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Aquatic hyphomycete strains from metal-contaminated and reference streams might respond differently to future increase in temperature

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Abstract: Aquatic hyphomycetes, a group of polyphyletic fungi, have been reported in streams contaminated with metals. This tolerance to metal contamination however can result in limited performance and limited ability to cope with additional environmental change. The predicted increase in water temperature, as a consequence of global warming, will have an additional effect on many streams. The sensitivity to temperature of strains of three aquatic hyphomycete species isolated from a metal-contaminated stream and an uncontaminated stream was assessed by determining their radial growth and activity (conidial production, oxygen consumption, mycelial biomass accumulation, fine particulate organic matter [FPOM] production, and microbial induced leaf mass loss) at 13 C (present water temperature in autumn) and at 18 C (predicted water temperature under global warming). Growth and reproductive activity generally were depressed for the strains isolated from the metal-contaminated stream when compared with those isolated from the unpolluted stream. These differences however were not translated into differences in FPOM production and leaf-litter mass loss, indicating that the strains isolated from the contaminated stream can decompose leaf litter similar to those of the reference stream. The 5 C increase in temperature stimulated fungal activity and litter decomposition, irrespective of species and strain. This might have strong effect on aquatic food-web and ecosystem functioning under global warming because increases in litter decomposition might lead to food shortage for higher trophic levels. The sensitivity to temperature depended on the response variable, species and strain. FPOM production was the variable most sensitive to temperature across strains and species and that for which temperature sensitivities differed most between strains. Fungal tolerance to metal contamination affects the extent to which its functions are stimulated by an increase in temperature, constituting an additional cost of metal tolerance.

Key words: climate warming, fungal activity, fungal radial growth, leaf decomposition, temperature sensitivity

Introduction

Woodland streams derive most of their energy and carbon from terrestrial organic matter supplied by the surrounding riparian vegetation (Vannote et al. 1980, Mulholland et al. 2001). The decomposition of this organic matter is at the base of aquatic food webs and is ruled mainly by aquatic hyphomycetes, a group of polyphyletic fungi. This group of decomposers mineralize the leaf litter, incorporate it into its own biomass (mycelia and conidia), enhance litter palatability to invertebrate shredders and convert it into fine particulate organic matter to be used downstream by filter-feeders and collectors (Graça et al. 2001, Hieber and Gessner 2002, Baldy et al. 2007, Chung and Suberkropp 2009). Aquatic hyphomycetes therefore are important intermediates between dead organic matter and secondary production, being key actors on nutrient and carbon cycling.

The activity of aquatic hyphomycetes, and consequently ecosystem functioning, can be affected by anthropogenic activities such as mining (Niyogi et al. 2003, Lecerf and Chauvet 2008). Metal contamination has been demonstrated to negatively affect growth, reproductive activity and litter decomposition by aquatic hyphomycetes (Abel and Bärlocher 1984; Berminghan et al. 1996; Miersch et al. 1997; Niyogi et al. 2002; Duarte et al. 2004, 2008; but see Medeiros et al. 2008). However the presence of aquatic hyphomycetes in heavily contaminated waters (Sridhar et al. 2000, 2001; Krauss et al. 2001) suggests some degree of acclimatization or adaptation to these harsh conditions. This is supported in reports of higher tolerance to metals of strains isolated from contaminated than from noncontaminated streams (Chamier and Tipping 1997, Miersch et al. 1997, Fernandes et al. 2011) and evidences of morphologic (colony shape and coloration and conidia shape and size) and enzymatic differences between strains (Braha et al. 2007, Ferreira et al. 2010). This tolerance to metal contamination however can cause limited performance (e.g. reduced growth, activity) and limited ability to cope with additional environmental

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change because tolerance to metals might require energy; cost of tolerance has been observed for benthic invertebrates (Courtney and Clements 2000, Kashian et al. 2007) and fish (Weis and Weis 1989).

