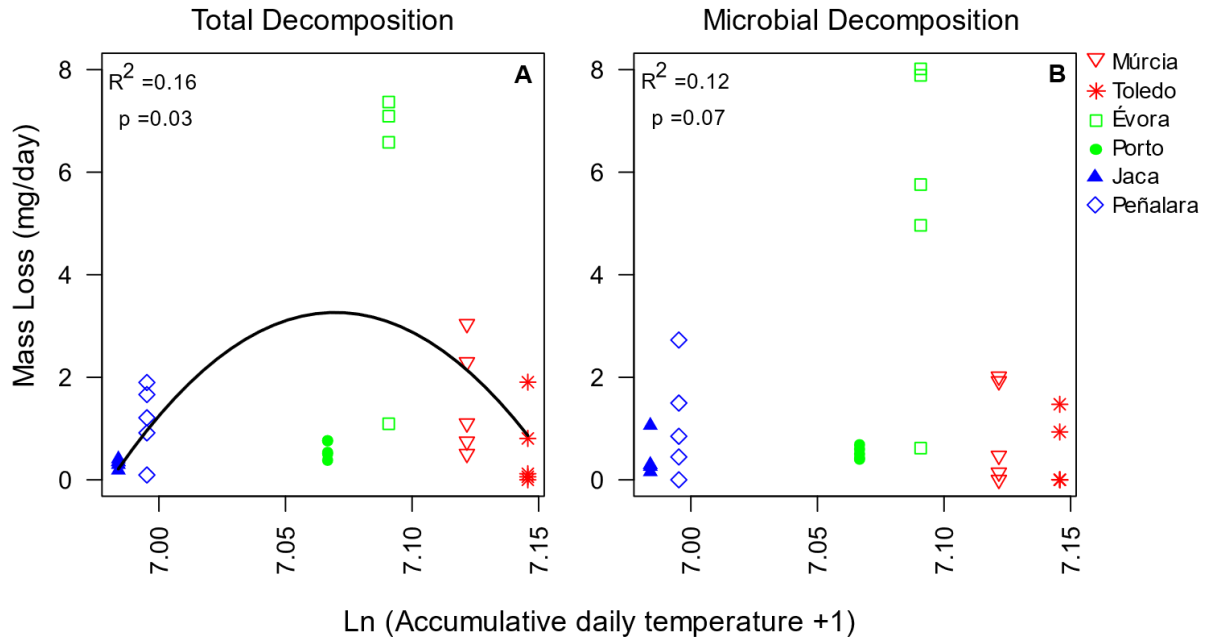
Figure 18- Relation between accumulative daily temperature and mass loss in total decomposition (A) and microbial decomposition (B).
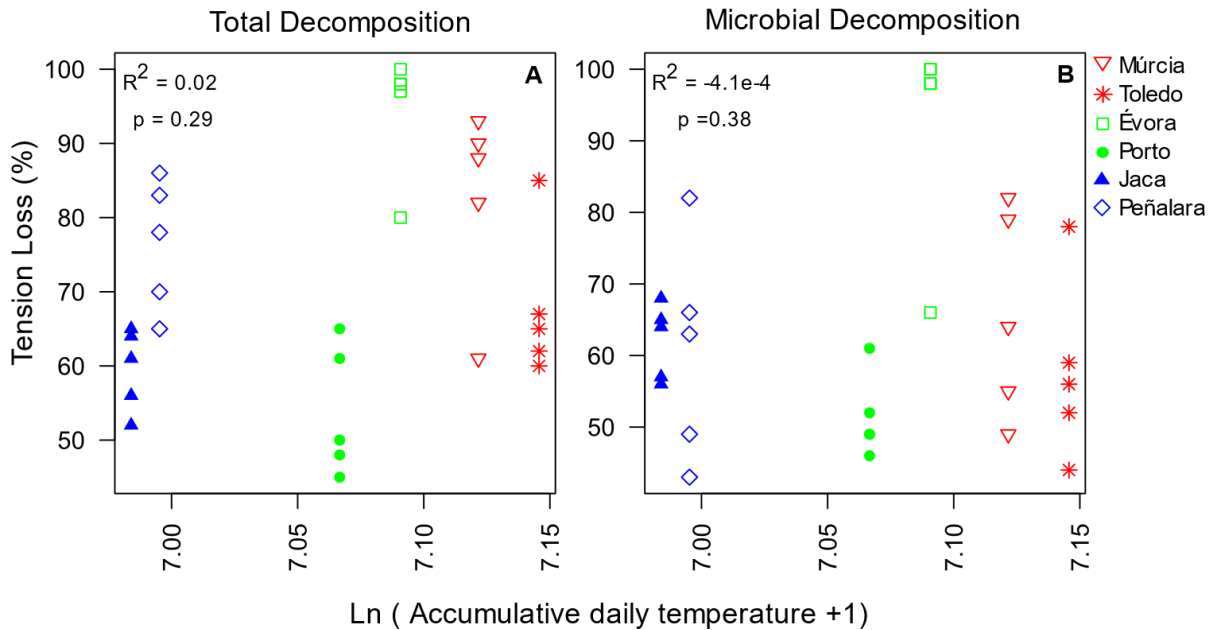
Figure 19- Relation between accumulative daily temperature and mass loss in total decomposition (A) and microbial decomposition (B).

In the relation between Cond and weight loss between TD (A) and MD (B), in both treatments, the model showed that there is more mass loss in the intermediate values of

Cond and less in the extremes (Figure 20A) TD: $R^2 = 0.19$, p-value < 0.05; Figure 20B) MD: $R^2 = 0.19$, p-value < 0.05). The relation between Cond and tension loss between TD (A) and MD (B), in TD the model showed that there is more tension loss in intermediate values of conductivity (Figure 21A) $R^2 = 0.22$, p-value < 0.05). MD did not show a clear pattern between the 2 variables (Figure 21B) $R^2 = 0.09$, p-value > 0.05).
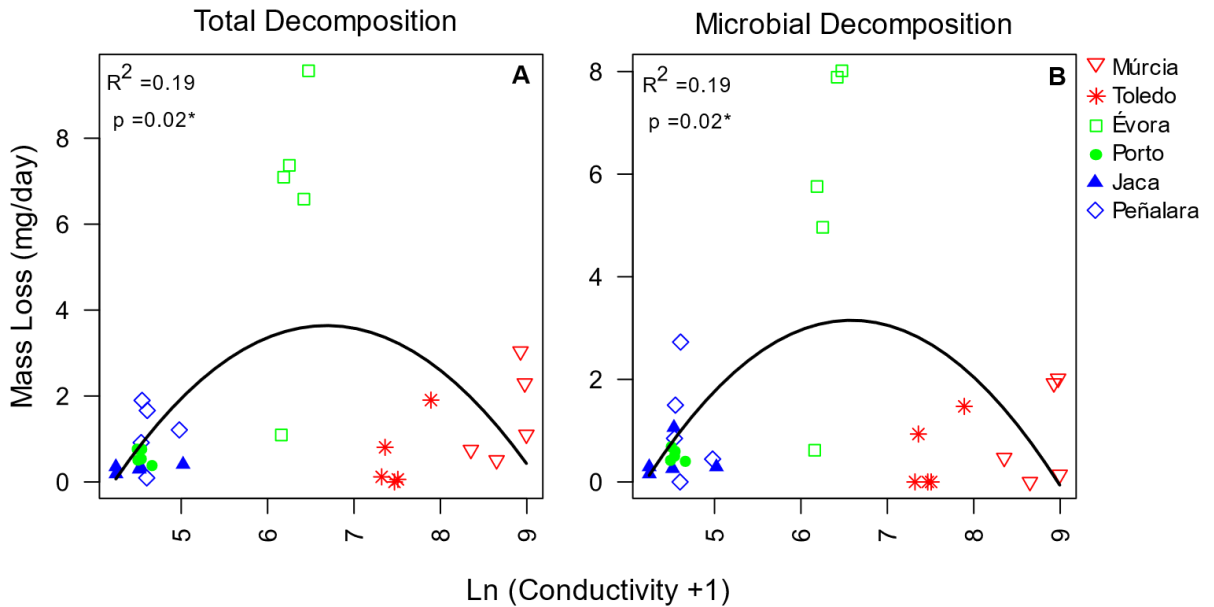
Figure 20- Relation between conductivity and mass loss in total decomposition (A) and microbial decomposition (B).
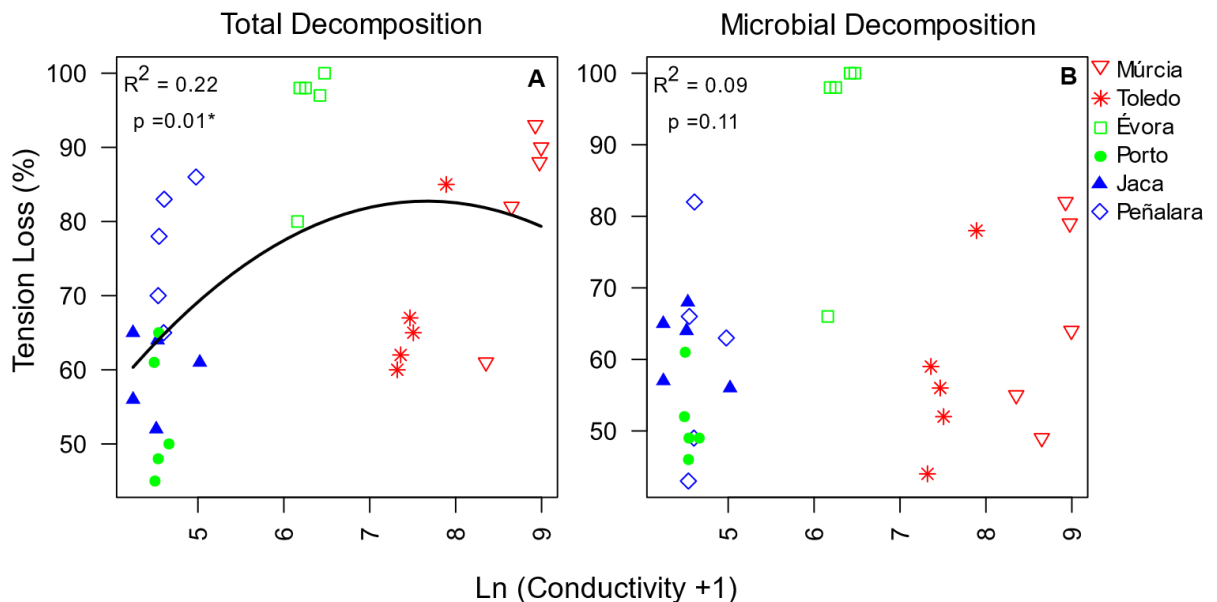
Figure 21- Relation between conductivity and Tension loss in total decomposition (A) and microbial decomposition (B).

Decomposition and macroinvertebrates in mesocosms

The variable selection done by AIC showed that the variable that better explained variation in mass loss and tension loss was the abundance of gatherers (Figure 22).

In most of the location's gatherers where present with higher relative abundances than most of the other function feeding groups, where Toledo was the location with the highest abundance (74.27%. Table 6) and Porto was the location with the lowest gatherer abundance (2.10%. Table 6). For more detailed information on the abundance on each of the species found in the mesocosms check Appendix 4, Table 1.

