

# Intraspecific traits change biodiversity effects on ecosystem functioning under metal stress

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**Abstract** Studies investigating the impacts of biodiversity loss on ecosystem processes have often reached different conclusions, probably because insufficient attention has been paid to some aspects including (1) which biodiversity measure (e.g., species number, species identity or trait) better explains ecosystem functioning, (2) the mechanisms underpinning biodiversity effects, and (3) how can environmental context modulates biodiversity effects. Here, we investigated how species number (one to three species) and traits of aquatic fungal decomposers (by replacement of a functional type from an unpolluted site by another from a metal-polluted site) affect fungal production (biomass accumulation) and plant litter decomposition in the presence and absence of metal stress. To examine the putative mechanisms that explain biodiversity effects, we determined the contribution of each fungal species to the total biomass produced in multicultures by real-time PCR. In the absence of metal, positive diversity effects were observed for fungal production and leaf decomposition as a result of species complementarity. Metal stress decreased diversity effects on leaf decomposition in assemblages containing the functional type from the unpolluted site, probably due to competitive interactions between fungi. However, dominance effect maintained positive diversity

effects under metal stress in assemblages containing the functional type from the metal-polluted site. These findings emphasize the importance of intraspecific diversity in modulating diversity effects under metal stress, providing evidence that trait-based diversity measures should be incorporated when examining biodiversity effects.

**Keywords** Intraspecific diversity · Ecosystem processes · Leaf decomposition · Aquatic fungi · Freshwaters

## Introduction

Since the early 1990s, a large number of studies in ecology have focused on the question of how biodiversity matters for ecosystem functioning. Most results point to positive effects of species richness on ecosystem processes (Hooper et al. 2005; Gamfeldt and Hillebrand 2008; Pascoal and Cássio 2008; Reiss et al. 2009) and conclusions have been supported by meta-analyses with different trophic groups (producers, herbivores, detritivores and predators) from aquatic and terrestrial ecosystems (Balvanera et al. 2006; Cardinale et al. 2006). However, some questions that still need to be further addressed to better understand how biodiversity modulates ecosystem functioning are (1) which diversity measure (e.g., gene, trait or species) better predicts ecosystem functioning (Hughes and Stachowicz 2004; Reusch et al. 2005; Gamfeldt and Källström 2007), (2) which are the mechanisms behind biodiversity effects (Loreau and Hector 2001; Fox 2005; Hector et al. 2009), and (3) how environmental change modulates the effects of diversity on ecosystem processes.

It has been assumed that positive effects of diversity on ecosystem functioning can be partitioned into two components: complementarity effects due to facilitative

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interactions between species or resource partitioning, and selection effects due to the greater probability that a diverse community includes dominant species with particular traits (Loreau and Hector 2001). However, species with particular traits can perform better than expected in multicultures either due to competitive replacement of other species or due to the expression of their traits without negative impacts to others (Fox 2005; Hector et al. 2009). Therefore, a tripartite model, including the “so-called” (1) dominance effect, when species with particular traits dominate at expense of others, (2) trait-dependent complementarity, when species with particular traits dominate in multicultures but not at the expense of others, and (3) trait-independent complementarity, when species performances in multicultures increase independently of their traits and not at the expense of others (equivalent to the complementarity defined by Loreau and Hector 2001), would best describe the mechanisms underpinning net biodiversity effects (Fox 2005).

Over the last few years, the use of microbial assemblages as model systems in ecology has been increasing mainly because microorganisms are ubiquitous, have short generation times, and are easy to manipulate under controlled conditions (Jessup et al. 2004). Moreover, they are key players in several environmental services, like nutrient recycling, water purification and carbon sequestration (Ducklow 2008); so, it becomes essential to understand how changes in microbial diversity can influence the underlying ecosystem processes (Reiss et al. 2010).

In freshwaters, microorganisms together with invertebrates are the biotic drivers of plant litter decomposition. Freshwater microbes are sensitive to environmental changes, and shifts in microbial assemblages on plant litter have been associated with alterations in the available resources (leaf litter quality, Marks et al. 2009; Kominoski et al. 2009; inorganic nutrients, Ferreira et al. 2006), temperature (Fernandes et al. 2009b) or water quality (Sridhar et al. 2001; Duarte et al. 2009). A polyphyletic group of fungi known as aquatic hyphomycetes are the major microbial decomposers of plant litter (Pascoal and Cássio 2004; Das et al. 2007); they produce a vast array of hydrolytic enzymes able to degrade plant cell-wall polysaccharides, and they act as intermediaries between plant litter and higher trophic levels (Bärlocher 2005).