The predicted increase in water temperature, as a consequence of global warming (Eaton and Scheller 1996), will be an additional modulating factor to many streams already facing other stressors (e.g. nutrient enrichment, metal contamination). Increases in temperature have been reported to stimulate growth and sporulation of aquatic hyphomycete species (Chauvet and Suberkropp 1998, Rajashekar and Kaveriappa 2000, Dang et al. 2009, Ferreira and Chauvet 2011), which can translate into accelerated decomposition (Ferreira and Chauvet 2011). The extent to which fungal strains are sensitive to increases in temperature however might depend on their tolerance to other existing stressors (e.g. metals), which might limit the ability to cope with additional environmental change. The ability of metal-tolerant strains to maintain ecosystem processes therefore will depend on their tolerance to both metals and warming being positively correlated (Vinebrooke et al. 2004). The physiological mechanism responsible for temperature tolerance most probably is the production of heat-shock proteins (HSPs) whose role is to promote the stability or recovery of the original structure of proteins, minimizing in this way the tendency for altered proteins to interact inappropriately (Feder and Hofmann 1999). Because metal contamination has been shown to induce the production of HSPs in several organisms, aquatic fungi included (Miersch and Grancharov 2008), the tolerant strains might have an advantage when facing increases in temperature (Feder and Hofmann 1999). However this might have additional costs if HSPs are diverted from their role in development regulation to healing, if HPSs concentrations become toxic or if the synthesis and degradation of HSPs consume large amounts of energy and nutrients and/or take cell components needed for the processing of other molecules (Feder and Hofmann 1999). The response of distinct fungal species to increases in temperature has been shown (Chauvet and Suberkropp 1998, Rajashekar and Kaveriappa 2000, Dang et al. 2009), but intraspecific differences still were not assessed.

In this study we evaluated the sensitivity of growth and activity of strains of aquatic hyphomycete species isolated from two streams contrasting in metal concentration (a reference stream and a stream receiving mine drainage from an abandoned uranium mine) to a 5 C increase as expected in global warming (Eaton and Scheller, 1996). We anticipate that (i) aquatic hyphomycete strains isolated from the contaminated stream will have slower growth and lower activity than reference strains and (ii) conspecific strains will differ in the extent to which their activity will be stimulated by an increase in temperature.

MATERIALS AND METHODS

The sensitivity to temperature of strains of three aquatic hyphomycete species isolated from a metal-contaminated and a reference streams was assessed by determining their radial growth and activity (conidial production, oxygen consumption, mycelial biomass accumulation, fine particulate organic matter [FPOM] production and microbial-induced leaf-mass loss) at 13 C and 18 C. These temperatures were chosen to simulate present water temperature in temperate mountain streams in autumn and water temperature under global warming. In some areas of the world air temperature is predicted to increase up to 7 C (e.g. Portugal; Miranda et al. 2002) and so an increase by 5 C in water temperature for temperate streams is realistic (Eaton and Scheller 1996). In the laboratory, incubations took place in temperature-controlled rooms, under a 12 h light : 12 h dark photoperiod, and each of the 12 treatments (3 species × 2 strains × 2 temperatures) was replicated threefold.

Aquatic hyphomycete species and strains.—Three species of aquatic hyphomycetes (*Articulospora tetracladia* Ingold, *Fontanospora fusiramosa* Marvanová and *Varicosporium elodeae* Kegel) were isolated from single conidia released from submerged leaf litter collected from two streams with contrasting metal concentration: a stream historically contaminated by uranium mine drainage (Ribeira da Pantanha, 40°59′N, 8°00′W) located near the abandoned uranium mines of Urgeiriça (Nelas, central Portugal) and a reference stream (Ribeira de Alhões, 40°30′N, 7°52′W) (Carvalho et al. 2006, 2007; Ferreira et al. 2010). The isolates were grown 15–30 d in malt extract agar medium (Difco, 20 g MEA L^{-1} distilled water) at 20 C before use.

Conidial suspensions (<1 d old) were produced at 18 C by agitation (100 rpm) of agar plugs taken from the leading edge of growing colonies in 100 mL Erlenmeyer flasks filled with 25 mL nutrient solution (75.5 mg $CaCl_2$, 10 mg $MgSO_4.7H_2O$, 0.5 g 3-morpholinopropanesulfonic acid, 5.5 mg K_2HPO_4 and 100 mg KNO_3 per liter sterile distilled water). An aliquot of each specific conidial suspension, based on conidial numbers, was used to inoculate each microcosm (see below).

Radial growth.—Radial growth of species and strains was assessed in 9 cm diam Petri dishes filled with 20 mL MEA media. Petri dishes were inoculated in the center with one agar plug (3 mm diam) cut from the leading edge of a growing colony with a flamed cork borer under aseptic conditions. Colony diameter was measured (±0.5 mm) at two perpendicular directions every 3–6 d until the colony was ~1 cm from dish edges (Rajashekar and Kaveriappa 2000, Treton et al. 2004).