In both cases, the model showed there is a linear relation between decomposition and abundance of gatherer species (Mass loss: $R^2 = 0.21$, p-value < 0.05; Tension loss: $R^2 = 0.45$, p-value < 0.05).
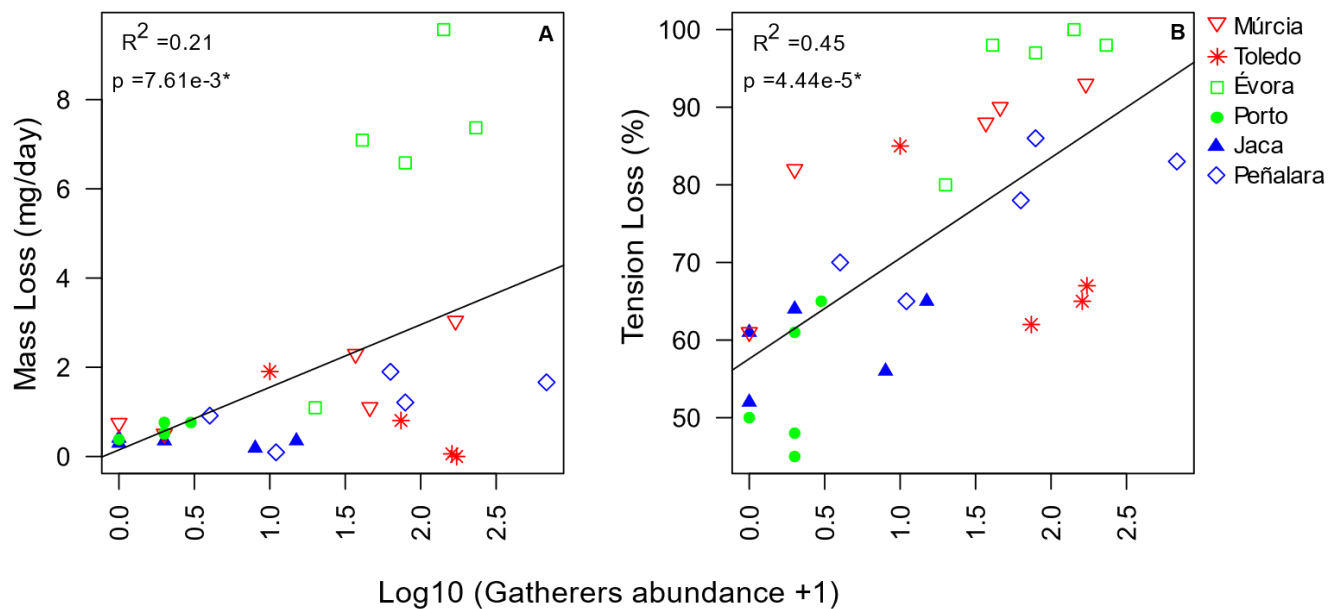
Figure 22- Relation between the abundance of gatherers and mass loss (A) and tension loss (B).

Table 6- Abundance of Functional Feeding groups in the mesocosms used for this experiment in all locations (Mean abundances and standard deviation).

Functional Feeding Groups	Location					
	Múrcia		Toledo		Évora	
	Mean Abundance	Abundance (%)	Mean Abundance	Abundance (%)	Mean Abundance	Abundance (%)
Active filter feeders	21.45 ± 15.64	7.01	2.18 ± 4.61	0.23	7.18 ± 9.93	2.50
Gatherers/Collectors	167.00 ± 313.87	54.59	582.36 ± 1091.45	61.07	266.64 ± 326.82	92.70
Grazers	95.27 ± 118.14	31.14	16.45 ± 28.39	1.73	6.27 ± 12.32	2.18
Passive filter feeders	0.00 ± 0.00	0.00	0.00 ± 0.00	0.00	0.00 ± 0.00	0.00
Predators	11.36 ± 11.72	3.71	340.91 ± 433.10	35.75	6.91 ± 6.57	2.40
Shredders	10.82 ± 34.21	3.54	11.73 ± 18.12	1.23	0.64 ± 1.72	0.22
	Porto		Jaca		Peñalara	
Functional Feeding Groups	Mean Abundance	Abundance (%)	Mean Abundance	Abundance (%)	Mean Abundance	Abundance (%)
Active filter feeders	0.00 ± 0.00	0.00	0.7 ± 1.00	2.94	3.3 ± 3.98	1.32
Gatherers/Collectors	53.40 ± 65.95	41.17	4.2 ± 5.10	17.65	160.90 ± 212.28	64.59
Grazers	34.20 ± 26.77	26.37	0.70 ± 1.55	2.94	3.4 ± 2.62	1.36
Passive filter feeders	0.00 ± 0.00	0.00	0.10 ± 0.30	0.42	0.00 ± 0.00	0.00
Predators	35.80 ± 18.36	27.60	12.50 ± 9.21	52.52	24.10 ± 17.37	9.67
Shredders	6.30 ± 7.77	4.86	5.6 ± 5.28	23.53	57.40 ± 70.97	23.04

Discussion

No significant differences were found between rates of decomposition in natural ponds and mesocosms, with both having similar decomposition rates. Decomposition varied between different biogeographical regions, with Évora being the region with the highest decomposition. It was also shown that macroinvertebrates play an important role in the decomposition of organic matter and that gatherer species were the major players in this ecosystem service, despite of the low abundance of species that are considered real decomposers (e.g. shredders). In this study it was hypothesized and demonstrated that decomposition would vary across different biogeographical regions, that macroinvertebrates are important in the decomposition of organic matter and that there were no differences between natural ponds and mesocosms.

Mesocosms versus natural ponds

In this study, there were no significant differences between the two aquatic systems used: mesocosms and natural ponds. Based on what has been shown by other experiments artificial ponds are not very different from natural ones, supporting the same biodiversity that natural system support, with small differences being found in environmental variables (Céréghino *et al.*,2008; Ruggiero *et al.*,2008; Le Viol *et al.*, 2009). Mesocosms, experimental enclosures with a thousand litters, were used as artificial ponds in this study. Mesocosms can be used to test community and ecosystem-level responses to change (Stewart et al., 2013; for more detailed explanation on the mesocosm used in this experiment check Methods' subsection Study area).

Several mesocosms experiments, used at this scale, showed that they can reproduce the key elements of community structure and ecosystem functioning of small pond ecosystems (Jones *et al.*, 2002; McKee *et al.*, 2003; Ventura *et al.*, 2008; Yvon-Durocher *et al.*, 2010). In Yvon-Durocher *et al.* (2010), the main conclusion reached was that the data provided by their mesocosms could serve as a good baseline for understanding the mechanisms that control the effects of temperature on the metabolic balance of ecosystems. Although caution is needed when extrapolating such data from mesocosms to natural systems due to the great complexity and diversity in biotic and abiotic factors that can be found influencing the dynamic of these ecosystems. Since the mesocosms used in this study were inoculated with soil from nearby ponds (see Methods' subsection

Study area), it was expected that there wouldn't be many differences between both systems.

Differences among regions

Decomposition rates differed between the standardized aquatic mesocosms across different locations. Previous studies have shown that warmer climates promote higher decomposition rates than colder climates (e.g. Young *et al.*, 2008). Similar to other studies, temperature was found to be one of the main factors responsible for the different decomposition rates observed in this experiment (Webster and Benfield, 1986; Costantini *et al.*, 2009; Goodman *et al.*, 2010; Vysná *et al.*, 2014; Martínez *et al.*, 2015; Santonja *et al.*, 2017). High temperatures can promote an increase in microbial decomposition (Young *et al.*, 2008; Goodman *et al.*, 2010; Vysná *et al.*, 2014), and has also been known to affect diversity and community composition of macroinvertebrates (Burgmer *et al.*, 2006) by effecting the physiological processes of species, which may impact timing of life history events and trophic interactions (Ward, 1992). Brucet *et al.* (2012) found a greater diversity and abundance in colder and temperate climates, with Diptera being the most abundant group.

Another important factor in this experiment was conductivity. There is no consensus on the effects of this environmental factor on this ecosystem service. While there are studies that showed that high levels of conductivity led to an increase in decomposition (Weston et al., 2006; Craft, 2007; Morrissey *et al.*, 2014), there are others that showed a decrease in this function (Rejmánkoná & Houdková, 2006; Roache *et al.*, 2006; Neubauer, 2012). Conductivity is found to be strongly dependent of temperature (Hayashi, 2004) and can be related to the nutrient availability in the water (Stevens *et al.,* 1995), in this case we can see the same thing as Weston *et al.* (2006) and Craft (2007), where high conductivity increased decomposition. A possible explanation for this can be found in Morrissey *et al.* (2014) were it was concluded that, conductivity affected the composition of the microbial community, which, in conjugation with other abiotic factors, stimulated extracellular enzymes and increased the decomposition of organic matter. Conductivity might also have indirect effects on decomposition by effecting the distribution of microbial community and structure of macroinvertebrate communities (Young *et al.*, 2008). As shown in Brucet *et al.* (2012), conductivity has a negative effect on macroinvertebrate

diversity and abundance although, some groups are not affected (e.g. Odonata; Polychaeta) and some have a high tolerance to high conductivity levels (e.g. Diptera).

As mentioned above (method subsection Statistical analysis), the loss of tensile strength appears to be more sensitive to small differences than mass loss (Tiegs *et al.,* 2007). This leads to, environmental variables explaining differences in decomposition at the regional scale, while at a local scale microbial community composition seem to explain differences in decomposition more evidently, at least for the case of mass loss. The possible explanation for this is that the microbiological community is more sensitive to small changes in the environmental variables, that would only be detected at a local level. This can lead to more efficient decomposers being benefited in one pond, which leads to an increase in their abundance, but be impaired in another, which can lead to the absence of this species, due to a difference in temperature or conductivity. For example, Dang *et al.* (2009) found that variations of 8ºC in temperature would benefit a species of decomposing fungi that is more efficient than the other microbiological decomposer, which lead to faster decomposition rates.

The role of macroinvertebrates

Significant differences were found between total decomposition (decomposition by microbial and macroinvertebrates communities; TD) and only microbial decomposition (MD), with TD showing higher levels of decomposition, which can be related to the importance that macroinvertebrates have in the decomposition process. Although cotton strips have been found to be a less palatable than leaf litter as a food resource (Tiegs *et al.,* 2013), cotton strip palatability will improve with colonization and conditioning by the microbial community (Graça, 2002; Tiegs *et al.,* 2013). Studies that used the litter bag experiment have shown different results, some studies showing that there were no significant differences between TD and MD, where others defended that the role of macroinvertebrates was neglectable (Stockley *et al.,* 1998; Lamed, 2000; Benstead *et al.,* 2009; MacKenzie *et al.,* 2013; Raposeiro *et al.,* 2014; Ferreira *et al.,* 2016). While other experiments found higher levels of decomposition in TD (Imbert & Pozo, 1989; Howe & Suberkropp, 1994; Graça & Canhoto, 2006; Tiegs *et al.,* 2007; Jacob *et al.,* 2010), showing a higher importance of macroinvertebrates in the decomposition of organic matter.

It should be expected that in biogeographical regions with stronger environmental filters, higher temperatures in the south of Spain and colder temperatures in the mountain regions, MD should be more important, due to the stresses that are placed on the macroinvertebrate communities (Brucet *et al.,* 2012). On the other hand, in temperate regions TD would be more important due to their high diversity in terms of macroinvertebrates. However, this was not observed, decomposition was higher in TD in almost all the regions with strong environmental filters and the temperate locations showed higher MD. Although temperate locations had the highest diversity of macroinvertebrates, not all of these species had a relevant role in the decomposition of organic matter.