The effects of aquatic fungal diversity on leaf litter decomposition have been described as positive or neutral, depending on the environmental context. A decrease in diversity of aquatic fungi in streams affected by changes in riparian vegetation (Bärlocher and Graça 2002; Lecerf et al. 2005) or moderate pollution (Raviraja et al. 1998; Pascoal et al. 2005a) was not accompanied by a reduction in leaf decomposition rates (Raviraja et al. 1998; Bärlocher

and Graça 2002; Lecerf et al. 2005, Pascoal et al. 2005a). However, in severely metal-polluted streams with impoverished aquatic hyphomycete assemblages (Sridhar et al. 2000), leaf decomposition proceeded at low rates (Sridhar et al. 2001). A positive relationship between aquatic fungal diversity and leaf decomposition has been observed in manipulative experiments (Bärlocher and Corkum 2003; Treton et al. 2004; Duarte et al. 2006; Raviraja et al. 2006; but see Dang et al. 2005). However, the positive diversity effects on leaf decomposition were less pronounced under metal stress (Pascoal et al. 2010), suggesting that traits that determine how a particular species affects ecosystem processes can differ from traits that determine how that species responds to an environmental stressor. Moreover, it is expected that performances might be determined by individual traits, which can vary within a species. Hence, a trait-based biodiversity measure should better reflect the potential for assemblages' performance than does species richness per se (Reiss et al. 2009).

Most studies examining the relationships between microbial diversity and ecological processes have been limited by difficulties in tracking individual species performances within assemblages (Bärlocher and Corkum 2003; Duarte et al. 2006). DNA-based approaches have the potential to overcome this problem (Pascoal et al. 2010), but they rely on polymerase chain reaction (PCR) and bias such as preferential amplification can occur (Kanagawa 2003). Real-time PCR is currently the most promising technique to circumvent this limitation because it can accurately quantify the amount of DNA in a sample (Kubista et al. 2006). Therefore, real-time PCR may be useful to determine the contribution of each species to the total assemblage performance (e.g., Kennedy et al. 2007) and to discriminate the mechanisms behind microbial diversity effects on ecosystem functioning.

In this study, we manipulated a pool of three widespread aquatic hyphomycete species including two functional types of a highly productive species, one from an unpolluted site and another from a metal-polluted site, to clarify (1) if lower richness of aquatic hyphomycetes reduces leaf decomposition, (2) if diversity effects are modulated by intraspecific traits, (3) the mechanisms behind diversity effects (complementarity and dominance), and (4) if diversity effects change under metal stress. We hypothesized that under metal stress intraspecific traits would change biodiversity effects on ecosystem functioning due to the expression of unique traits to cope with metal stress. If so, genetic diversity might be more important than species richness to maintain ecosystem functions under changing environmental conditions. The measured endpoints were leaf mass loss, and fungal production as ergosterol accumulation and DNA content (estimated by real-time PCR).

## Materials and methods

### Fungal species

We selected three widespread aquatic hyphomycete species in Portugal: *Flagellospora curta* J. Webster (UMB-39.01; FC39), *Tricladium splendens* Ingold (UMB-100.01; TS100) and two isolates of *Articulospora tetraccladia* Ingold (UMB-61.01, AT61; and UMB-72.01, AT72). The functional type AT72 was isolated from foam collected in the Maceira stream, an unpolluted stream at the Peneda-Gerês National Park (northwest Portugal). The other fungi were isolated from decomposing leaves collected in the Este River near the city of Braga (northwest Portugal), at a site impacted by metals including cadmium (Cd) (Soares et al. 1999; Pascoal et al. 2005a).

Pure cultures of aquatic hyphomycetes on 2% malt extract agar were prepared for microcosm inoculation.

### Microcosm setup

In October 2007, leaves of *Alnus glutinosa* (L.) Gaertn. (alder) were collected immediately before abscission and dried at room temperature. The leaves were leached in deionised water for 2 days, and cut into 12-mm-diameter disks. Sets of 25 disks were placed in 150-mL Erlenmeyer flasks and autoclaved (120°C, 20 min). To each flask, 70 mL of filtered (filter paper; Macherey–Nagel, Düren, Germany) and autoclaved stream water was added aseptically. Physical and chemical analysis of the stream water indicated circumneutral pH (6.34), low conductivity (40  $\mu\text{S cm}^{-1}$ , Multiline F/set 3 no. 400327; WTW, Weilheim, Germany), moderate concentrations of nitrate (0.3  $\text{mg L}^{-1}$   $\text{NO}_3^-$ -N, HACH kit, program 351) and phosphate (0.05  $\text{mg L}^{-1}$   $\text{PO}_4^{3-}$ -P, HACH kit, program 490), and low concentration of nitrite (0.002  $\text{mg L}^{-1}$   $\text{NO}_2^-$ -N, HACH kit, program 371) and ammonium (<0.01  $\text{mg L}^{-1}$   $\text{NH}_4^+$ -N, HACH kit, program 385).