Fungal activity.—Alder leaves, microcosms and experimental setup. Alder (*Alnus glutinosa* [L.] Gaertner) leaves were collected just after abscission and dried at room temperature

until needed. Just before use leaves were sprayed with distilled water and leaf disks were cut with a 12 mm diam cork borer. Batches of 20 leaf disks were dried at 105 C, weighed (±0.1 mg) to determine initial dry mass (DM), placed inside glass tubes with 10 mL distilled water and autoclaved (20 min at 121 C). Six batches of 20 leaf disks were given the same treatment and were used to create a correction factor for mass loss due to leaching during sterilization and in microcosms (see below). Sterilized 100 mL Erlenmeyer flasks (hereafter called microcosm) were displayed in rooms acclimatized to 13 C and 18 C, were filled with 40 mL nutrient solution and the corresponding leaf disks and agitated on an orbital shaker (100 rpm) 24 h to allow further leaching. Nutrient solution was replaced and the microcosms inoculated with 3000 conidia of a single isolate (day 0). For the first 2 h the microcosms were agitated for 20 min periods interspaced with 20 min periods to aid conidia settlement and attachment to litter disks; afterward microcosms were agitated continuously until the end of the experiment. The nutrient solution was replaced after 24 h and then every 2 d for the duration of the experiment (33 d). All microcosms were killed at day 33 for conidial and leaf-disks sampling. All manipulations of microcosms took place under aseptic conditions.

Conidial production. Nutrient solutions were replaced 15 times during incubation. The correspondent conidial suspensions from each microcosm were accumulated in 1.5 L jars and preserved with 37% formalin (2 mL formalin per date). At day 33 conidial suspensions were placed in 50 mL Falcon tubes, preserved with 2 mL 37% formalin and the volume adjusted to 45 mL with distilled water. When preparing filters for conidia counting Triton X-100 (0.5%) was added to the suspension, stirred to ensure a uniform distribution of conidia, and an aliquot was filtered (Millipore SMWP, pore size 5 μm, Millipore Corp., Bedford, Massachusetts). Filters were stained with 0.05% trypan blue in lactic acid (60%) and spores were counted under a compound microscope at 250× (Bärlocher 2005). For cumulative samples (until day 31) results were expressed as total number of conidia g^{-1} leaf DM. At day 33 sporulation rates were expressed as number conidial g^{-1} leaf DM day^{-1}.

Oxygen consumption. A subset of five leaf disks from each microcosm was used to determine fungal oxygen consumption at day 33 with a closed six-channel dissolved oxygen measuring system (Strathkelvin 929 System, North Lanarkshire, Scotland) connected to a computer. The oxygen electrodes were calibrated against a 0% O_2 solution (2% sodium sulfite in 0.01 M sodium borate) and a 100% O_2 saturated nutrient solution at the target temperature. Leaf disks were incubated in 3 mL chambers containing 100% O_2 saturated nutrient solution, with constant stirring, kept at the target temperatures, 13 or 18 C, by circulation of water originating from a temperature-controlled water bath. After 1 h trial disks were enclosed in small sterile plastic zippered bags and promptly frozen at −20 C for later assessment of remaining DM and ergosterol extraction. Oxygen consumption was determined by the difference in the oxygen concentration in the sample and the control over a 20 min interval during which oxygen consumption over time was linear, corrected for the chamber's volume, time and disks mass. Results were expressed as mg O_2 g^{-1} leaf DM h^{-1}.

Mycelial biomass. Leaf disks were lyophilized, promptly weighed (±0.1 mg) to determine DM (g), and used for ergosterol extraction, which can be used as a surrogate of mycelial biomass (Gessner 2005). Leaf disks were placed in tightly closed tubes with 10 mL KOH/methanol in a water bath (80 C) for 30 min. The extract was purified by solid-phase extraction (Waters Sep-Pak Vac RC tC_{18} cartridges, Waters Corp., Massachusetts) (Gessner 2005). Ergosterol was quantified by high performance liquid chromatography (HPLC) by measuring absorbance at 282 nm. The HPLC system (Dionex DX-120, Sunnyvale, California) was equipped with the LiChroCART 250-4 LiChrospher 100 RP-18 (5 μm) column (Merk, Darmstadt, Germany), maintained at 30 C. The mobile phase was 100% methanol, flowing at 1.4 mL min^{-1}. Ergosterol was converted into mycelial biomass assuming 5.5 μg ergosterol mg^{-1} mycelial DM (Gessner and Chauvet 1993), and results were expressed as mg mycelial biomass g^{-1} leaf DM.

FPOM production. FPOM production over 31 d was determined for each sample by filtration of 800 mL of the cumulated suspension through ignited, pre-weighed fiberglass filters (Millipore APFF, pore size 0.7 μm, Millipore Corp., Bedford, Massachusetts). Filters were dried at 105 C for 24 h and reweighed (±0.1 mg). FPOM production was calculated after correction for the conidial mass produced; total conidial mass was estimated by multiplying the total number of specific conidia produced by its average mass calculated from biovolume data (Bärlocher and Schweizer 1983) and assuming 70% water content. Results were expressed as mg FPOM g^{-1} initial leaf DM.

Leaf-mass loss. The other 15 leaf disks from each microcosm were enclosed in small zippered plastic bags, immediately frozen at −20 C, lyophilized, and promptly weighed (±0.1 mg) to determine DM (g). The leaf mass loss (%) was calculated after summing the mass of all disks, corrected for the mycelial biomass.

