Effects of increased temperature and aquatic fungal diversity on litter decomposition

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Abstract

Climate warming and biodiversity loss are two major factors threatening freshwaters. Aquatic hyphomycetes are fungi that play a key role in organic matter turnover in streams. To assess the impacts of temperature increase and aquatic hyphomycete diversity on plant-litter decomposition, we manipulated fungal assemblage composition at two levels of diversity (four and eight species) under ambient temperature of 16 ºC and two regimes of temperature increase differing in 8 ºC: abrupt versus gradual increase from 16 to 24 ºC. The effects were evaluated on leaf-litter decomposition, fungal biomass production and fungal reproduction. The increase in temperature affected the structure of fungal assemblages and leaf decomposition more than fungal biomass or reproduction. Although evidence for some redundancy between fungal species was found, assemblage composition emerged as the major factor controlling fungal biomass and reproduction under different temperature regimes.

Key words: plant-litter decomposition, aquatic hyphomycetes, biodiversity, warming.

Introduction

Climate change is of great concern among the multitude of factors that are threatening biodiversity, ecosystem functioning and the services they provide (Dudgeon *et al.* 2006; Vitousek *et al.* 1997; Vörösmarty *et al.* 2010). Climate change is represented by phenomena like the increase of i) mean global temperature, ii) diurnal temperature oscillations, and iii) frequency and magnitude of severe weather events, like drought periods or intense rainfalls (Easterling *et al.* 2000; Jentsch *et al*. 2007). These events, in turn, can affect the behaviour and distribution of species and the structure of communities (Walther *et al.* 2005) because each species within communities can migrate, adapt or perish under climate change conditions (Perkins *et al.* 2010). Temperature is a key factor determining the activity of organisms in ecosystems (Friberg *et al.* 2009), with higher temperatures stimulating biological activities at least within physiological limits (Bergfur & Friberg 2012). As a result, changes in temperature can have repercussions in key ecosystem processes like organic matter decomposition (Hobbie 1996; Cornelissen *et al.* 2007).

In temperate forested streams, the input of plant litter from the riparian trees is the main energy and carbon source to aquatic biota (Webster & Benfield 1986). Aquatic hyphomycetes are key drivers of plant-litter decomposition in these ecosystems (Bärlocher 1992); they produce extracellular enzymes that degrade plant litter, improve litter palatability to invertebrate shredders, release fine particulate organic matter used by invertebrate collectors, and release inorganic nutrients to primary producers (Suberkropp 1998; Bärlocher 2005).

The functional impacts of biodiversity loss have been the focus of increasing research in ecology. A positive relationship between fungal species richness and leaf decomposition has often been found (Bärlocher & Corkum 2003; Treton *et al.* 2004; Duarte *et al.* 2006;

Pascoal *et al.* 2010; Fernandes *et al.* 2011) due to facilitative interactions and resource-use complementarity (Fernandes *et al.* 2011). Moreover, there is evidence that the functional impacts of biodiversity loss largely depend on the identity of species that are lost or gained from the system (Duarte *et al.* 2006; Pascoal *et al.* 2010). This means that traits of certain species may have a greater impact on ecosystem processes than species diversity *per se* (Pascoal & Cássio 2008). However, it appears that environmental context modulates the impacts of fungal diversity on litter decomposition (Bärlocher & Corkum 2003; Pascoal *et al.* 2010; Fernandes *et al.* 2011).

Temperature affects the distribution (Suberkropp 1984; Wood-Eggenschwiler & Bärlocher 1985), growth and reproduction (Chauvet & Suberkropp 1998; Rajashekhar & Kaveriappa 2000; Dang *et al.* 2009; Fernandes *et al.* 2009) of aquatic hyphomycetes. An increase in temperature generally increases metabolic rates (Sokolova & Lannig 2008) probably explaining the accelerated leaf decomposition driven by aquatic fungi at higher temperatures (Dang *et al.* 2009; Fernandes *et al.* 2009, Ferreira & Chauvet 2011).