The abundance of gatherer species of macroinvertebrates was a factor that influenced decomposition in this experiment, where higher abundances were related with higher decomposition rates. Some of the studies mentioned above (e.g. Raposeiro *et al.,* 2014; Ferreira *et al.,* 2016) stated that the reason why they found no difference between treatments might be related to the fact that there were no shredding macroinvertebrates present in the ecosystem. Which is similar to what was found in this experiment, where there were very low abundances of true shredders in the mesocosms. Other studies defend that in the absence of shredding macroinvertebrates, other species with different feeding types might take the role of shredders (Chergui & Patteo, 1991; Lock, 1993; Graça, 2001). In Chergui & Patteo (1991) it was shown that in the absence of trichopteran and plecopteran in a Morocco stream there was a higher shredding affect by the gastropods species *Melanopsis praemorsa* and *Physa acuta*. Other studies also showed that the abundance of shredding macroinvertebrate was as important as the abundance of gatherers (Alvarez *et al.,* 2001), where it was found that species of the Chironomidae subfamily, which are mostly classified as gatherers, to be responsible for the decomposition of the organic matter. Similar to what was found by Abelho (2008), that consistently found a high abundance of Chironomidae subfamily when compared to the abundance of shredders. In Silveira *et al.* (2013) it was determined that the Chironomidae larvae species of *Chironomus*, *Polypedilum*, and *Tanytarsus*, which are all gatherer macroinvertebrates, were the major species involved in decomposition, with the *Chironomus* species being more associated with the late stages of decomposition and the *Tanytarsus* species more associated with the beginning stages of decomposition.

Limitations/Future directions

Despite the valuable information generated by this study, we acknowledge that there is still room for some improvements and further experiments to be done to expand our understanding of decomposition across different systems and scales. One important limitation in this study is the narrow temporal window of observation through which decomposition was measured. An obvious improvement to this methodology would be the use of multiple time points (i.e. deploy additional cotton strips for different time periods) to obtain accurate decomposition rates like it is done in most of the studies (e.g. Tiegs *et al.*, 2007; Ferreira *et al.*, 2016), but a single time point is adequate if effort must be minimized (Young *et al.*, 2008).

Another area that should be taken into account is that, while cotton strips have proven to be a good method to measure decomposition (Boulton & Quinn, 2000; Tiegs *et al.*, 2007; Tiegs *et al.*, 2013), it only serves as a *proxy* for decomposition of leaves, so a similar study should be conducted using leaves, that were picked from trees from a single location and air-dried before being placed in the field (Boulton & Boon, 1991; Young *et al.*, 2008). Another solution for the fact that cotton strips have a simpler chemical composition when compared with leaves, could be to conduct a new experiment related to the chemical composition of litter used in this experiment. In this type of experiment a decomposition and consumption tablet (DECOTAB), which consists of a high concentration of cellulose powder embedded in an agar matrix (Kampfraath *et al.*, 2012), could be used instead. This material allows for a manipulation of its chemical composition, which allows to test how macroinvertebrates react to the presence of pesticides, for example.

Environmental variables like conductivity, dissolved oxygen, pH, chlorophyll and turbidity were only measured at the start of the experiment, which may lead to errors due to changes in these variables during the time the experiment was being conducted. Most of the studies (e.g. Ferreira *et al.*, 2016) measured environmental variables at the beginning and at the end of the experiment. Studies that used the litter bag experiment would collect the macroinvertebrates found inside the mesh bags, this way its possible to determine which species were inside the litter bags and determine their abundance (Silveira *et al.,* 2013; Biasi *et al.*, 2013; Leite-Rossi *et al.*, 2015). This study might have done the same. In some of the locations used in this study there are external factors that need to be considered, for example locations with trees nearby had an increase in leaf

litter availability, which gives an increase in availability of organic matter in these mesocosm and may affect the decomposition process. In future studies, leaf fall should be quantified by placing a container or a net on top of the ponds and leaving it there for a fixed time to determine leaf fall rates (Boulton & Boon, 1991).

Multiple macroinvertebrate taxa undergo ontogenetic diet shifts (Merritt, *et al.,* 2008; Rosi-Marshall *et al.,* 2016). To determine which group of macroinvertebrates were important for the decomposition of organic matter functional feeding groups (FFG) were used. FFG's are a classification based on the organism's mode of feeding (Cummins, 1973) and not the actual food resources that are being consumed. Despite this fact, FFG classifications may help understand the form of food resources consumed but cannot be counted as a measure of the identity of the food resources consumed (Rosi-Marshall & Wallace, 2002; Rosi-Marshall *et al.,* 2016). One way to overcome this would be to determine what each macroinvertebrate species is eating, this could be done by analysing gut-content (Cummins, 1973, Rosi-Marshall *et al.,* 2016) or by doing something similar to Holgerson *et al.* (2016), who labelled the organic material with a known isotope marker and then used a stable isotope analysis to "follow" the isotope through the food web.

Finally, when working in regions with different climates, which affect decomposition (Young *et al.*, 2008), the thermal gradient must be considered. The team at CIBIO-UE will continue the experiment at the Iberian Ponds facilities where a warming experiment will be conducted in which some mesocosms will be warmed to assess the impacts of climate change in freshwater ecosystems, like other studies (Mckee *et al.,* 2003; Liboriussen *et al.,* 2004; Yvon-Durocher *et al.,* 2010; Fey *et al.,* 2015).

Conclusion

Freshwater ecosystems, which are the focus of this thesis, are amongst the most threatened ecosystems in the world (Szöllosi-Nagy *et al.,* 1998; Saunders *et al.,* 2002; Dudgeon *et al.,* 2005; Higgins *et al.,* 2005), having decreased dramatically during the last century (Saunders *et al.,* 2002; Oertli *et al.,* 2005; Le Viol *et al.,*2009). Addressing this challenge requires developing a better understanding how these ecosystems function. Declines in biodiversity have been estimated to be far greater in freshwater than in terrestrial ecosystems (Frissell et al., 1996; Sala et al., 2000), with the increase in extinction risks when compared to other systems (Ricciardi *et al.,* 1999; Saunders *et al.,* 2002) making them highly vulnerable. The results showing similarities in decomposition of organic matter between mesocosms and natural ponds present us with opportunities to use experimental systems to further investigate how natural ecosystems function. Furthermore, the fact that decomposition varied between regions, may reflect, not only, differences in the environmental variables (as shown in this thesis), but shed some light on potential consequences of major environmental threats (e.g. over exploitation; water pollution; flow modification; destruction or degradation of habitat; and, invasion by exotic species; De Meester *et al.,* 2005; Dudgeon *et al.,* 2005; Oertli *et al.,* 2005; Declerck *et al.,* 2006; Céréghino *et al.,* 2007). The role macroinvertebrates and how a group of aquatic organisms can have such a key role in this crucial ecosystem service, reinforces the need for continued research on the function of species in the ecosystem and how the potential loss of those species (and their functions), might harm the essential processes needed to maintain and conserve natural ecosystem services.

Acknowledgements

References

Abelho, M. (2008). Effects of Leaf Litter Species on Macroinvertebrate Colonization during Decomposition in a Portuguese Stream. *International Review of Hydrobiology*, 93(3), 358–371.

Abreu, I., Ribeiro, H., & Cunha, M. (2003). An Aeropalynological study of the Porto region (Portugal). *Aerobiologia*, 19(3–4), 235–241.

Alba-Tercedor, J. (1997). *Curso sobre Efemerópteros. Universidade de Coimbra (Portugal).*

Allan, J. D. & Castillo, M. M. (2007). 7- *Detrital energy sources. Stream Ecology: Structure and Function of Running Waters. pp. 135–161. Springer*, Dordrecht, The Netherlands.

Alonso-Sarría, F., Martínez-Hernández, C., Romero-Díaz, A., Cánovas-García, F., & Gomariz-Castillo, F. (2016). Main Environmental Features Leading to Recent Land Abandonment in Murcia Region (Southeast Spain). *Land Degradation and Development*, 27(3), 654–670.

Alvarez, S., Rico, E., Guerrero, M. C., & Montes, C. (2001). Decomposition of Juncus maritimus in Two Shallow Lakes of Doñana National Park. *International Review of Hydrobiology*, 86(4), 541–554.

Andersen, T., Cranston, P. S., Epler, J. H. (2013). *Chironomidae of the Holarctic Region: Keys and Diagnoses, Part 1: Larvae .Insect Systematics and Evolution Supplements*

Asensi, A., Aguiar, C., Sánchez-Mata, D., & Monteiro-Henriques, T. (2011). Flora and Vegetation of Iberian Ultramafics Excursion Guide. *Seventh International Conference on Serpentine Ecology*, 5–11.

Askew, R. (2004). *The Dragonflies of Europe. Harley Books.*

Bates, D., Maechler, M., Bolker, B., Walker, W. (2015). Fitting Linear Mixed-Effects Models Using lme4. *Journal of Statistical Software*, 67(1), 1-48.

Benstead J. P., March J. G., Pringle C. M., Ewel K. C. & Shor J. W. (2009). Biodiversity and ecosystem function in species-poor communities: community structure and leaf litter breakdown in a Pacific island stream. *Journal of the North American Benthological Society*, 28, 454–465.

Biasi, C., Tonin, A. M., Restello, R. M., & Hepp, L. U. (2013). The colonisation of leaf litter by Chironomidae (Diptera): The influence of chemical quality and exposure duration in a subtropical stream. *Limnologica*, 43(6), 427–133.

Brönmark C, Hansson LA. (2002). Environmental issues in lakes and ponds: current state and perspectives. *Environmental Conservation* 29: 290–307.

Brooks, S. & Cham, S. (2014). *Field Guide to the Dragonflies and Damselflies of Great Britain and Ireland. British Wildlife Publishing.*

Brooks, S. J., Langdon, P. G. & Heiri, O. (2008). *The identification and use of Palearctic Chironomidae larvae in palaeoecology. QRA Technical Guide No. 10. Journal of Paleolimnology*, 40 (2), 751–753.

Boulton, A., & Boon, P. (1991). A review of methodology used to measure leaf litter decomposition in lotic environments: time to turn over an old leaf? *Marine and Freshwater Research*, 42(1981), 1.

Boulton, A. J., & Quinn, J. M. (2000). A simple and versatile technique for assessing cellulose decomposition potential in floodplain and riverine sediments. *Arch. Hydrobiol.*, 150(1), 133–151.

Boyero, L., Pearson, R. G., Gessner, M. O., Barmuta, L. A., Ferreira, V., Graça, M. A. S. (2011). A global experiment suggests climate warming will not accelerate litter decomposition in streams but might reduce carbon sequestration. *Ecology Letters*, 14, 289–294.

Brucet, S., Boix, D., Nathansen, L. W., Quintana, X. D., Jensen, E., Balayla, D., Jeppesen, E. (2012). Effects of temperature, salinity and fish in structuring the macroinvertebrate community in shallow lakes: Implications for effects of climate change. *PLoS ONE*, 7(2).

Bunn, S. E., & Davies, P. M. (2000). Biological processes in running waters and their implications for the assessment of ecological integrity. *International Conference on Assessing the Ecological Integrity of Running Waters*, 61–70.

Bunn, S. E., Davies, P. M., & Mosisch, T. D. (1999). Ecosystem measures of river health and their response to riparian and catchment degradation. *Freshwater Biology*, 41, 333–345.

Burgmer, T., Hillebrand, H., & Pfenninger, M. (2006). Effects of climate-driven temperature changes on the diversity of freshwater macroinvertebrates. *Oecologia*, 151(1), 93–103.

Cardinale, B. J., Srivastava, D. S., Duffy, J. E., Wright, J. P., Downing, A. L., Sankaran, M., & Jouseau, C. (2006). Effects of biodiversity on the functioning of trophic groups and ecosystems. *Nature,* 443(7114), 989–992.

Céréghino, R., Biggs, J., Oertli, B., & Declerck, S. (2007). The ecology of European ponds: Defining the characteristics of a neglected freshwater habitat. *Hydrobiologia*, 597(1), 1–6.

Céréghino, R., Ruggiero, A., Marty, P., & Angélibert, S. (2008). Biodiversity and distribution patterns of freshwater invertebrates in farm ponds of a south-western French agricultural landscape. *Hydrobiologia*, 597(1), 43–51.

Cham, S. (2012). *Field Guide to the Larvae and Exuviae of British Dragonflies: Damselflies (Zgypotera) and Dragonflies (Anisoptera). British Drangonfly Society*

Chergui, H. and Pattee, E. (1991). An experimental study of the breakdown of submerged leaves by hyphomycetes and invertebrates in Morocco. *Freshwater Biology* 26: 97–110.