Sterilized (Filtropur S, 0.2  $\mu\text{m}$ ; SARSTEDT, Rio de Mouro, Portugal) stock solution of Cd was added to 44 microcosms to a final concentration of 1.5  $\text{mg L}^{-1}$  Cd (chloride salt; Sigma). This Cd concentration led to a 50% inhibition of biomass produced by the most sensitive species among five aquatic hyphomycetes (Azevedo and Cássio 2010), collected from the same contaminated site as most fungi used in this study. In metal-polluted streams, Cd in the water column can reach 3.2  $\text{mg L}^{-1}$  (Sridhar et al. 2000), making the Cd concentration used in our study environmentally relevant. An additional 44 microcosms without added Cd were used as controls.

Microcosms were inoculated with agar plugs, collected from the edge of 19-day-old colonies of the four fungi, in monoculture or in all possible combinations (4 replicates

per treatment), never mixing the strains of the same species. Inoculation of single species microcosms was done with a 6-mm-diameter plug. For multiple-species microcosms, the total inoculum size was maintained and divided equally among species.

The microcosms were incubated for 35 days on a shaker (120 rpm; Certomat BS 3, Melsungen, Germany) at 16°C, under artificial light, and stream water was changed every 5 days. At the end of the experiment, leaf disks were used to determine leaf mass loss, fungal biomass and fungal DNA content.

### Fungal biomass

Fungal biomass on leaves was estimated from ergosterol concentration according to Gessner (2005). Briefly, lipids were extracted from sets of 6 freeze-dried leaf disks by heating (80°C, 30 min) in 8  $\text{g L}^{-1}$  of KOH–methanol. Ergosterol was purified by solid-phase extraction and quantified at 282 nm by high performance liquid chromatography (HPLC; Beckmann Gold System, Brea, CA, USA) on a LiChrospher RP18 column, using methanol as the mobile phase (33°C, 1.4  $\text{mL min}^{-1}$ ).

To convert ergosterol concentration into fungal biomass, specific conversion factors for each fungal isolate were determined by measuring ergosterol concentration in fungal mycelia along the exponential growth phase in liquid medium (1% malt extract, 140 rpm, 16°C). The ergosterol was extracted from ca. 5 mg of freeze-dried mycelia and quantified as above. The conversion factors were  $3.1 \pm 0.3$ ,  $3.4 \pm 0.3$ ,  $3.7 \pm 0.4$  and  $10.3 \pm 0.2$   $\mu\text{g ergosterol mg}^{-1}$  fungal dry mass for TS100, AT72, AT61 and FC39, respectively (values are mean  $\pm$  SEM, 5 sampling times, 3 replicates,  $n = 15$ ). Previous experiments with *A. tetraccladia* (AT61) and *F. curta* (FC39) showed that Cd exposure did not significantly affect ergosterol concentration (unpublished results).

### Fungal DNA quantification by real-time polymerase chain reaction

Real-time PCR uses fluorescent probes to monitor the DNA amplification allowing us to accurately quantify the amount of initial DNA template of each fungus in mixtures. To establish standard curves relating DNA amounts and Ct values (i.e., the number of cycles at which the fluorescence value exceeds the background fluorescence and crosses the threshold, Kubista et al. 2006) for each fungal isolate, DNA from pure cultures (ca. 5 mg of fungal mycelia grown in 1% malt extract during 20 days) was extracted using the UltraClean Soil DNA kit (MoBio Laboratories, Solana Beach, CA, USA). The ITS2 region of each fungal rDNA was amplified with the primer pair ITS3 and ITS4 (White

et al. 1990; Duarte et al. 2009). PCR products were purified with GenElute™ PCR Clean-Up kit (Sigma–Aldrich, Sintra, Portugal), according to the manufacturer instructions, and sequenced at the Centre of Molecular and Environmental Biology, University of Minho, Portugal (ABI PRISM® 310 Genetic Analyzer; Applied Biosystems, Foster City, CA, USA). DNA sequences were submitted to GenBank (<http://www.ncbi.nlm.nih.gov/Genbank/>) with the accession numbers: AT61, GU938614; AT72, GU938615; FC39, GU938616; and TS100, GU938617. Species-specific primer pairs were designed using the Primer Express 2.0 software (Applied Biosystems) (Online Resource Table S1).