Although previous studies had evaluated the effects of temperature or fungal diversity on litter decomposition in aquatic ecosystems, their combined effects were not tested yet. Here, we manipulated the assemblage composition of aquatic hyphomycetes at two levels of diversity (four species and eight species) under two regimes of temperature differing in 8 ºC: abrupt versus gradual increase from 16 to 24 ºC. We hypothesize that: (a) the increase in temperature will stimulate fungal activity leading to an increase of litter decomposition, but the magnitude of the effect may depend on how fast temperature increase; and (b) traits of certain fungal species may be of greater importance than species number to maintain ecological processes under different temperature conditions. The impacts of temperature, fungal species composition and

diversity were evaluated on three functional aspects: leaf-litter decomposition, fungal biomass production and fungal reproduction.

Methods

Fungal species and growth

The aquatic hyphomycetes species used were: *Alatospora acuminata* Ingold (UMB – 140.01; AA), *Anguillospora filiformis* Greath. (UMB – 66.01; AF), *Articulospora tetracladia* Inglod (UMB – 72.01; AT), *Flagellospora curta* J.Webster (UMB – 39.01; FC), *Heliscus lugdunensis* Sacc. and Thérry (UMB – 159.01; HL), *Lunulospora curvula* Ingold (UMB – 108.01; LC), *Tetracladium marchalianum* De Wild. (UMB – 239.02; TM), *Tricladium splendens* Ingold (UMB – 100.01; TS) and *Varicosporium elodeae* W. Kegel (UMB – 142.01; VE). Fungi were isolated from single spores collected from leaves, twigs and foam in streams of Northwest Portugal, and are maintained at the Centre of Molecular and Environmental Biology, Department of Biology, University of Minho.

For microcosm experiments, fungi were grown on 2% malt extract agar, at 16 ºC, during 20-25 days.

Microcosm setup and experimental design

Air-dried leaves of *Alnus glutinosa* L. Gaertn. (alder) were leached in deionised water for 48 h and cut into 12 mm diameter disks. Sets of 20 disks were autoclaved (120 ºC, 20 minutes) and placed, aseptically, in 150 ml Erlenmeyer flasks containing 80 ml of filtered and autoclaved stream water. The stream water was collected in a low-order stream and had slightly acidic pH (6.49 ± 0.01), low conductivity (44 ± 1 µS cm^{-1}), low nutrient concentrations (1.1 ± 0.1 mg l^{-1} $N\text{-}NO_3^-$, 0.008 ± 0.001 mg l^{-1} $N\text{-}NO_2^-$, 0.02 ±

0.01 mg l^{-1} $P\text{-}PO_4^{3-}$, <0.01 mg l^{-1} $N\text{-}NH_3$), and low chemical oxygen demand (COD, 12 ± 1 mg l^{-1}).

Microcosms were inoculated with a total of 2 agar plugs with 6 mm diameter collected from the edge of fungal colonies in the following diversity treatments: two combinations of low diversity (four fungal species) and two combinations of high diversity (eight fungal species) as shown in Table 1. The total inoculum size in microcosms was maintained and divided equally among all species. Fungal assemblages were exposed during 28 days to the following temperature treatments: i) ambient temperature of 16 ºC, ii) an abrupt increase of 8 ºC, (from 16 to 24 ºC) after 21 days, and iii) a gradual increase of 2-3 ºC (from 16 to 24 ºC) every 7 days, in a total of 48 microcosms (two diversity levels, two fungal assemblages, three temperature treatments, and four replicates). The microcosms were maintained under shaking (120 rpm; Certomat BS 3, Melsungen, Germany). Every 7 days, the stream water was renewed and the discarded water was used to estimate fungal sporulation, as described below. At the end of the experiment, leaf disks were used to determine the remaining dry mass and fungal biomass.

Fungal biomass and sporulation

Fungal biomass was estimated from ergosterol concentration on leaves (Gessner 2005). Sets of eight leaf disks were placed in K-OH methanol (8 g L^{-1}) and heated (80 ºC for 30 min) for lipid extraction. The ergosterol was purified by solid phase extraction and quantified by high performance liquid chromatography (HPLC, Beckman Gold System Brea, CA, USA), using a LiChrospher RP18 column (250 X 4 mm, Merck). The system was run isocratically with HPLC-grade methanol at 1.4 ml min^{-1} and 33 °C. Ergosterol peaks were detected at 282 nm, and series of ergosterol standards in isopropanol

(Sigma) were used to estimate ergosterol concentration in the samples.