Clapcott, J. E. & Barmuta, L. A. (2010). Metabolic patch dynamics in small headwater streams: exploring spatial and temporal variability in benthic processes. *Freshwater Biology*, 55, 806–824.

Collares-Pereira, M. J., Cowx, I. G., Ribeiro, F., Rodrigues, J. A., & Rogado, L. (2000). Conservation of endangered fish species in the face of water resourece development schemes in the Guadiana, Portugal: harmony of the incompatible. *Management and Ecology of River Fisheries*, (30), 428–438.

Collinson, N. H., Biggs, J., Corfield, A., Hodson, M. J., Walker, D., Whitfield, M., & Williams, P. J. (1995). Temporary and permanent ponds: An assessment of the effects of drying out on the conservation value of aquatic macroinvertebrate communities. *Biological Conservation*, *74*(2), 125–133.

Costantini, M. L., Rossi, L., Fazi, S., & Rossi, D. (2009). Detritus accumulation and decomposition in a coastal lake (Acquatina–southern Italy). *Aquatic Conservation: Marine and Freshwater Ecosystems,* 19(December), 566–574.

Craft, C. (2007). Freshwater input structures soil properties, vertical accretion, and nutrient accumulation of Georgia and US tidal marshes. *Limnology and Oceanography*, 52, 1220–1230

Cummins, K. W. (1973). Trophic relations of aquatic insects. *Annual Review of Entomology* 18:183–206.

Cummins, K. W., & Klug, M. J. (1979). Feeding ecology of stream invertebrates. *Annual Review of Ecology and Systematics*, 10, 147–172.

Dang, C. K., Schindler, M., Chauvet, E., & Gessner, M. O. (2009). Temperature oscillation coupled with fungal community shifts pi can modulate warming effects on litter decomposition. *Ecology*, 90(1), 122–131.

Davies, B., Biggs, J., Williams, P., Whitfield, M., Nicolet, P., Sear, D., Maund, S. (2008). Comparative biodiversity of aquatic habitats in the European agricultural landscape. Agriculture, *Ecosystems and Environment*, 125, 1–8.

Declerck, S., De Bie, T., Ercken, D., Hampel, H., Schrijvers, S., Van Wichelen, J., Gillard, V., Mandiki, R., Losson, B., Bauwens, D., Keijers, S., Vyverman, W., Goddeeris, B., De Meester, L., Brendonck, L., & Martens, K. (2006). Ecological characteristics of small ponds: associations with land-use practices at different spatial scales. *Biological Conservation* 131: 523–532.

DeLong, D. C. J. (1996). Defining Biodiversity. *Wildlife Society Bulletin*, 24(4), 738–749.

De Meester, L., Declerck, S., Stoks, R., Louette, G., Van de Meutter, F., De Bie, T., Michels E., & Brendonck, L. (2005). Ponds and pools as model systems in conservation biology, ecology and evolutionary biology. *Aquatic Conservation: Marine and Freshwater Ecosystems* 15:715–726.

Dudgeon, D., Arthington, A. H., Gessner, M. O., Kawabata, Z., Naiman, R. J., Knowler, D. J., & Le, C. (2005). Freshwater biodiversity : importance , threats , status and conservation challenges, 81(2006), 163–182.

Fairchild, G. W., Cruz, J., Faulds, A. M., Short, A. E. Z., & Matta, J. F. (2003). Microhabitat and landscape influences on aquatic beetle assemblages in a cluster of temporary and permanent ponds. *Journal of the North American Benthological Society*, *22*(2), 224–240.

Fellows, C. S., Clapcott, J. E., Udy, J. W., Bunn, S. E., Harch, B. D., Smith, M. J., & Davies, P. M. (2006). Benthic metabolism as an indicator of stream ecosystem health. *Hydrobiologia*, 572(1), 71–87.

Ferreira, V., & Guérold, F. (2017). Leaf litter decomposition as a bioassessment tool of acidification effects in streams: Evidence from a field study and meta-analysis. *Ecological Indicators,* 79(January), 382–390.

Ferreira, V., Raposeiro, P. M., Pereira, A., Cruz, A. M., Costa, A. C., Graça, M. A. S., & Gonçalves, V. (2016). Leaf litter decomposition in remote oceanic island streams is driven by microbes and depends on litter quality and environmental conditions. *Freshwater Biology*, 61(5), 783–799.

Fey, S. B., Mertens, A. N., Beversdorf, L. J., McMahon, K. D., & Cottingham, K. L. (2015). Recognizing cross-ecosystem responses to changing temperatures: Soil warming impacts pelagic food webs. *Oikos*, 124(11), 1473–1481.

Florencio, M., Díaz-Paniagua, C., Serrano, L., & Bilton, D. T. (2011). Spatio-temporal nested patterns in macroinvertebrate assemblages across a pond network with a wide hydroperiod range. Oecologia, 166(2), 469–483.

Frissell, C. A., & Bayles, D. (1996). Ecosystem management and the conservation of aquatic biodiversity and ecological integrity. *American water resources association*, 32(2).

Fritz, M. K., Fulton, S., Johnson, R. B., Barton, D. C., Jack, D. J., Word, A. D., & Burke, A. R. (2011). An assessment of cellulose filters as a standardized material for measuring litter breakdown in headwater streams. *Ecohydrology*, 4(February), 469–476.

Garcia-Palacios, P., McKie, B. G., Handa, I. T., Frainer, A., & Hättenschwiler, S. (2016). The importance of litter traits and decomposers for litter decomposition: A comparison of aquatic and terrestrial ecosystems within and across biomes. *Functional Ecology*, 30, 819–829.

Garcia-Pausas, J., Casals, P., Camarero, L., Huguet, C., Sebastià, M. T., Thompson, R., & Romanyà, J. (2007). Soil organic carbon storage in mountain grasslands of the Pyrenees: Effects of climate and topography. *Biogeochemistry*, 82(3), 279–289.

Gessner, M.O. & Chauvet, E., 2002. A case for using litter breakdown to assess functional stream integrity. *Ecological Applications,* 12, 498–510.

Graça, M. A. S. (2001). The role of invertebrates on leaf litter decomposition in streams-a review. *International Review of Hydrobiology*, 86, 383–393.

Graça, M.A.S., Canhoto, C. (2006). Leaf litter processing in low order streams. *Limnetica* 25, 1–10.

Goodman, K. J., Baker, M. A., & Wurtsbaugh, W. A. (2010). Mountain lakes increase organic matter decomposition rates in streams. *Journal of the North American Benthological Society*, 29(2), 521–529.

Handa, I.T., Aerts, R., Berendse, F., Berg, M.P., Bruder, A., Butenschoen, O. et al. (2014). Consequences of biodiversity loss for litter decomposition across biomes. *Nature*, 509, 218–221.

Hayashi, M. (2004). Temperature-electrical conductivity relation of water for environmental monitoring and geophysical data inversion. Environmental Monitoring and Assessment, 96(1–3), 119–128.

Hernández, D., Fernández, J. M., Plaza, C., & Polo, A. (2007). Water-soluble organic matter and biological activity of a degraded soil amended with pig slurry. *Science of the Total Environment*, 378(1–2), 101–103.

Higgins, J. V. (2005). A Freshwater Classification Approach for Biodiversity Conservation Planning. *Conservation Biology*, *19*(2), 432–445.

Holgerson, M. A., Post, D. M., & Skelly, D. K. (2016). Reconciling the role of terrestrial leaves in pond food webs: A whole-ecosystem experiment. *Ecology*, 97(7), 1771–1782.

Hooper, D. U., Chapin, F. S., Ewel, J. J., Hector, A., Inchausti, P., Lavorel, S., Lawton, J. H., Lodge, D. M., Loreau, M., Naeem, S., Schmid, B., Set¨ LA, H., Sysmstad, A. J., Vandemeer, J., & Wardle, D. A. (2005). Effects of biodiversity on ecosystem functioning: a consensus of current knowledge. *Ecological Monographs* 75: 3–35.

Howe, M. J. & Suberkkropp, K. (1994). Effects of isopod (Lierceus sp.) feeding on aquatic hyphomycetes colonizing leaves in a stream. *Archiv fur Hydrobiologie*, 130: 93–103.

Imbert, J. B. & Pozo, J. (1989). Breakdown of four-leaf litter species and associated fauna in a Basque country forested stream. *Hydrobiologia* 182: 1–14.

Jacob, M., Viedenz, K., Polle, A., Thomas, F.M., (2010). Leaf litter decomposition intemperate deciduous forest stands with a decreasing fraction of beech (Fagussylvatica). *Oecologia* 164, 1083–1094.

Jones, J. I., Young, J. O., Eaton, J. W. & Moss, B. (2002). The influence of nutrient loading dissolved inorganic carbon and higher trophic levels on the interaction between submerged plants and periphyton. *J. Ecol.* 90, 12–24.

Joyce, P., & Wotton, R. S. (2008). Shredder fecal pellets as stores of allochthonous organic matter in streams. *Journal of the North American Benthological Society,* 27, 521–528.

Kampfraath, A. A., Hunting, E. R., Mulder, C., Breure, A. M., Gessner, M. O., Kraak, M. H. S., & Admiraal, W. (2012). DECOTAB: a multipurpose standard substrate to assess effects of litter quality on microbial decomposition and invertebrate consumption. *Freshwater Science*, 31(4), 1156–1162.

Larned S.T. (2000) Dynamics of coarse riparian detritus in a Hawaiian stream ecosystem: a comparison of drought and post-drought conditions. *Journal of the North American Benthological Society*, 19, 215–234.

Langton, P. H. (1984). *A key to pupal exuviae of British Chironomidae. March, Cambridgeshire. United Kingdom.*

Latter, P. M., & Walton, D. W. H. (1988) The cotton strip assay for cellulose decomposition studies in soil: history of the assay and development. Symposium, I. T. E.

Leite-Rossi, L. A., Saito, V. S., Cunha-Santino, M. B., & Trivinho-Strixino, S. (2015). How does leaf litter chemistry influence its decomposition and colonization by shredder Chironomidae (Diptera) larvae in a tropical stream? *Hydrobiologia*, 771(1), 119–130.

Le Viol, I., Mocq, J., Julliard, R., & Kerbiriou, C. (2009). The contribution of motorway stormwater retention ponds to the biodiversity of aquatic macroinvertebrates. *Biological Conservation*, *142*(12), 3163–3171.

Liboriussen, L., Landkildehus, F., Meerhoff, M., & Bramm, M. E. (2004). Limnology and Oceanography Methods Abstracts Global warming : Design of a flow-through shallow lake mesocosm climate experiment. *Limnology And Oceanography Methods*, 2005–2005.

Lock, M. A. (1993). Attached microbial communities in rivers. – pp. 113–138 – In: T. E. FORD (ed.), *Aquatic microbiology: an ecological approach. Blackwell Scientific Publications, Boston.*

MacKenzie R.A., Wiegner T.N., Kinslow F., Cormier N. & Strauch A.M. (2013) Leaf-litter inputs from an invasive nitrogen-fixing tree influence organic-matter dynamics and nitrogen inputs in a Hawaiian river. *Freshwater Sciences*, 32, 1036–1052.

Martínez, A., Pérez, J., Molinero, J., Sagarduy, M., & Pozo, J. (2015). Effects of flow scarcity on leaf-litter processing under oceanic climate conditions in calcareous streams. *Science of the Total Environment*, 503–504, 251–257.

Mayer-Pinto, M., Matias, M. G., & Coleman, R. A. (2016). The interplay between habitat structure and chemical contaminants on biotic responses of benthic organisms. *PeerJ*, 4, e1985.

Mcintyre, P. B., L. E. Jones, A. S. Flecker, & M. J. Vanni. 2007. Fish extinctions alter nutrient recycling in tropical freshwaters. *Proceedings of the National Academy of Sciences of the United States of America* 104:4461–4466.