Data treatment.—Colony diameter over time was compared between strains and temperatures for each species by repeated measures ANOVA. Growth rate (mm d^{-1}) was determined from the slope of the linear regression fitted to the colony diameter data over time (with free intercept). Cumulative conidial production, sporulation rates, oxygen consumption rates, mycelial biomass, FPOM production and leaf mass loss were compared between strains and temperatures for each species by two-way ANOVAs. The sensitivities to temperature (Q_{10}) of radial growth rates, cumulative conidial production, FPOM production and leaf mass loss, which are variables integrated over time, were calculated as $(V_{18}/V_{13})^{(10/[18-13])}$, being V_{18} and V_{13} the target variable at 18 and 13 C respectively. Data were log transformed when necessary to achieve homocedasticity, and Tukey's test was used for posthoc multiple comparisons. Analyses were performed with Statistica 7 software.

RESULTS

Radial growth.—Colony diameter increased linearly over time (FIG. 1). Growth rates were significantly lower for the strain isolated from the contaminated stream than for the reference strain for *A. tetracladia*

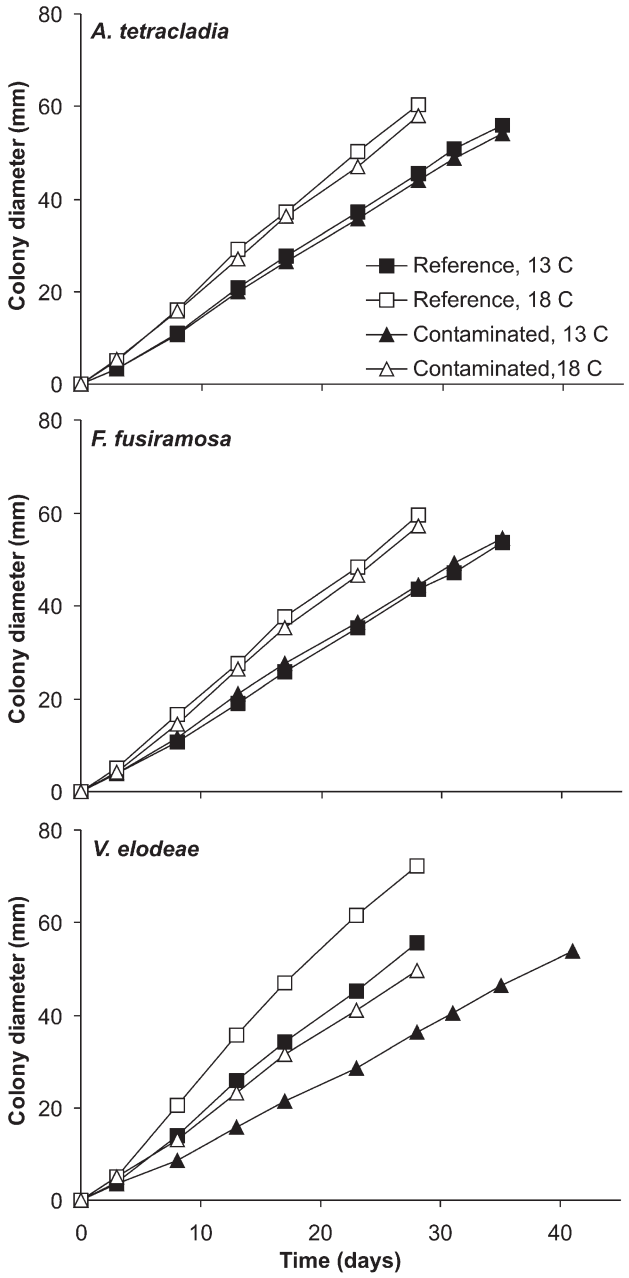

FIG. 1. Growth of *A. tetracladia*, *F. fusiramosa* and *V. elodeae* strains isolated from a reference and a metal contaminated stream in MEA medium at 13 and 18 C. SE bars are smaller than the symbols.

and *F. fusiramosa* at 18 C (Tukey's test, $P = 0.006$ for both species) and for *V. elodeae* at both temperatures ($P < 0.001$) (TABLE I). An increase of 5 C significantly stimulated growth rates of both strains for all species (Tukey's test, $P < 0.001$) (TABLE I). The Q_{10} temperature coefficient was similar across species and strains (1.72–1.90, TABLE I).