Conidial suspensions were mixed with 0.5 % Triton X-100 and appropriate volumes of each replicate were filtered (5 µm pore size, Millipore, Billerica, MA, USA). Conidia retained on filters were stained with 0.05 % cotton blue in lactic acid and the filters were scanned under a light microscope (400 X, Leica Biomed, Heerbrug, Switzerland) for conidial types and numbers.

Leaf mass loss

Sets of eight leaf disks from each replicate microcosm were freeze-dried (Christ alpha 2–4, B. Braun, Germany) for 48 h and weighed (± 0.01 mg). Non-inoculated leaf disks were used to determine the initial weight of the disks.

Data analyses

To test the effects of temperature, species richness and assemblage composition on leaf mass loss, fungal biomass and conidial production, a three-way nested ANOVA was performed (Underwood 1997). Additionally, a two-way nested ANOVA was used to assess the effects of temperature and species richness at the ambient temperature (16 ^{o}C).

To achieve normal distribution and homoscedasticity, fungal biomass data were ln-transformed (Zar 1996). Statistical analyses were performed with Statistica 8.0 for Windows (Statsoft, Inc, Tulsa, OK, USA).

Results

Leaf mass loss

Mass loss of alder leaves driven by fungal assemblages with different species composition within two levels of diversity (four and eight species) varied from 14 to 29 % in microcosms kept at ambient temperature (16 ºC) for 28 days (Fig 1A). The exposure of fungal assemblages to increased temperatures led to leaf mass losses between 30 and 37 % (Fig 1A). Overall, leaf mass loss increased with the increase in temperature, but was not affected by species number or composition (three-way nested ANOVA; $P < 0.0001$, $P = 0.4287$ and $P = 0.1867$, respectively; Table 2). However, at ambient temperature, leaf mass loss was affected by assemblage composition (two-way nested ANOVA; $P = 0.0437$).

Fungal biomass and sporulation

At the end of the experiment, fungal biomass on alder leaves varied between 264 and 423 µg ergosterol g^{-1} leaf dry mass for all species combinations and temperature regimes, except in microcosms with high diversity and without *Articulospora tetracladia* (All – AT) kept at ambient temperature (16 ºC), in which fungal biomass reached 664 µg ergosterol g^{-1} leaf dry mass (Fig 1B). Assemblage composition nested within species number significantly affected fungal biomass, so did the interaction between temperature and assemblage composition (three way-nested ANOVA, $P = 0.0098$ and $P = 0.0052$, respectively; Table 2), but species number or temperature regimes had no significant effect (three way-nested ANOVA, $P = 0.056$ and $P = 0.309$, respectively; Table 2).

After 28 days, the highest sporulation rate (5.3×10^6 conidia mg^{-1} leaf dry mass day^{-1}) occurred in decomposing leaves with the highest fungal biomass, i.e. in microcosms kept at ambient temperature containing the assemblage All-AT, while the lowest sporulation rate was found in treatments with low diversity containing *Flagellospora*

curta, *Tricladium splendens*, *Tetracladium marchalianum* and *Anguillospora filiformis* (Fig 1C). Species number and assemblage composition significantly affected fungal sporulation (three way-nested ANOVA, $P < 0.0001$ for both cases; Table 2). Temperature did not affect fungal sporulation (three way-nested ANOVA, $P = 0.1816$; Table 2), but interactions between temperature and species number or assemblage composition were significant (three way-nested ANOVA; $P = 0.0394$ and $P = 0.0085$, respectively; Table 2).

Structure of fungal assemblages

The analysis of the structure of aquatic hyphomycete assemblages on decomposing leaves showed the dominance of one sporulating species (Table 3). At low diversity level, *Heliscus lugdunensis* or *T. splendens* contributed with more than 70 % to the total released conidia. The dominance pattern was not affected by temperature, but the contribution of *Lunulospora curvula* or *A. filiformis* increased at least two-times in treatments with a gradual increase of temperature (from 16 to 24 ºC).

In assemblages with high fungal diversity, the dominant species was *H. lugdunensis*, contributing at least with 52.2 % to the total conidial production (Table 3). The species *Alatospora acuminata* and *Varicosporium elodeae* were well represented with contributions ranging from 8.8 to 19.5 %. Again, the dominance species pattern did not change under different temperature regimes, but the gradual increase in temperature increased almost two- and five-times the contribution of *T. splendens* and *L. curvula* to the total conidial production, respectively. On the contrary, the contribution of *A. acuminata* decreased to near half the value found at the ambient temperature. *A. tetracladia* contributed with 6 to 12% to the total conidial production with lower values found in microcosms in which the temperature was gradually increased.