McKee, D., Atkinson, D., Collings, S. E., Eaton, J. W., Gill, A. B., Harvey, I., Moss, B. (2003). Response of Freshwater Microcosm Communities to Nutrients, Fish and Elevated Temperature dring Winter and Summer, *Limnology and oceanography* 48(2), 707–722.

Merritt, R. W., Cummins, K. W. & Berg, M. B. (2008). *An introduction to the aquatic insects of North America. Kendall/Hunt, Dubuque, Iowa.*

Morrissey, E. M., Gillespie, J. L., Morina, J. C., & Franklin, R. B. (2014). Salinity affects microbial activity and soil organic matter content in tidal wetlands. *Global Change Biology*, 20(4), 1351–1362.

Neubauer SC (2012) Ecosystem responses of a tidal freshwater marsh experiencing saltwater intrusion and altered hydrology. *Estuaries and Coasts*, 36, 491–607.

Newman, S., Kumpf, H., Laing, A., & Kennedy, W. C. (2001). Decomposition response to phosphorus enrichment in an Everglades (USA) slough. Biogeochemistry 54:229–250.

Noss, R. F., & Cooperrider, A. Y.. 1994. Saving nature's legacy: protecting and restoring biodiversity. *Island Press,Washington, D.C.* 416pp.

Niyogi, D. K., Harding, J. S., & Simon, K. S. (2013). Organic matter breakdown as a measure of stream health in New Zealand streams affected by acid mine drainage. *Ecological Indicators*, 24, 510–517.

Oertli, B., Biggs, J., Céréghino, R., Grillas, P., Joly, P., & Lachavanne, J. B. (2005). Conservation and monitoring of pond biodiversity1: Introduction. *Aquatic Conservation: Marine and Freshwater Ecosystems,* 15(6), 535–540.

Oksanen, A., Blanchet, F. G., Friendly, M., Kindt, R., Legendre, P., Mcglinn, D., Szoecs, E. (2018). vegan: Community Ecology Package. R package version 2.4-6. https://CRAN.R-project.org/package=vegan

Palacios, D., de Andrés, N., & Luengo, E. (2003). Distribution and effectiveness of nivation in Mediterranean mountains: Peñalara (Spain). Geomorphology, 54(3–4), 157–178.

Pekel, J., Cottam, A., Gorelick, N., & Belward, A. S. (2016). High-resolution mapping of global surface water and its long-term changes. Nature, 1–19.

Pereira, J. S., Mateus, J. A., Aires, L. M., Pita, G., Pio, C., David, J. S., T. A. Pac¸o1, & A. R. (2007). Net ecosystem carbon exchange in three contrasting Mediterranean ecosystems - the effect of drought. *Biogeosciences*, 4(5), 791–802.

Petersen, R.C. & Cummins, K.W. (1974) Leaf processing in a woodland stream. *Freshwater Biology*, 4, 343–368.

Piggott, J. J., Niyogi, D. K., Townsend, C. R., & Matthaei, C. D. (2015). Multiple stressors and stream ecosystem functioning: Climate warming and agricultural stressors interact to affect processing of organic matter. *Journal of Applied Ecology,* 52(5), 1126–1134.

Pyrovetsi, M., & Papastergiadou, E. (1992). Biological conservation implications of water level fluctuations in a wetland of international importance: Lake Kerkini, Makedonia, Greece. *Environmental Conservation,* 19(3), 235–244.

Raposeiro P.M., Martins G.M., Moniz I., Cunha A., Costa A.C. & Gonçalves V. (2014) Leaf litter decomposition in remote oceanic islands: the role of macroinvertebrates vs. microbial decomposition of native vs. exotic plant species. *Limnologica*, 45, 80–87.

Reis, A. R., Lourenço, J. M. M., Parker, A. & Alencoão, A. (2014). Estimative of sediment yield in mountainous catchments: a pilot study in a meso-scale catchment of the Douro Basin, Northern Portugal. *Comunicações Geologicas* T. 101, 101(Especial II), 1051–1054.

Rejmánková E., & Houdková, K. (2006) Wetland plant decomposition under different nutrient conditions: what is more important, litter quality or site quality? *Biogeochemistry*, 80, 245–262.

Ricciardi, A., Neves, R. J., & Rasmussen, J. B. (1999). Extinction rates of North American freshwater fauna. *Conservation Biology* 13:1–3

Roache M. C., Bailey P. C. & Boon P. I. (2006) Effects of salinity on the decay of the freshwater macrophyte, Triglochin procerum. *Aquatic Botany*, 84, 45–52.

Rosi-Marshall, E. J., Vallis, K. L., Baxter, C. V., & Davis, J. M. (2016). Retesting a prediction of the River Continuum Concept: autochthonous versus allochthonous resources in the diets of invertebrates. *Freshwater Science*, 35(2), 534–543.

Rosi-Marshall, E. J., & Wallace, J. B. (2002). Invertebrate food webs along a stream resource gradient. *Freshwater Biology* 47:129–141.

Ruggiero, A., Céréghino, R., Figuerola, J., Marty, P., & Angélibert, S. (2008). Farm ponds make a contribution to the biodiversity of aquatic insects in a French agricultural landscape. *Comptes Rendus - Biologies*, 331(4), 298–308.

Sala, O. E. (2000). Global Biodiversity Scenarios for the Year 2100. Science, 287(5459), 1770–1774.

Santonja, M., Pellan, L., & Piscart, C. (2017). Macroinvertebrate identity mediates the effects of litter quality and microbial conditioning on leaf litter recycling in temperate streams. Ecology and Evolution, (August 2017), 1–12.

Saunders, D. L., Meeuwig, J. J., & Vincent, a C. J. (2002). Freshwater Protected Areas: Strategies for Conservation Áreas Protegidas de Agua Dulce: Estrategias para la Conservación. *Conservation Biology*, 16(1), 30–41.

Sayer, C., Andrews, K., Shilland, E., Edmonds, N., Edmonds-Brown, R., Patmore, I., … Axmacher, J. (2012). The role of pond management for biodiversity conservation in an agricultural landscape. *Aquatic Conservation: Marine and Freshwater Ecosystems,* 22(5), 626–638.

Silva-Junior, E.F., Moulton, T.P., Boëchat, I.G. & Gücker, B. (2014) Leaf decomposition and ecosystem metabolism as functional indicators of land use impacts on tropical streams. *Ecological Indicators*, 36, 195–204.

Silveira, L. S. da, Martins, R. T., Silveira, G. A. da, Grazul, R. M., Lobo, D. P., & Alves, R. da G. (2013). Colonization by Chironomidae Larvae in Decomposition Leaves of Eichhornia azurea in a Lentic System in Southeastern Brazil. *Journal of Insect Science*, 13(20), 1–13.

Sowa, R. (1975). What is Cloeon dipterum (Linnaeus, 1761)? *Ent. Scand.*, 6, 215–223.

Stewart, R. I. A., Dossena, M., Bohan, D. A., Jeppesen, E., Kordas, R. L., Ledger, M. E., Woodward, G. (2013). Mesocosm Experiments as a Tool for Ecological Climate-Change Research. Advances in Ecological Research (1st ed., Vol. 48). *Elsevier Ltd.*

Stevens, R. J., O'brick, C. J., & Carton, O. T. (1995). Estimating nutrient content of animal slurries using electrical conductivity. Journal of Agricultural Science, 125, 233–238.

Stockley, R. A., Oxford, G. S., & Ormond, R. F. G. (1998). Do invertebrates matter? Detrital processing in the River Swale-Ouse. – *Science of the total environment*, 210–211: 427–435.

Strayer, D. L., & Dudgeon, D. (2010). Freshwater biodiversity conservation : recent progress and future challenges. The North American Benthological Society, 29(February), 344–358.

Szöllosi-Nagy, A., Najlis, P., & Björklund, G. (1998). Assessing the world's freshwater resources. *Nature and Resources* 34(1):8–18.

Tachet, H., Richoux, P., Bournard, M., & Usseglio-Polatera, P. (2010). *Invertébrés d'eau douce: systématique, biologie, écologie*. CNRS Éditions, Paris, France

Tiegs, S. D., Clapcott, J. E., Griffiths, N. A., & Boulton, A. J. (2013). A standardized cotton-strip assay for measuring organic-matter decomposition in streams. *Ecological Indicators*, *32*, 131–139.

Tiegs, S. D., Langhans, S. D., Tockner, K., & Gessner, M. O. (2007). Cotton strips as a leaf surrogate to measure decomposition in river floodplain habitats. *Journal of the North American Benthological Society*, *26*(1), 70–77.

Ward, J.V. (1992). Temperature. In: Ward J.V. (ed) Aquatic insect ecology. *Wiley, London*, pp 201–231

Webster, J.R. & Benfield, E.F. (1986). Vascular plant breakdown in freshwater ecosystems. *Annual Review of Ecology and Systematics*, 17, 567–594.

Weston NB, Dixon RE & Joye SB (2006) Ramifications of increased salinity in tidal freshwater sediments: geochemistry and microbial pathways of organic matter mineralization. *Journal of Geophysical Research: Biogeosciences*, 111, 2005–2012

Williams, P., Whitfield, M., Biggs, J., Bray, S., Fox, G., Nicolet, P., & Sear D. (2004). Comparative biodiversity of rivers, streams, ditches and ponds in an agricultural landscape in Southern England. *Biological Conservation* 115: 329–341.

Wilson, R. S., & Ruse, L. P. (2005). A Guide to the Identification of Genera of Chironomid Pupal Exuviae Occurring in Britain and Ireland (including Common Genera from Northern Ireland). *Freshwater Biological Assn.*

Valverde, P., de Carvalho, M., Serralheiro, R., Maia, R., Ramos, V., & Oliveira, B. (2015). Climate change impacts on rainfed agriculture in the Guadiana river basin (Portugal). *Agricultural Water Management*, 150, 35–45.

Vaughn, C. C., Spooner, D. E., & Galbraith, H. S. (2007). Context dependent species identity effects within a functional group of filter-feeding bivalves. Ecology 88:1654–1662.

Ventura, M., Liboriussen, L., Lauridsen, T., Sondergaard, M. & Jeppesen, E. (2008). Effects of increased temperature and nutrient enrichment on the stoichiometry of primary producers and consumers in temperate shallow lakes. *Freshwater Biology* 53, 1434–1452.

Vysná, V., Dyer, F., Maher, W., & Norris, R. (2014). Cotton-strip decomposition rate as a river condition indicator - Diel temperature range and deployment season and length also matter. *Ecological Indicators*, 45, 508–521.

Young, R. G., Matthaei, C. D., & Townsend, C. R. (2008).Organic matter breakdown and ecosystem metabolism: functional indicators for assessing river ecosystem health. *Journal of the North American Benthological Society* 27:605–625.

Yvon-Durocher, G., Jones, J. I., Trimmer, M., Woodward, G., & Montoya, J. M. (2010). Warming alters the metabolic balance of ecosystems. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 365(1549), 2117–2126.