Fungal activity.—Conidial production. Cumulative conidial production over 31 d was very high, varying between 2.9 and 51.7×10^6 conidia g^{-1} initial leaf DM across treatments (FIG. 2A). There was a tendency for the strains isolated from the contaminated stream to produce fewer conidia than the reference strains, although significant differences were found only for *F. fusiramosa* at 13 C (Tukey's test, $P = 0.024$) (FIG. 2A, TABLE II). An increase in temperature tended to stimulate conidial production in all cases, except *F. fusiramosa* reference strain that reduced conidial production by 31% when temperature was raised from 13 C to 18 C (Tukey's test, $P = 0.001$) (FIG. 2A, TABLE II). Temperature coefficients (Q_{10}) were 0.48–2.29 across treatments, being lower for the *A. tetracladia* strain from the contaminated stream than for the reference strain, while for *F. fusiramosa* and *V. elodeae* the opposite was true (TABLE III).

Sporulation rates at day 33 were low and did not significantly differ between strains and temperatures for *A. tetracladia* (two-way ANOVA, $P > 0.221$). For *F. fusiramosa* sporulation rate was significantly lower for the strain from the contaminated stream when incubated at 13 C than at 18 C (Tukey's test, $P = 0.005$) and then for the reference strain when incubated at 13 C ($P = 0.020$). *V. elodeae* strain isolated from the contaminated stream and incubated at 13 C had the highest sporulation rate (Tukey's test, $P < 0.034$) (FIG. 2B, TABLE II).

Oxygen consumption and mycelial biomass. Oxygen consumption and mycelial biomass at day 33 generally were similar between strains incubated at the same temperature (Tukey's test, $P > 0.075$ and $P > 0.150$ respectively); only *V. elodeae* strain isolated from the contaminated stream had lower respiration and biomass than the reference strain when incubated at 13 C ($P = 0.034$ and $P = 0.002$ respectively). An increase in temperature stimulated oxygen consumption by both strains for all species (Tukey's test, $P < 0.020$), while it led to a decrease in mycelial biomass for the *V. elodeae* reference strain ($P = 0.024$) (FIG. 2C, D; TABLE II).

FPOM production. FPOM production was similar between strains incubated at the same temperature (Tukey's test, $P > 0.162$). An increase in temperature stimulated FPOM production by both strains for all species (Tukey's test, $P < 0.029$) (FIG. 2E, TABLE II). The sensitivity to temperature of FPOM production was lower for the *F. fusiramosa* and *V. elodeae* strains isolated from the contaminated stream than for the correspondent reference strains; for *A. tetracladia* sensitivity to temperature was higher for the strain from the contaminated stream (TABLE III).

Leaf-mass loss. Fungal-induced leaf mass loss did not significantly differ between strains and temperatures for *V. elodeae* (two-way ANOVA, $P > 0.084$). For *A. tetracladia* and *F. fusiramosa* mass loss was similar

TABLE I. Growth of *A. tetracladia*, *F. fusiramosa* and *V. elodeae* strains isolated from a reference and a metal contaminated stream in MEA medium at 13 and 18 C; letters indicate differences at $P < 0.05$ for Tukey multiple comparisons

Species and strain origin[a]	Temperature (C)	Growth rate (mm d^{-1})	SE	R^2	P	Tukey's test	Q_{10}
A. tetracladia							
Reference	13	1.654	0.014	0.998	<0.001	a	
Reference	18	2.203	0.021	0.998	<0.001	b	1.77
Contaminated	13	1.594	0.013	0.998	<0.001	a	
Contaminated	18	2.087	0.015	0.999	<0.001	c	1.72
F. fusiramosa							
Reference	13	1.560	0.012	0.999	<0.001	a	
Reference	18	2.149	0.043	0.993	<0.001	b	1.90
Contaminated	13	1.593	0.013	0.998	<0.001	a	
Contaminated	18	2.086	0.020	0.998	<0.001	c	1.72
V. elodeae							
Reference	13	2.026	0.023	0.998	<0.001	a	
Reference	18	2.679	0.046	0.994	<0.001	b	1.75
Contaminated	13	1.334	0.020	0.994	<0.001	c	
Contaminated	18	1.798	0.041	0.990	<0.001	d	1.82

[a] GenBank accession numbers: *A. tetracladia*, Reference: GQ152144.1; *A. tetracladia*, Contaminated: GQ152145.1; *V. elodeae*, Reference: GQ152148.1; *V. elodeae*, Contaminated: GQ152149.1.

between strains incubated at the same temperature (Tukey's test, $P > 0.137$), and was stimulated by an increase in temperature ($P < 0.024$) (FIG. 2F, TABLE II). The sensitivity to temperature of fungal-induced leaf decomposition was lower for the strain isolated from the contaminated stream than for the reference strain for *A. tetracladia*, while the opposite was observed for *F. fusiramosa* and *V. elodeae* (TABLE III).