Effect size

The effect size (i.e. how much each treatment deviated from the grand mean) of increasing temperature was positive on leaf mass loss (up to 46 % differences between ambient and elevated temperature) but negative on fungal reproduction and fungal biomass (up to 23 and 29 % differences, respectively; Fig. 2A). The effect size of species number was positive on fungal reproduction (up to 47 % differences) but effect size was minor for leaf mass loss and fungal biomass (Fig. 2B).

Discussion

In our study, leaf-litter decomposition was more affected by temperature than by identity or number of aquatic hyphomycete species. Even though faster decomposition was found at higher temperatures, which agrees with previous studies (Dang *et al.* 2009; Fernandes *et al.* 2009; Ferreira & Chauvet 2011), no differences were observed between an abrupt and a gradual increase of 8 ºC, probably because the aquatic hyphomycete species used here were isolated in temperate streams that can reach high temperatures in the warm season (16 – 21 ºC in spring; Pascoal & Cássio 2004). Most aquatic hyphomycetes show maximum growth between 15 and 25 ºC (Suberkropp 1984; Sridhar & Bärlocher 1993), which was the range of temperatures used in our study. Because temperature enhances chemical reactions and biological activities (Brown *et al.* 2004; Bergfur & Friberg 2012), the increase in leaf decomposition may be the result of a stimulation of exoenzyme activities of aquatic hyphomycete species. Indeed, other studies found a stimulation of leaf decomposition by increased temperature (Fernandes *et al.* 2009; Ferreira & Chauvet 2011).

Although temperature is one of the most important factors affecting fungal activity, we were not able to detect any significant effect of temperature on overall fungal biomass produced. This was not expected since temperature was the factor that most stimulated leaf decomposition by fungi. Increased temperature differentially affects microbial biomass production and respiration, with a negative effect on growth efficiency often found (Apple *et al.* 2006; Gonzalo *et al.* 2007). Thus, it is conceivable that temperature had decreased growth efficiency, i.e. ratio biomass produced / (biomass produced + respiration) of overall fungal assemblage, and this would contribute to the absence of fungal biomass stimulation under increased temperatures. Unfortunately, we did not measure fungal respiration to further clarify this question. Moreover, the individual contribution of aquatic hyphomycetes to the whole assemblage biomass is difficult to track (but see Pascoal *et al.* 2010 and Fernandes *et al*. 2011), making it difficult to assess temperature effects on species interactions within fungal assemblages.

In our study, there were no significant differences in leaf decomposition between fungal assemblages with high (eight species) and low (four species) diversity. This result agrees with that reported for stream microcosms with up to eight fungal species on leaf litter (Dang *et al.* 2005), but not with the positive fungal diversity effects on decomposition found when manipulating one to five species (Bärlocher & Corkum 2003; Duarte *et al.* 2006; Pascoal *et al.* 2010). Even in streams with highly diverse fungal assemblages (up to 26 species; Pascoal & Cássio 2004; Bärlocher *et al.* 2005), three to five fungal species are generally dominant on decomposing leaves. Altogether, our data suggest functional redundancy among aquatic hyphomycete species, indicating that processes can be maintained at relatively low levels of diversity.

In our experiment at 16 ºC, the composition of fungal assemblages affected leaf-litter decomposition. Also, overall fungal biomass was affected by assemblage composition,

but not by species number, indicating that certain species have traits that may have a greater influence on ecosystems processes than species number *per se* (Bärlocher & Corkum 2003; Duarte *et al*. 2006; Treton *et al*. 2004; Pascoal & Cássio 2008). Data from literature also indicate that responses of fungal assemblages to temperature depend on which species are present (Dang *et al.* 2009). Our data from conidial production corroborate this observation; certain species such as *L. curvula* were stimulated by elevated temperature while others such as *A. tetracladia* were inhibited. However, collective fungal sporulation was not affected by temperature. These findings agree with those obtained by Dang *et al.* (2009), who found that assemblage responses reflect the response of dominant species, which were not affected by temperature in our study. Some studies emphasise that early fungal colonizers will determine the development and activity of fungal assemblages on decomposing leaves (Ferreira & Chauvet 2011; Sridhar *et al.* 2009). In our study, the temperature was only increased after 7 days of fungal colonization, which may partially explain the absence of temperature effects on species dominance patterns.