Appendix 1
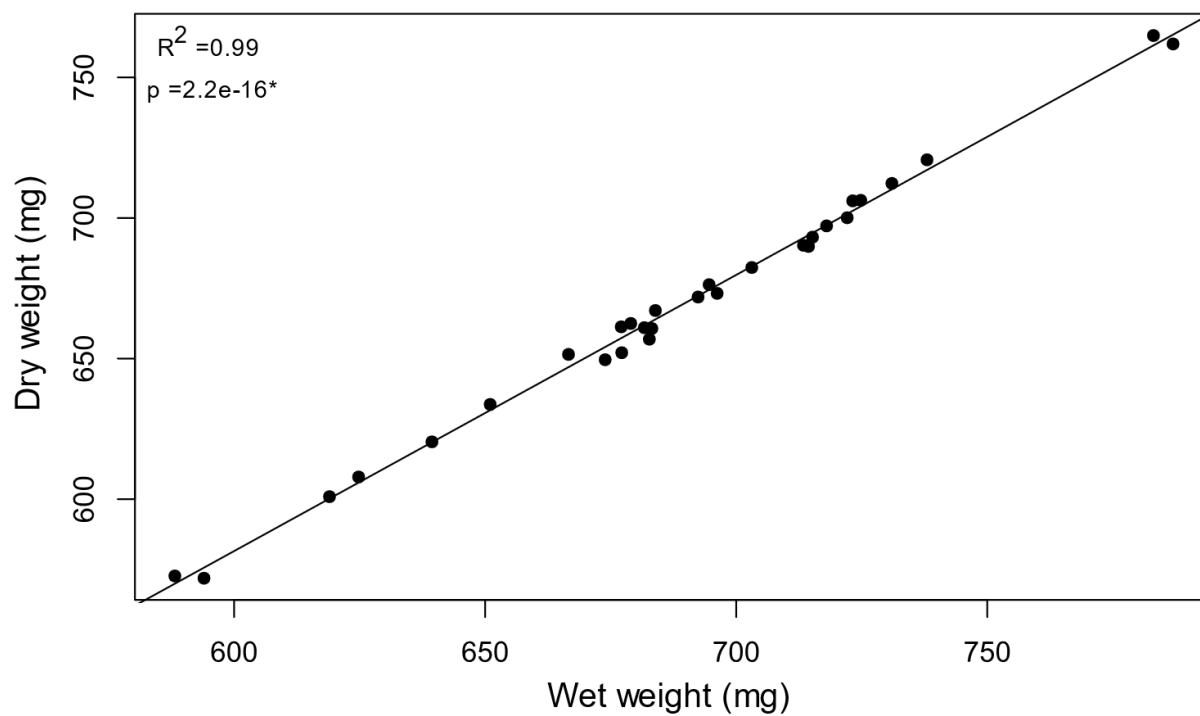
Figure 1- Relation between wet and dry weight (mg) of the cotton strips. There is a high correlation between both variables that allows for an estimation of the dry weight of the cotton strips using the formula Y = 0,982*X+(-0,00778).

Appendix 2

Table 1- Functional feeding groups of macroinvertebrate species found in the mesocosms. aff- Active filter feeders; gat- Gatherers/Collectors; gra- Grazers; pff- Passive filter feeders; pre- Predators; shr- Shredders.

Taxa	Group	Kingdom	Phylum	Class	Order	Family	Genus	Feeding
Anthomyiidae.sp	Macroinvertebrates	Animalia	Arthropoda	Insecta	Diptera	Anthomyiidae		pre
Neo.Culex_territans	Macroinvertebrates	Animalia	Arthropoda	Insecta	Diptera	Culicidae	*Culex*	aff
Aedes.ochlerotatus.sp.nymph	Macroinvertebrates	Animalia	Arthropoda	Insecta	Diptera	Culicidae	*Ochlerotatus*	aff
Aedes.Ochlerotatus.sp	Macroinvertebrates	Animalia	Arthropoda	Insecta	Diptera	Culicidae	*Ochlerotatus*	aff
Aeshna.sp	Macroinvertebrates	Animalia	Arthropoda	Insecta	Odonata	Aeshnidae	*Aeshna*	pre
Agabus.sp.larvae	Macroinvertebrates	Animalia	Arthropoda	Insecta	Coleoptera	Dytiscidae	*Agabus*	pre
Anacaena.globulus	Macroinvertebrates	Animalia	Arthropoda	Insecta	Coleoptera	Hydrophilidae	*Anacaena*	gat
Anax. sp	Macroinvertebrates	Animalia	Arthropoda	Insecta	Odonata	Aeshnidae	*Anax*	pre
Berosus.sp.larvae	Macroinvertebrates	Animalia	Arthropoda	Insecta	Coleoptera	Hydrophilidae	*Berosus*	pre
Brachythemis.leucosticta	Macroinvertebrates	Animalia	Arthropoda	Insecta	Odonata	Libellulidae	*Brachythemis*	pre
Caenis.luctuosa	Macroinvertebrates	Animalia	Arthropoda	Insecta	Ephemeroptera	Caenidae	*Caenis*	gat
Ceratopogonidae.sf	Macroinvertebrates	Animalia	Arthropoda	Insecta	Diptera	Ceratopogonidae		pre
Ceratopogonidae.sf.nymph	Macroinvertebrates	Animalia	Arthropoda	Insecta	Diptera	Ceratopogonidae		pre
Chaoborus.flavicans	Macroinvertebrates	Animalia	Arthropoda	Insecta	Diptera	Chaoboridae	*Chaoborus*	pre
Chaoborus.sp.nymph	Macroinvertebrates	Animalia	Arthropoda	Insecta	Diptera	Chaoboridae	*Chaoborus*	pre
Chironomidae.sf.nymph	Macroinvertebrates	Animalia	Arthropoda	Insecta	Diptera	Chironomidae		gat
Chironomus.sp	Macroinvertebrates	Animalia	Arthropoda	Insecta	Diptera	Chironomidae	*Chironomus*	gat
Chironomus.gr.plumosis	Macroinvertebrates	Animalia	Arthropoda	Insecta	Diptera	Chironomidae	*Chironomus*	gat
Chironomus.sp.nymph	Macroinvertebrates	Animalia	Arthropoda	Insecta	Diptera	Chironomidae	*Chironomus*	gat
Cloeon.sp.adult	Macroinvertebrates	Animalia	Arthropoda	Insecta	Ephemeroptera	Baetidae	*Cloeon*	gra
Cloeon.gr.Dipterum	Macroinvertebrates	Animalia	Arthropoda	Insecta	Ephemeroptera	Baetidae	*Cloeon*	gra

Table 1- Functional feeding groups of macroinvertebrate species found in the mesocosms. aff- Active filter feeders; gat- Gatherers/Collectors; gra- Grazers; pff- Passive filter feeders; pre- Predators; shr- Shredders.

Taxa	Group	Kingdom	Phylum	Class	Order	Family	Genus	Feeding
Cloeon.gr.Simile	Macroinvertebrates	Animalia	Arthropoda	Insecta	Ephemeroptera	Baetidae	*Cloeon*	gra
Cloeon.inscriptum	Macroinvertebrates	Animalia	Arthropoda	Insecta	Ephemeroptera	Baetidae	*Cloeon*	gra
Cloeon.schoenemundi	Macroinvertebrates	Animalia	Arthropoda	Insecta	Ephemeroptera	Baetidae	*Cloeon*	gra
Colymbetes.sp	Macroinvertebrates	Animalia	Arthropoda	Insecta	Coleoptera	Dytiscidae	*Colymbetes*	pre
Copelatus.sp	Macroinvertebrates	Animalia	Arthropoda	Insecta	Coleoptera	Dytiscidae	*Copelatus*	pre
Cordulegaster.boltoni	Macroinvertebrates	Animalia	Arthropoda	Insecta	Odonata	Cordulegastridae	*Cordulegaster*	pre
Cordulia.aenea	Macroinvertebrates	Animalia	Arthropoda	Insecta	Odonata	Corduliidae	*Cordulia*	pre
Corixa.sp	Macroinvertebrates	Animalia	Arthropoda	Insecta	Hemiptera	Corixidae	*Corixa*	gat
Corixinae.sf.larvae	Macroinvertebrates	Animalia	Arthropoda	Insecta	Hemiptera	Corixidae		gat
Corynoneura.sp	Macroinvertebrates	Animalia	Arthropoda	Insecta	Diptera	Chironomidae	*Corynoneura*	gra
Cricotopus.sp	Macroinvertebrates	Animalia	Arthropoda	Insecta	Diptera	Chironomidae	*Cricotopus*	gra
Crocothemis.erythraea	Macroinvertebrates	Animalia	Arthropoda	Insecta	Odonata	Libellulidae	*Crocothemis*	pre
Culex.pipiens	Macroinvertebrates	Animalia	Arthropoda	Insecta	Diptera	Culicidae	*Culex*	aff
Culex.sp	Macroinvertebrates	Animalia	Arthropoda	Insecta	Diptera	Culicidae	*Culex*	aff
Culicinae.sf.nymph	Macroinvertebrates	Animalia	Arthropoda	Insecta	Diptera	Culicidae		aff
Cymatia.sp	Macroinvertebrates	Animalia	Mollusca	Gastropoda		Ranellidae	*Cymatia*	pre
Dasyhelea.sp	Macroinvertebrates	Animalia	Arthropoda	Insecta	Diptera	Ceratopogonidae	*Dasyhelea*	pre
Diplacodes.lefebvrii	Macroinvertebrates	Animalia	Arthropoda	Insecta	Odonata	Libellulidae	*Diplacodes*	pre
Dixa.sp.nymph	Macroinvertebrates	Animalia	Arthropoda	Insecta	Diptera	Dixidae	*Dixa*	pff
Dixella.sp	Macroinvertebrates	Animalia	Arthropoda	Insecta	Diptera	Dixidae	*Dixella*	gat
Dorytomus.longimanus	Macroinvertebrates	Animalia	Arthropoda	Insecta	Coleoptera	Curculionidae	*Dorytomus*	shr
Dytiscinae.sf.	Macroinvertebrates	Animalia	Arthropoda	Insecta	Coleoptera	Dytiscidae		pre
Dytiscus.sp.larvae	Macroinvertebrates	Animalia	Arthropoda	Insecta	Coleoptera	Dytiscidae	*Dytiscus*	pre

Table 1- Functional feeding groups of macroinvertebrate species found in the mesocosms. aff- Active filter feeders; gat- Gatherers/Collectors; gra- Grazers; pff- Passive filter feeders; pre- Predators; shr- Shredders.

Taxa	Group	Kingdom	Phylum	Class	Order	Family	Genus	Feeding
Elmidae.f	Macroinvertebrates	Animalia	Arthropoda	Insecta	Coleoptera	Elmidae		gra
Ephydridae.f	Macroinvertebrates	Animalia	Arthropoda	Insecta	Diptera	Ephydridae		gra
Ischnura.pumilio	Macroinvertebrates	Animalia	Arthropoda	Insecta	Odonata	Coenagrionidae	*Ischnura*	pre
Gerris.sp	Macroinvertebrates	Animalia	Arthropoda	Insecta	Hemiptera	Gerridae	*Gerris*	pre
Gyraulus.sp	Macroinvertebrates	Animalia	Mollusca	Gastropoda		Planorbidae	*Gyraulus*	gra
Haliplus.sp.larvae	Macroinvertebrates	Animalia	Arthropoda	Insecta	Coleoptera	Haliplidae	*Haliplus*	pre
Haplotaxidae.f	Macroinvertebrates	Animalia	Annelida	Clitellata	Haplotaxida	Haplotaxidae		gat
Helophorus.sp	Macroinvertebrates	Animalia	Arthropoda	Insecta	Coleoptera	Helophoridae	*Helophorus*	shr
Hydrobius.sp.larvae	Macroinvertebrates	Animalia	Arthropoda	Insecta	Coleoptera	Hydrophilidae	*Hydrobius*	pre
Hydrochus.sp.	Macroinvertebrates	Animalia	Arthropoda	Insecta	Coleoptera	Hydrochidae	*Hydrochus*	shr
Hydrometra.sp	Macroinvertebrates	Animalia	Arthropoda	Insecta	Hemiptera	Hydrometridae	*Hydrometra*	pre
Hydrophilus.sp	Macroinvertebrates	Animalia	Arthropoda	Insecta	Coleoptera	Hydrophilidae	*Hydrophilus*	pre
Hydroporus.sp.adult	Macroinvertebrates	Animalia	Arthropoda	Insecta	Coleoptera	Dytiscidae	*Hydroporus*	pre
Hydroporus.sp.larvae	Macroinvertebrates	Animalia	Arthropoda	Insecta	Coleoptera	Dytiscidae	*Hydroporus*	pre
Hygrobia.hermanii	Macroinvertebrates	Animalia	Arthropoda	Insecta	Coleoptera	Hygrobiidae	*Hygrobia*	pre
Hygrotus.sp.	Macroinvertebrates	Animalia	Arthropoda	Insecta	Coleoptera	Dytiscidae	*Hygrotus*	pre
Laccobius.sp.larvae	Macroinvertebrates	Animalia	Arthropoda	Insecta	Coleoptera	Hydrophilidae	*Laccobius*	pre
Laccophilus.sp	Macroinvertebrates	Animalia	Arthropoda	Insecta	Coleoptera	Dytiscidae	*Laccophilus*	pre
Leucorrhinia.rubicunda	Macroinvertebrates	Animalia	Arthropoda	Insecta	Odonata	Libellulidae	*Leucorrhinia*	pre
Libelluilidae	Macroinvertebrates	Animalia	Arthropoda	Insecta	Odonata	Libellulidae		pre
Libellula depressa	Macroinvertebrates	Animalia	Arthropoda	Insecta	Odonata	Libellulidae	*Libellula*	pre
Libellula quadrimaculata	Macroinvertebrates	Animalia	Arthropoda	Insecta	Odonata	Libellulidae	*Libellula*	pre
Macropelapia.sp	Macroinvertebrates	Animalia	Arthropoda	Insecta	Diptera	Chironomidae	*Macropelapia*	pre