DISCUSSION

Aquatic hyphomycetes in streams contaminated with metals might be affected physiologically because energy may be diverted from basic functions to create metal tolerance. Metal tolerance in fungi might be achieved by several processes, including reduction in metal uptake, increase in metal excretion, metal immobilization (e.g. cell-wall adsorption, extracellular binding to polysaccharides, intracellular sequestration by binding to proteins, vacuolar localization) (reviewed by Gadd 1993, 2007), which might require energy. In fact growth and reproductive activity generally were depressed for the strains isolated from the metal-contaminated stream when compared with those isolated from an unpolluted stream. This seems to indicate genetic adaptation of aquatic hyphomycetes to metal contamination because these observations were made in metal-free media. *V. elodeae* isolated from the contaminated stream grew better in solid medium prepared with contaminated stream water than when reference water was used, according to Ferreira et al. (2010), which further supports the claim of fungal tolerance to metal contamination. It is not possible however to anticipate to which metal(s) the strain isolated from the contaminated stream was tolerant given that the mine drainage is composed of a complex mixture of metals dominated by uranium, iron and copper (Ferreira et al. 2010). Studies also reported differences in enzymatic activity and growth of strains isolated from streams differing in metal concentration when incubated in the absence of metals (Miersch et al. 1997, Braha et al. 2007). In addition differences in the way strains from the contaminated stream and reference stream responded to the increase in temperature might be related with previous tolerance to metals and production of HSPs (Miersch and Grancharov 2008); for instance the increase in temperature reduced the conidial production by *F. fusiramosa* reference strain but not by the strain from contaminated stream while for *A. tetracladia* warming stimulated conidial production by the strain from contaminated stream but not by the reference strain. The differences in growth and activity between conspecific strains however were not translated into differences in FPOM production and leaf-litter mass loss, indicating that the strains isolated from the metal-contaminated stream have capabilities to decompose leaf litter similar to those of the reference stream. However this is far from saying that fungal communities at metal-polluted streams are as able to maintain ecosystem functioning as those in unpolluted streams. Metal contamination often leads to a reduction in fungal richness (Bermingham et al.