Aquatic hyphomycetes release conidia to complete their entire life cycle, and so, low conidial production may compromise successfully colonization of new substrata and, ultimately, threatening species survival. In our study, fungal reproductive output increased with diversity, and differences in conidial production between fungal assemblages were lower at high than at low diversity levels. This suggests that high fungal diversity decrease variability of reproductive activity of the whole assemblage, with implications for community stability when facing environmental changes as shown by Pascoal *et al.* (2010).

Overall results showed faster leaf decomposition under increased temperature, but without differences between an abrupt and a gradual increase in temperature. Under

different temperature regimes, assemblage composition came out as the major factor controlling fungal biomass and reproduction, while fungal diversity was only critical to maintain reproduction. Although evidence for some redundancy between fungal species was found, it appears that the loss of certain species may lead to measurable losses in ecological functions of fungal assemblages under different temperature regimes. However, results obtained here should be interpreted with caution, because impacts may differ at longer time scales (Van Ruijven & Berendse 2005), and other factors associated with global change may act in combination (Hoffman *et al.* 2003; Przeslawski *et al.* 2005; Ferreira & Chauvet 2011), which overall impacts are still difficult to predict.

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Table 1 – Fungal species combinations in microcosms at high and low fungal diversity levels

Diversity level	Species combinations
High	*A. acuminata + A. filiformis + F. curta + H. lugdunensis + L. curvula + T. marchalianum + T. splendens + V. elodeae*
	A. acuminata + A. filiformis + A. tetracladia + F. curta + H. lugdunensis + T. marchalianum + T. splendens + V. elodeae
Low	*A. tetracladia + H. lugdunensis + L. curvula + V. elodeae*
	A. filiformis + F. curta + T. marchalianum + T. splendens

Table 2 – Three-way nested ANOVAs of the effects of temperature, species number and assemblage composition (identity), nested within species number, on leaf mass loss, fungal biomass and fungal sporulation.

Parameter	Treatment	Df	F	P
Leaf mass loss	Temperature	2	16.99	<0.0001
	Species number	1	0.64	0.4287
	Identity {species number}	2	1.76	0.1867
	Temperature X species number	2	0.31	0.7358
	Temperature X identity {species number}	4	1.81	0.1474
	Error	36		
Fungal biomass	Temperature	2	3.13	0.0560
	Species number	1	1.06	0.3090
	Identity {species number}	2	5.28	0.0098
	Temperature X species number	2	1.40	0.2606
	Temperature X identity {species number}	4	4.43	0.0052
	Error	36		
Fungal sporulation	Temperature	2	1.79	0.1816
	Species number	1	22.67	<0.0001
	Identity {species number}	2	50.42	<0.0001
	Temperature X species number	2	3.54	0.0394
	Temperature X identity {species number}	4	4.02	0.0085
	Error	36		

Table 3 – Relative percentage contribution of aquatic hyphomycete species to the total conidia released, after 28 days in microcosms under the following temperature regimes: i) kept at 16 ºC during 28 days (ambient temperature), ii) an abrupt increase of 8 ºC, from 16 to 24 ºC, after 21 days (abrupt), and iii) a gradual increase from 16 to 24 ºC with increments of 2-3 ºC after each 7 days (gradual). Mean values, n=4.