Table 1- Functional feeding groups of macroinvertebrate species found in the mesocosms. aff- Active filter feeders; gat- Gatherers/Collectors; gra- Grazers; pff- Passive filter feeders; pre- Predators; shr- Shredders.

Taxa	Group	Kingdom	Phylum	Class	Order	Family	Genus	Feeding
Meladema.coriacea	Macroinvertebrates	Animalia	Arthropoda	Insecta	Coleoptera	Dytiscidae	*Meladema*	pre
Meladema.sp.larvae	Macroinvertebrates	Animalia	Arthropoda	Insecta	Coleoptera	Dytiscidae	*Meladema*	pre
Mesovelia.vittigera	Macroinvertebrates	Animalia	Arthropoda	Insecta	Hemiptera	Mesoveliidae	*Mesovelia*	pre
Micronecta.sp	Macroinvertebrates	Animalia	Arthropoda	Insecta	Hemiptera	Corixidae	*Micronecta*	gat
Micropsectra.sp	Macroinvertebrates	Animalia	Arthropoda	Insecta	Diptera	Chironomidae	*Micropsectra*	gat
Microvelia.sp	Macroinvertebrates	Animalia	Arthropoda	Insecta	Hemiptera	Veliidae	*Microvelia*	pre
Naididae.f	Macroinvertebrates	Animalia	Annelida	Clitellata	Haplotaxida	Naididae		gat
Nebrioporus.sp	Macroinvertebrates	Animalia	Arthropoda	Insecta	Coleoptera	Dytiscidae	*Nebrioporus*	pre
Notonecta.sp	Macroinvertebrates	Animalia	Arthropoda	Insecta	Hemiptera	Notonectidae	*Notonecta*	pre
Ochthebius.sp.adult	Macroinvertebrates	Animalia	Arthropoda	Insecta	Coleoptera	Hydraenidae	*Ochthebius*	gra
Oligochaeta	Macroinvertebrates	Animalia	Arthropoda	Insecta				gat
Orthetrum.nitidinerve	Macroinvertebrates	Animalia	Arthropoda	Insecta	Odonata	Libellulidae	*Orthetrum*	pre
Orthocladiinae.sf	Macroinvertebrates	Animalia	Arthropoda	Insecta	Diptera	Chironomidae		gra
Orthocladiinae.sf.nymph	Macroinvertebrates	Animalia	Arthropoda	Insecta	Diptera	Chironomidae		gra
Osmylus.fulvicephalus	Macroinvertebrates	Animalia	Arthropoda	Insecta	Neuroptera	Osmylidae	*Osmylus*	pre
Oxygastra.curtisii	Macroinvertebrates	Animalia	Arthropoda	Insecta	Odonata	Corduliidae	*Oxygastra*	pre
Palpomyiini.tr	Macroinvertebrates	Animalia	Arthropoda	Insecta	Diptera	Ceratopogonidae		pre
Physa.sp	Macroinvertebrates	Animalia	Mollusca	Gastropoda		Physidae	*Physa*	gra
Planorbarius.dufourii	Macroinvertebrates	Animalia	Mollusca	Gastropoda	Hygrophila	Planorbidae	*Planorbarius*	gra
Planorbidae.f	Macroinvertebrates	Animalia	Mollusca	Gastropoda	Hygrophila	Planorbidae		gra
Plea.minutissima	Macroinvertebrates	Animalia	Arthropoda	Insecta	Hemiptera	Pleidae	*Plea*	pre
Psectrocladius.octomuculatus.nymph	Macroinvertebrates	Animalia	Arthropoda	Insecta	Diptera	Chironomidae	*Psectrocladius*	shr
Psectrocladius.limbatellus.nymph	Macroinvertebrates	Animalia	Arthropoda	Insecta	Diptera	Chironomidae	*Psectrocladius*	shr

Table 1- Functional feeding groups of macroinvertebrate species found in the mesocosms. aff- Active filter feeders; gat- Gatherers/Collectors; gra- Grazers; pff- Passive filter feeders; pre- Predators; shr- Shredders.

Taxa	Group	Kingdom	Phylum	Class	Order	Family	Genus	Feeding
Psectrocladius.psilopterus	Macroinvertebrates	Animalia	Arthropoda	Insecta	Diptera	Chironomidae	*Psectrocladius*	shr
Psectrocladius.limbatellus	Macroinvertebrates	Animalia	Arthropoda	Insecta	Diptera	Chironomidae	*Psectrocladius*	shr
Riolus.sp.	Macroinvertebrates	Animalia	Arthropoda	Insecta	Coleoptera	Elmidae	*Riolus*	gra
Sciomyzidae.f	Macroinvertebrates	Animalia	Arthropoda	Insecta	Diptera	Sciomyzidae		pre
Sigara.sp	Macroinvertebrates	Animalia	Arthropoda	Insecta	Hemiptera	Corixidae	*Sigara*	gat
Stagnicola.sp	Macroinvertebrates	Animalia	Mollusca	Gastropoda		Lymnaeidae	*Stagnicola*	gra
Sympetrum sanguinem	Macroinvertebrates	Animalia	Arthropoda	Insecta	Odonata	Libellulidae	*Sympetrum*	pre
Sympetrum.striolatum	Macroinvertebrates	Animalia	Arthropoda	Insecta	Odonata	Libellulidae	*Sympetrum*	pre
Sympetrum.pro.parte	Macroinvertebrates	Animalia	Arthropoda	Insecta	Odonata	Libellulidae	*Sympetrum*	pre
Sympterum.flaveolum	Macroinvertebrates	Animalia	Arthropoda	Insecta	Odonata	Libellulidae	*Sympetrum*	pre
Sympetrum.nigrescens	Macroinvertebrates	Animalia	Arthropoda	Insecta	Odonata	Libellulidae	*Sympetrum*	pre
Sympetrum.vulgatum	Macroinvertebrates	Animalia	Arthropoda	Insecta	Odonata	Libellulidae	*Sympetrum*	pre
Sympterum.fonscolombii	Macroinvertebrates	Animalia	Arthropoda	Insecta	Odonata	Libellulidae	*Sympetrum*	pre
Tanypodinae.sf	Macroinvertebrates	Animalia	Arthropoda	Insecta	Diptera	Chironomidae		pre
Tanypodinae.sf.nymph	Macroinvertebrates	Animalia	Arthropoda	Insecta	Diptera	Chironomidae		pre
Tanytarsini.tr	Macroinvertebrates	Animalia	Arthropoda	Insecta	Diptera	Chironomidae		gat
Tanytarsus.mendax	Macroinvertebrates	Animalia	Arthropoda	Insecta	Diptera	Chironomidae	*Tanytarsus*	gat
Tanytarsini.tr.nymph	Macroinvertebrates	Animalia	Arthropoda	Insecta	Diptera	Chironomidae		gat
Tubificidae.f	Macroinvertebrates	Animalia	Annelida	Clitellata	Haplotaxida	Tubificidae		gat
Yola.sp.larvae	Macroinvertebrates	Animalia	Arthropoda	Insecta	Coleoptera	Dytiscidae	*Yola*	pre

Appendix 3
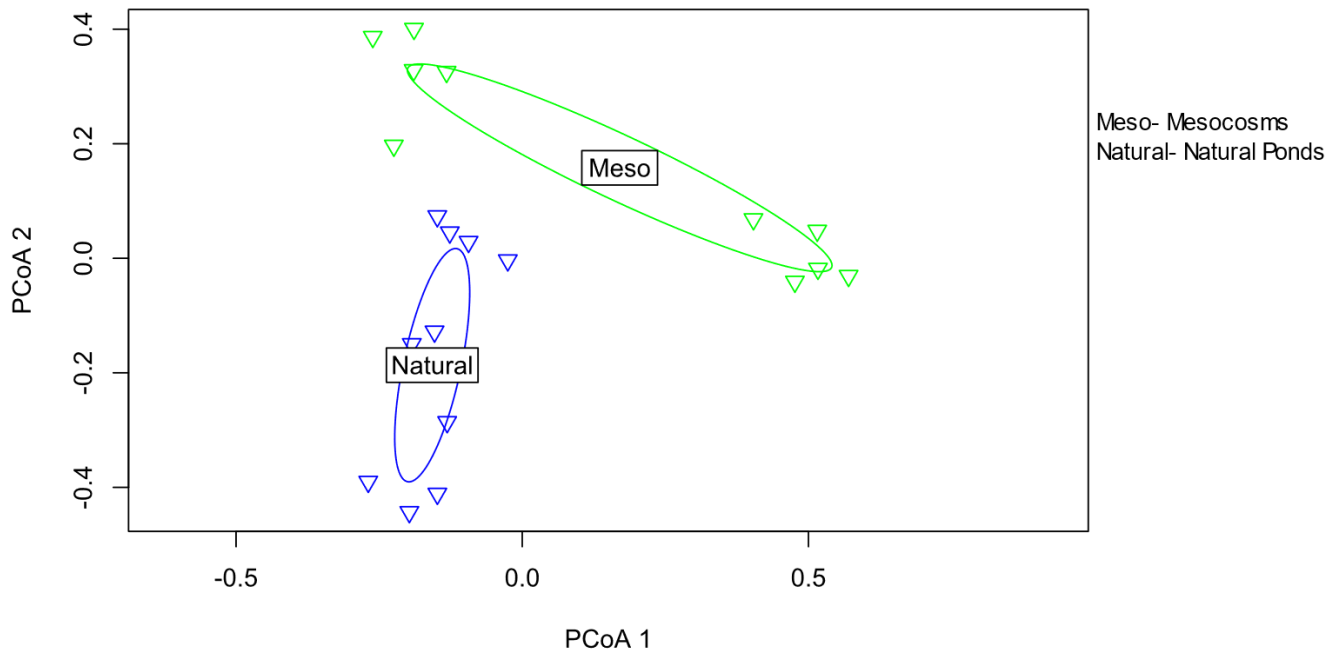
Figure 1- Principal coordinate analysis (PCoA) of the macroinvertebrate community in mesocosms ("Meso") and natural ponds ("Natural"). Each symbol indicates a pond that was used in Évora and Porto for this experiment in both systems adding to a total of 10 ponds, 5 ponds per region.