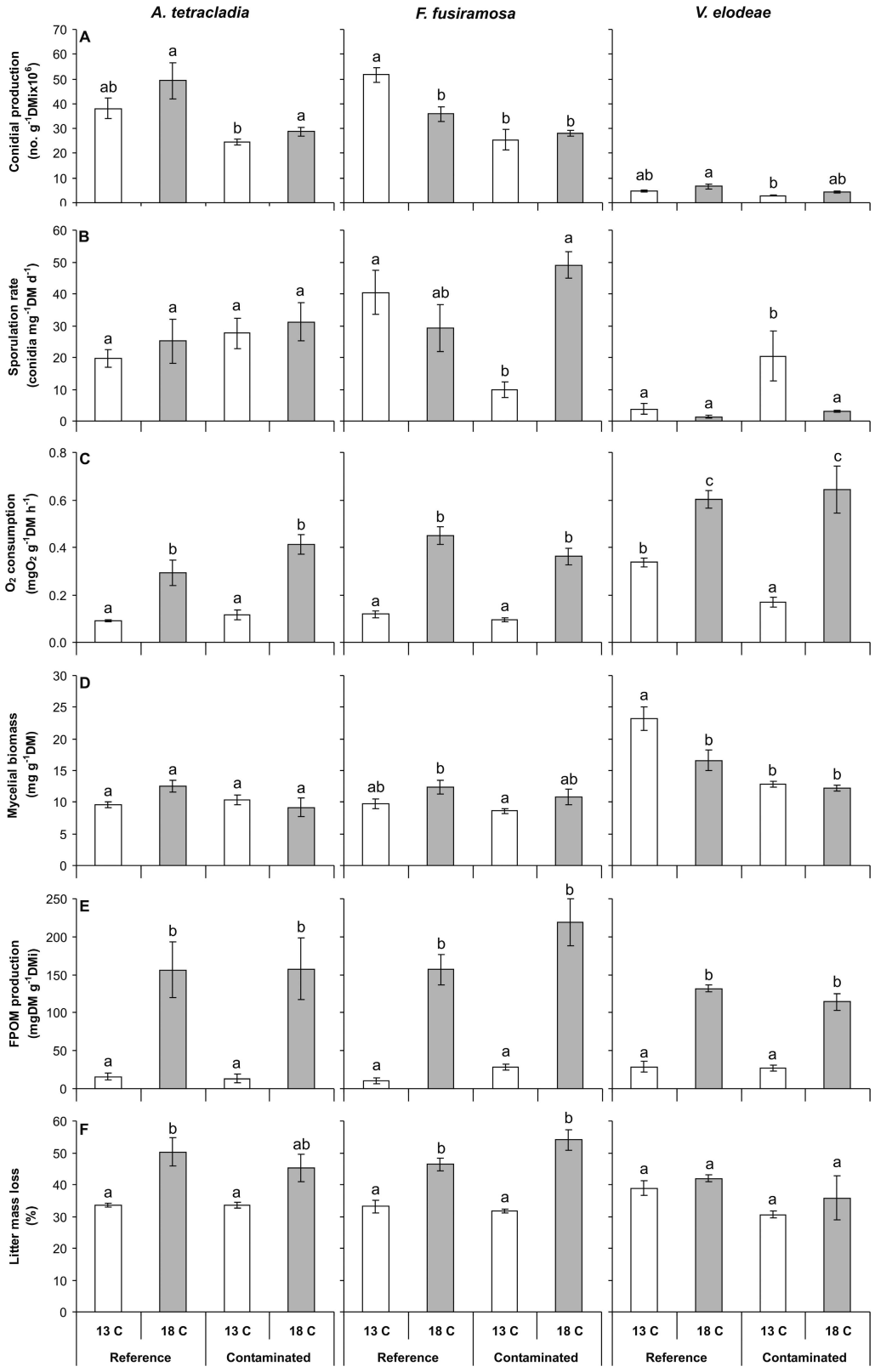

FIG. 2. A. Cumulative conidial production. B. Sporulation rate. C. Oxygen consumption rate. D. Mycelial biomass. E. FPOM production. F. Induced leaf-mass loss by *A. tetracladia*, *F. fusiramosa* and *V. elodeae* strains isolated from a reference and a metal contaminated stream and incubated at 13 and 18 C.

TABLE II. Summary for two-way ANOVAs performed on cumulative conidial production, sporulation rate, oxygen consumption rate, mycelial biomass, FPOM production and leaf-mass loss induced by *A. tetracladia*, *F. fusiramosa* and *V. elodeae* strains isolated from a reference and a metal contaminated stream and incubated at 13 and 18 C. Significant *P* values are in boldface

		Cumulative conidial production		Sporulation rate		O_2 consumption		Mycelial biomass		FPOM production		Leaf-mass loss	
	df	F	*P*	F	*P*	F	*P*	F	*P*	F	*P*	F	*P*
A. tetracladia													
Intercept	1	266.325	<0.001	95.247	<0.001	160.857	<0.001	447.354	<0.001	38.615	<0.001	1402.792	<0.001
Strain	1	3.179	0.112	1.761	0.221	3.993	0.081	1.785	0.218	<0.001	0.985	0.628	0.451
Temperature	1	15.707	**0.004**	0.732	0.417	48.050	**<0.001**	0.781	0.403	26.495	**0.001**	20.020	**0.002**
Strain × temperature	1	0.675	0.435	0.030	0.866	1.623	0.238	4.165	0.076	0.005	0.946	0.619	0.454
Error	8												
F. fusiramosa													
Intercept	1	547.256	<0.001	133.608	<0.001	377.338	<0.001	515.419	<0.001	124.541	<0.001	2881.756	<0.001
Strain	1	4.866	0.058	0.931	0.363	4.196	0.075	2.188	0.177	4.741	0.061	2.125	0.183
Temperature	1	32.086	**<0.001**	6.307	**0.036**	127.959	**<0.001**	7.168	**0.028**	82.256	**<0.001**	65.716	**<0.001**
Strain × temperature	1	9.486	**0.015**	20.464	**0.002**	1.534	0.251	0.045	0.837	1.366	0.276	4.251	0.073
Error	8												
V. elodeae													
Intercept	1	208.518	<0.001	56.838	<0.001	334.562	<0.001	657.892	<0.001	427.781	<0.001	1178.999	<0.001
Strain	1	6.918	**0.030**	14.538	**0.005**	9.625	**0.015**	33.898	**<0.001**	1.803	0.216	3.902	0.084
Temperature	1	9.327	**0.016**	16.417	**0.004**	81.951	**<0.001**	8.284	**0.021**	170.217	**<0.001**	1.301	0.287
Strain × temperature	1	0.095	0.766	1.220	0.302	12.694	**0.007**	5.747	**0.043**	1.071	0.331	0.091	0.771
Error	8												

1996, Raghu et al. 2001, Niyogi et al. 2002, Sridhar et al. 2005, Baudoin et al. 2008, Solé et al. 2008), resulting in impoverished communities composed of tolerant or adapted species. These metal-induced tolerant communities are usually not able to maintain ecosystem functioning at the same level of those from unpolluted streams (Bermingham et al. 1996, Sridhar et al. 2005, Baudoin et al. 2008). Functional redundancy has been described among communities from reference streams and streams subject to organic pollution or changes in riparian vegetation (Bärlocher and Graça 2002, Pascoal et al. 2005, Ferreira et al. 2006) and more rarely suggested as a response to metal contamination (Pascoal et al. 2005, Gonçalves et al. 2011).