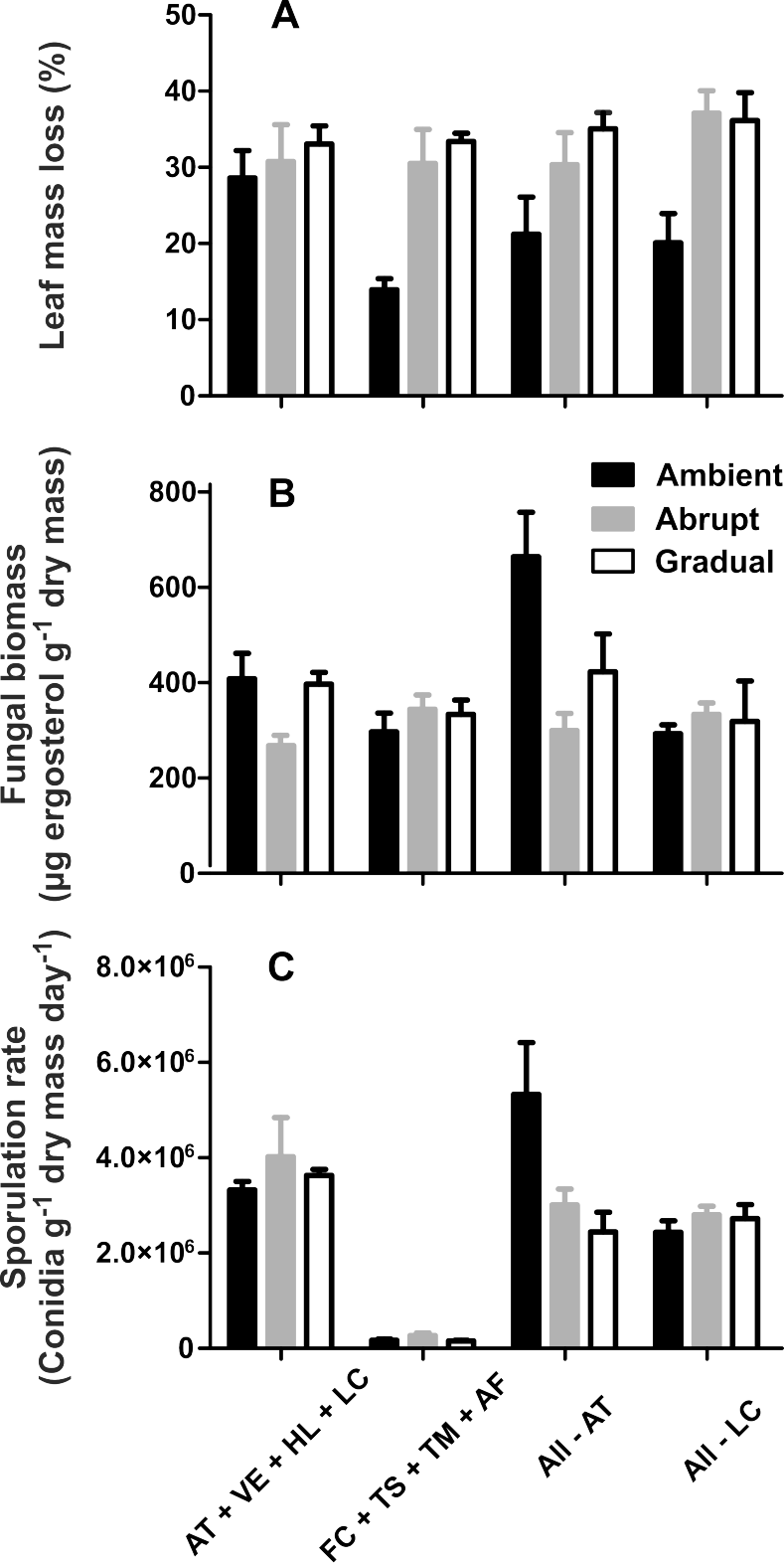
Species	Low diversity						High diversity					
	AT + VE + HL + LC			FC + TS + TM + AF			All – AT			All – LC		
	Ambient	Abrupt	Gradual	Ambient	Abrupt	Gradual	Ambient	Abrupt	Gradual	Ambient	Abrupt	Gradual
A. acuminata (AA)	-	-	-	-	-	-	14.9	11.7	8.8	11.1	10.2	9.6
A. filiformis (AF)	-	-	-	12.4	14.3	24.7	3.8	4.0	4.9	3.4	3.7	3.3
A. tetracladia (AT)	4.8	2.9	4.5	-	-	-	-	-	-	11.9	9.6	6.1
F. curta (FC)	-	-	-	1.9	2.0	1.6	0.0	0.3	0.0	0.0	0.0	0.0
H. lugdunensis (HL)	76.0	79.6	71.8	-	-	-	69.0	62.9	65.8	52.2	55.7	61.1
L. curvula (LC)	2.8	3.6	7.8	-	-	-	0.4	0.7	2.5	-	-	-
T. marchalianum (TM)	-	-	-	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
T. splendens (TS)	-	-	-	85.7	83.6	73.7	2.8	6.8	6.8	1.9	3.0	2.2
V. elodeae (VE)	16.4	13.9	15.9	-	-	-	9.1	13.6	11.2	19.5	17.8	17.7