Table 1- Anova between both systems and macroinvertebrate communities

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
System	1	6.37e-03	6.37e-03	2.13	0.16
Residuals	18	5.38e-02	2.99e-03		

Appendix 4

Table 1- Abundance of macroinvertebrate species found in the five mesocoms used in the decomposition experiment in each location.

| Species | Location | | | | | |
	Múrcia	Toledo	Évora	Porto	Jaca	Peñalara
Anthomyiidae.sp	0.73 ± 2.41	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Neo.Culex_territans	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.40 ± 1.26
Aedes.ochlerotatus.sp.nymph	2.82 ± 7.33	0.45 ± 1.51	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Aedes.Ochlerotatus.sp	14.82 ± 11.02	0.00 ± 0.00	4.27± 7.96	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Aeshna.sp	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Agabus.sp.larvae	0.00 ± 0.00	3.00 ± 5.18	3.27 ± 4.20	0.50 ± 1.27	1.60 ± 2.80	6.20 ± 7.19
Anacaena.globulus	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Anax.sp	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Berosus.sp.larvae	0.00 ± 0.00	0.18 ± 0.60	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.60 ± 1.07
Brachythemis.leucosticta	0.00 ± 0.00	0.09 ± 0.30	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Caenis.luctuosa	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.10 ± 0.32	0.00 ± 0.00	0.00 ± 0.00
Ceratopogonidae.sf	0.09 ± 0.30	101.82 ± 198.35	0.00 ± 0.00	0.20 ± 0.63	0.00 ± 0.00	0.00 ± 0.00
Ceratopogonidae.sf.nymph	0.00 ± 0.00	117.91 ± 174.02	0.09 ± 0.30	2.70 ± 6.13	0.10 ± 0.32	0.00 ± 0.00
Chaoborus.flavicans	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	4.40 ± 6.96	0.00 ± 0.00	0.00 ± 0.00
Chaoborus.sp.nymph	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.10 ± 0.32	0.00 ± 0.00	0.00 ± 0.00
Chironomidae.sf.nymph	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Chironomus.sp	132.18 ± 324.71	520.00 ± 1127.38	263.55 ± 339.60	23.80 ± 35.31	0.60 ± 0.97	10.30 ± 16.06
Chironomus.gr.plumosis	9.73 ± 32.26	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Chironomus.sp.nymph	2.27 ± 7.21	11.55 ± 17.90	2.55 ± 3.62	0.80 ± 1.87	0.10 ± 0.32	0.70 ± 1.34
Cloeon.sp.adult	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Cloeon.gr.Dipterum	1.36 ± 2.34	4.82 ± 9.73	0.00 ± 0.00	10.50 ± 15.57	0.00 ± 0.00	0.00 ± 0.00
Cloeon.gr.Simile	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

Table 1- Abundance of macroinvertebrate species found in the five mesocoms used in the decomposition experiment in each location.

Species	Location					
	Múrcia	**Toledo**	**Évora**	**Porto**	**Jaca**	**Peñalara**
Cloeon.inscriptum	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Cloeon.schoenemundi	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Colymbetes.sp	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Copelatus.sp	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.10 ± 0.32
Cordulegaster.boltoni	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Cordulia.aenea	0.00 ± 0.00	4.36 ± 14.47	0.00 ± 0.00	0.10 ± 0.32	0.00 ± 0.00	0.00 ± 0.00
Corixa.sp	0.00 ± 0.00	0.27 ± 0.65	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Corixinae.sf.larvae	0.00 ± 0.00	0.00 ± 0.00	0.18 ± 0.60	0.00 ± 0.00	0.00 ± 0.00	0.30 ± 0.95
Corynoneura.sp	0.00 ± 0.00	0.00 ± 0.00	3.18 ± 7.22	0.00 ± 0.00	0.00 ± 0.00	0.10 ± 0.32
Cricotopus.sp	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Crocothemis.erythraea	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.70 ± 2.21	0.00 ± 0.00	0.00 ± 0.00
Culex.pipiens	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.70 ± 1.64
Culex.sp	0.00 ± 0.00	1.73 ± 4.78	2.55 ± 5.32	0.00 ± 0.00	0.40 ± 0.70	0.60 ± 1.26
Culicinae.sf.nymph	3.82 ± 7.33	0.00 ± 0.00	0.36 ± 0.92	0.00 ± 0.00	0.30 ± 0.95	1.60 ± 3.50
Cymatia.sp	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Dasyhelea.sp	0.00 ± 0.00	58.18 ± 192.97	0.00 ± 0.00	0.00 ± 0.00	0.20 ± 0.63	0.00 ± 0.00
Diplacodes.lefebvrii	0.00 ± 0.00	1.82 ± 6.03	0.00 ± 0.00	1.50 ± 4.06	0.00 ± 0.00	0.00 ± 0.00
Dixa.sp.nymph	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.10 ± 0.32	0.00 ± 0.00
Dixella.sp	0.00 ± 0.00	0.00 ± 0.00	0.09 ± 0.30	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Dorytomus.longimanus	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Dytiscinae.sf.	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.10 ± 0.32	0.00 ± 0.00
Dytiscus.sp.larvae	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Elmidae.f	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.10 ± 0.32	0.00 ± 0.00
Ephydridae.f	2.45 ± 6.23	1.00 ± 2.49	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

Table 1- Abundance of macroinvertebrate species found in the five mesocoms used in the decomposition experiment in each location.

Species	Location					
	Múrcia	**Toledo**	**Évora**	**Porto**	**Jaca**	**Peñalara**
Ischnura.pumilio	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	1.10 ± 1.73	0.10 ± 0.32	0.00 ± 0.00
Gerris.sp	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.40 ± 0.70	0.50 ± 0.97
Gyraulus.sp	0.00 ± 0.00	0.73 ± 2.41	0.09 ± 0.30	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Haliplus.sp.larvae	0.00 ± 0.00	2.00 ± 4.65	0.09 ± 0.30	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Haplotaxidae.f	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Helophorus.sp	0.00 ± 0.00	11.64 ± 19.06	0.64 ± 1.80	6.30 ± 8.19	5.60 ± 5.56	57.40 ± 74.81
Hydrobius.sp.larvae	0.00 ± 0.00	0.00 ± 0.00	0.18 ± 0.60	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Hydrochus.sp.	0.00 ± 0.00	0.09 ± 0.30	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Hydrometra.sp	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Hydrophilus.sp	1.09 ± 3.62	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Hydroporus.sp.adult	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.10 ± 0.32	0.00 ± 0.00	0.00 ± 0.00
Hydroporus.sp.larvae	0.18 ± 0.60	19.73 ± 52.44	0.27 ± 0.90	0.00 ± 0.00	0.40 ± 0.52	1.10 ± 2.51
Hygrobia.hermanii	0.09 ± 0.30	2.09 ± 4.76	0.18 ± 0.60	0.30 ± 0.67	0.10 ± 0.32	6.40 ± 14.10
Hygrotus.sp.	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Laccobius.sp.larvae	0.00 ± 0.00	0.00 ± 0.00	0.27 ± 0.90	0.00 ± 0.00	0.00 ± 0.00	0.70 ± 1.57
Laccophilus.sp	0.00 ± 0.00	0.00 ± 0.00	0.09 ± 0.30	0.40 ± 1.26	0.00 ± 0.00	0.00 ± 0.00
Leucorrhinia.rubicunda	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Libelluilidae	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.20 ± 0.63	0.00 ± 0.00	0.00 ± 0.00
Libellula depressa	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Libellula quadrimaculata	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.20 ± 0.63	0.00 ± 0.00	0.00 ± 0.00
Macropelapia.sp	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	1.10 ± 3.48	0.00 ± 0.00
Meladema.coriacea	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Meladema.sp.larvae	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.20 ± 0.63	0.00 ± 0.00
Mesovelia.vittigera	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.10 ± 0.32	0.00 ± 0.00	0.30 ± 0.95

Table 1- Abundance of macroinvertebrate species found in the five mesocoms used in the decomposition experiment in each location.

Species	Location					
	Múrcia	**Toledo**	**Évora**	**Porto**	**Jaca**	**Peñalara**
Micronecta.sp	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Micropsectra.sp	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	27.60 ± 87.28
Microvelia.sp	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.30 ± 0.67	0.00 ± 0.00
Naididae.f	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	1.00 ± 2.54	0.00 ± 0.00	0.00 ± 0.00
Nebrioporus.sp	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Notonecta.sp	0.00 ± 0.00	1.45 ± 4.82	0.27 ± 0.90	2.90 ± 4.79	0.20 ± 0.42	0.40 ± 1.26
Ochthebius.sp.adult	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Oligochaeta	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	27.50 ± 58.33	0.10 ± 0.32	0.00 ± 0.00
Orthetrum.nitidinerve	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Orthocladiinae.sf	91.00 ± 123.63	9.82 ± 18.91	2.27 ± 5.42	22.80 ± 31.12	0.60 ± 1.58	3.30 ± 2.75
Orthocladiinae.sf.nymph	0.45 ± 1.51	0.09 ± 0.30	0.00 ± 0.00	0.50 ± 1.08	0.00 ± 0.00	0.00 ± 0.00
Osmylus.fulvicephalus	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Oxygastra.curtisii	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Palpomyiini.tr	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.90 ± 2.23	0.00 ± 0.00	0.00 ± 0.00
Physa.sp	0.00 ± 0.00	0.00 ± 0.00	0.36 ± 1.21	0.20 ± 0.42	0.00 ± 0.00	0.00 ± 0.00
Planorbarius.dufourii	0.00 ± 0.00	0.00 ± 0.00	0.18 ± 0.60	0.10 ± 0.32	0.00 ± 0.00	0.00 ± 0.00
Planorbidae.f	0.00 ± 0.00	0.00 ± 0.00	0.18 ± 0.60	0.10 ± 0.32	0.00 ± 0.00	0.00 ± 0.00
Plea.minutissima	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Psectrocladius.octomuculatus.nymph	0.09 ± 0.30	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Psectrocladius.limbatellus.nymph	10.00 ± 33.17	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Psectrocladius.psilopterus	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Psectrocladius.limbatellus	0.73 ± 2.41	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Riolus.sp.	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Sciomyzidae.f	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

Table 1- Abundance of macroinvertebrate species found in the five mesocoms used in the decomposition experiment in each location.

Species	Location					
	Múrcia	**Toledo**	**Évora**	**Porto**	**Jaca**	**Peñalara**
Sigara.sp	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Stagnicola.sp	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Sympetrum.sanguinem	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Sympetrum.striolatum	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.20 ± 0.63	0.00 ± 0.00	0.00 ± 0.00
Sympetrum.pro.parte	0.09 ± 0.30	12.91 ± 38.87	0.18 ± 0.40	0.20 ± 0.42	2.00 ± 4.45	0.00 ± 0.00
Sympterum.flaveolum	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Sympetrum.nigrescens	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Sympetrum.vulgatum	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.40 ± 1.26	0.00 ± 0.00	0.00 ± 0.00
Sympterum.fonscolombii	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Tanypodinae.sf	8.73 ± 9.09	13.73 ± 22.96	2.00 ± 2.14	18.50 ± 19.57	4.70 ± 4.72	7.50 ± 5.85
Tanypodinae.sf.nymph	0.36 ± 1.21	1.64 ± 3.20	0.00 ± 0.00	0.10 ± 0.32	0.70 ± 1.16	0.30 ± 0.67
Tanytarsini.tr	22.18 ± 69.31	42.00 ± 127.18	0.00 ± 0.00	0.20 ± 0.63	1.30 ± 3.77	42.30 ± 102.75
Tanytarsus.mendax	0.00 ± 0.00	7.00 ± 23.22	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Tanytarsini.tr.nymph	0.64 ± 1.29	1.55 ± 4.80	0.27 ± 0.90	0.00 ± 0.00	2.10 ± 4.46	79.70 ± 210.98
Tubificidae.f	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Yola.sp.larvae	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.30 ± 0.95	0.00 ± 0.00