TABLE III. Sensitivities to temperature (Q_{10}) of cumulative conidial production, FPOM production and induced leaf-mass loss by *A. tetracladia*, *F. fusiramosa* and *V. elodeae* strains isolated from a reference and a metal contaminated stream

Species and strain origin	Cumulative conidial production	FPOM production	Leaf-mass loss
A. tetracladia			
Reference	1.68	95.74	2.24
Contaminated	1.37	136.28	1.82
F. fusiramosa			
Reference	0.48	243.56	1.96
Contaminated	1.22	57.89	2.89
V. elodeae			
Reference	1.97	20.66	1.17
Contaminated	2.29	18.30	1.38

An increase by 5 C stimulated growth (1.3–1.4-fold), conidial production (0.7–1.5), oxygen consumption (1.8–3.8), FPOM production (4.3–15.6) and leaf-mass loss induced by aquatic hyphomycete (1.1–1.7) across strains and species, as expected because the increase in temperature was within thermal-tolerance limits and within these limits enzymatic activity is stimulated by increases in temperature (Chauvet and Suberkropp 1998, Rajashekar and Kaveriappa 2000, Dang et al. 2009, Ferreira and Chauvet 2011). The positive effect of the increase in temperature was not so evident for sporulation rate and mycelial biomass, usually highly

dynamic over time, probably because they were determined only at the last day. The sporulation and biomass peaks might have been attained earlier at 18 C so that at the last day dynamics of fungal biomass and sporulation already could be descreasing, while those at 13 C were still increasing (Ferreira and Chauvet 2011). From a fungi perspective it seems that stimulation of growth and/or reproductive activity is more advantageous than stimulation of respiration, FPOM production and/or litter mass loss. Therefore *A. tetracladia* and *F. fusiramosa* strains isolated from the contaminated stream were more favored by the increase in temperature that all others because these enhanced both growth and conidial production while all others only significantly increased the colonized area. Nevertheless an increase in fungal activity and litter decomposition due to future increase in temperature, as that simulated here, might have strong effects on aquatic food-web and ecosystem functioning. Increases in litter-mass loss promoted directly by fungal activity and indirectly by stimulated fungal growth attracting invertebrate shredders might lead to food shortage for higher trophic levels locally. However this might be counteracted by higher primary production of litter of decreased quality under a CO_2-enriched atmosphere (Stiling and Cornelissen 2007) or by antagonistic effects on fungal activity promoted by metals in water and increased temperature. Increased conidial and FPOM production will stimulate collectors and filter-feeders locally and possibly downstream (Bärlocher and Brendelberger 2004, Jonsson and Malmqvist 2005). These changes in food supply might result in changes in the community structure of benthic invertebrates, which can cascade to higher trophic levels.

The sensitivity to temperature depended on the response variables, species and strain. FPOM production was the variable most sensitive to temperature across strains and species and that for which temperature sensitivities differed most between strains. Less consistent was the temperature sensitivity for each strain; the increase in temperature generally stimulated the activities of both strains, but this stimulation was higher for the strains tolerant to metals than for the reference ones in six out of 12 situations; in opposition in six out of 12 situations this stimulation was lower than that observed for reference strains. This seems to indicate that fungal tolerance to metal contamination affects the extent to which its functions are stimulated by an increase in temperature, which might be an additional cost of metal tolerance, a tradeoff between tolerance to metals and sensitivity to temperature. The mechanism by which tolerance to metals modulates fungal sensitivity to increases in temperature is beyond the scope of this study, but it might relate to differential allocation of available energy or genetic constrains (Weis and Weis 1989). The way in which future increase in temperature will affect the ability of strains to tolerate metals is difficult to anticipate given that toxicity of some metals increase with increases in temperature (Cairns et al. 1975). However our results and interpretations are limited by the sample size because only three species, each with only two strains, were used.

In this study we showed intraspecific variability in aquatic hyphomycetes growth and activities, most probably a result from tolerance to metals as suggested previously (Miersch et al. 1997, Braha et al. 2007, Ferreira et al. 2010). However tolerance to metal contamination was achieved at the cost of reduced growth and reproductive activity and distinct responses to an increase in temperature as in a future climate warming scenario (i.e. cost of altered sensitivity to additional environmental change) when compared to strains from a reference stream. This tradeoff between tolerance to harsh conditions and altered physiology and sensitivity to additional environmental change makes it difficult to predict the response to climate change of fungal communities, and consequently microbial mediated litter decomposition, in metal-contaminated streams. Bottom-up effects nevertheless are expected in the affected stream food webs because fungi are key players in litter processing and affect feeding by shredders and the production of FPOM that is used by filter-feeders and collectors, crucial trophic links between detritus and predators.

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