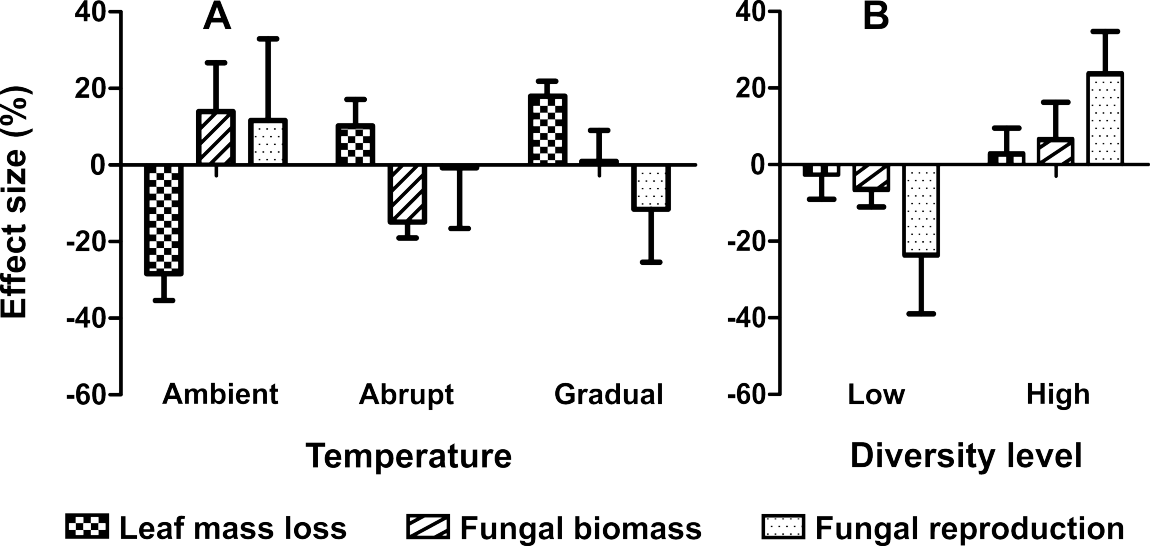
All – AT, all species except AT; All – LC, all species except LC-, absent in the treatment

Figure legends

Figure 1 - Effects of temperature on leaf mass loss **(A)**, fungal biomass **(B)** and fungal sporulation rate **(C)** in microcosms containing aquatic hyphomycete assemblages at two combinations of low diversity (four species) and two combinations of high diversity (eight species) exposed to the following temperature treatments: i) kept at constant temperature of 16 ºC for 28 days (Ambient); ii) an abrupt increase of temperature from 16 to 24ºC after 21 days (Abrupt), and iii) a gradual increase of temperature from 16 to 24ºC with increments of 2-3ºC every 7 days (Gradual). Microcosm experiment ran for 28 days. AA, *Alatospora acuminata*; AT, *Articulospora tetracladia*; VE, *Varicosporium elodeae*; HL, *Heliscus lugdunensis*; LC, *Lunulospora curvula*; FC, *Flagellospora curta*; TS, *Tricladium splendens*; TM, *Tetracladium marchalianum*; AF, *Anguillospora filiformis*; All-AT, all species except AT; and All – LC, all species except LC. M ± SD, n=4.

Figure 2 - Effect sizes of temperature **(A)** and diversity (**B)** treatments on leaf mass loss, fungal biomass and reproduction expressed as percentage of deviation from the grand mean.


A
Leaf mass loss (%)
50
40
30
20
10
0
B
Fungal biomass
(µg ergosterol g-1 dry mass)
800
600
400
200
0
Ambient
Abrupt
Gradual
C
Sporulation rate
(Conidia g-1 dry mass day-1)
8.0×10^6
6.0×10^6
4.0×10^6
2.0×10^6
0
AT + VE + HL + LC
FC + TS + TM + AF
All - AT
All - LC


A
B
Effect size (%)
40
20
0
-20
-40
-60
Ambient
Abrupt
Gradual
Low
High
Temperature
Diversity level
Leaf mass loss
Fungal biomass
Fungal reproduction