For real-time PCR reactions, we mixed 1 µL of DNA sample, 5 µL of 2× LightCycler® 480 SYBR Green I Master mix (Roche), 250 nM of each species-specific primer and nuclease-free water in a final volume of 10 µL. DNA amplification started with initial denaturation at 95°C for 10 min, followed by 45 cycles of denaturation at 95°C for 10 s, primer hybridization at 60°C during 20 s and extension at 72°C during 15 s.

DNA concentration was measured with a NanoDrop® ND-1000 Spectrophotometer (Thermo Scientific). DNA samples from each fungus were diluted at tenfold intervals from 10 ng to 1 pg, and duplicates of all dilutions were done to establish linear regressions between DNA amounts and Ct values. The coefficient of determination of all fits was >0.99 (Online Resource Table S2). Real-time PCR efficiencies ranged from 1.76 to 1.98 for AT61 and TS100, respectively (Online Resource Table S2). Negative controls were performed by testing all specific primers against all isolates, and no fluorescence of non-target template was observed in single-species treatments (not shown).

To assess the DNA content of each fungal species in microcosms, real-time PCR of DNA extracted from four leaf disks (1 disk from each replicate, pooled from 4 replicates) was done as described above. In all samples, no fluorescence of non-target template was observed (negative controls). Real-time PCR was done in a LightCycler®480 System (Roche) at the Unidad de Genómica Toñi Martín Gallardo, Parque Científico de Madrid, Spain.

#### Leaf dry mass

Sets of 18 leaf disks from each replicate microcosm were freeze-dried to constant mass (72 ± 24 h) and weighed (±0.001 g). Sets of leaf disks before fungal inoculation were used to estimate initial leaf dry mass.

#### Data analyses

Three-way nested ANOVAs were used to test the effects of Cd, species number and species identity (nested in species

number) as fixed factors on leaf mass loss and fungal biomass (Duarte et al. 2006; Pascoal et al. 2010). Differences between treatments were analysed by a Tukey's post-test (Zar 1996).

Linear regressions between log DNA amount and Ct values were done (Online Resource Table S2), with Prism 4.0 for Windows (GraphPad, San Diego, CA, USA). The Ct values of each fungal isolate obtained by real-time PCR were converted to DNA and were used to calculate the percentage of each isolate in multicultures as follows:

$$P_i = \frac{DNA_i}{\sum_{i=1}^N DNA_i},$$

where  $P_i$  is the percentage of species  $i$  in multicultures,  $N$  is the number of species and  $DNA_i$  is the DNA amount of species  $i$  assessed by real-time PCR. Ergosterol was converted to fungal biomass as:

$$B_t = \frac{E_t}{\sum_{i=1}^N (P_i \times F_i)},$$

where  $B_t$  is the total fungal biomass,  $E_t$  is the total ergosterol content,  $P_i$  is the percentage of species  $i$  in multiculture containing  $N$  species, and  $F_i$  is the ergosterol-to-biomass conversion factor of species  $i$ . Because leaves were the only carbon and energy source available for fungi, species contribution to leaf mass loss was assumed to be proportional to the biomass produced by individual fungal species in mixtures.

To discriminate the mechanisms behind net biodiversity effects, the tripartite partition model (Fox 2005) was applied to fungal biomass and leaf mass loss, as follows:

$$\Delta Y = NE(M)E(\Delta RY) + N\text{cov}\left(M, \frac{RY_O}{RYT_O} - RY_E\right) + N\text{cov}\left(M, RY_O - \frac{RY_O}{RYT_O}\right),$$

where the first term is the contribution of trait-independent complementarity, the second term is the contribution of dominance, and the final term is the contribution of trait-dependent complementarity to the net biodiversity effects ( $\Delta Y$ ). In the equation,  $N$  is the number of species in the mixture,  $E$  is unweighted arithmetic mean across all  $N$  species,  $M$  is the monoculture yield of each species, and  $\Delta RY$  is the difference between the observed ( $RY_O$ ) and expected ( $RY_E$ ) relative yields of species in the mixture.  $RY_O$  of a species is its yield in mixture divided by its yield in monoculture.  $RY_E$  of a species is the initial proportion of a species in the mixture.  $RYT_O$  is the sum of  $RY_O$  for all species in the mixture. Net biodiversity effects were square-root transformed with original signs preserved to

improve normality (Loreau and Hector 2001; Fox 2005), and tested against the null hypothesis that the values equalled 0 ( $t$  test) (Pascoal et al. 2010). The statistical analyses were performed with Statistica 6.0 for Windows (Statsoft, Tulsa, OK, USA).

## Results

### Leaf decomposition and fungal biomass on leaves

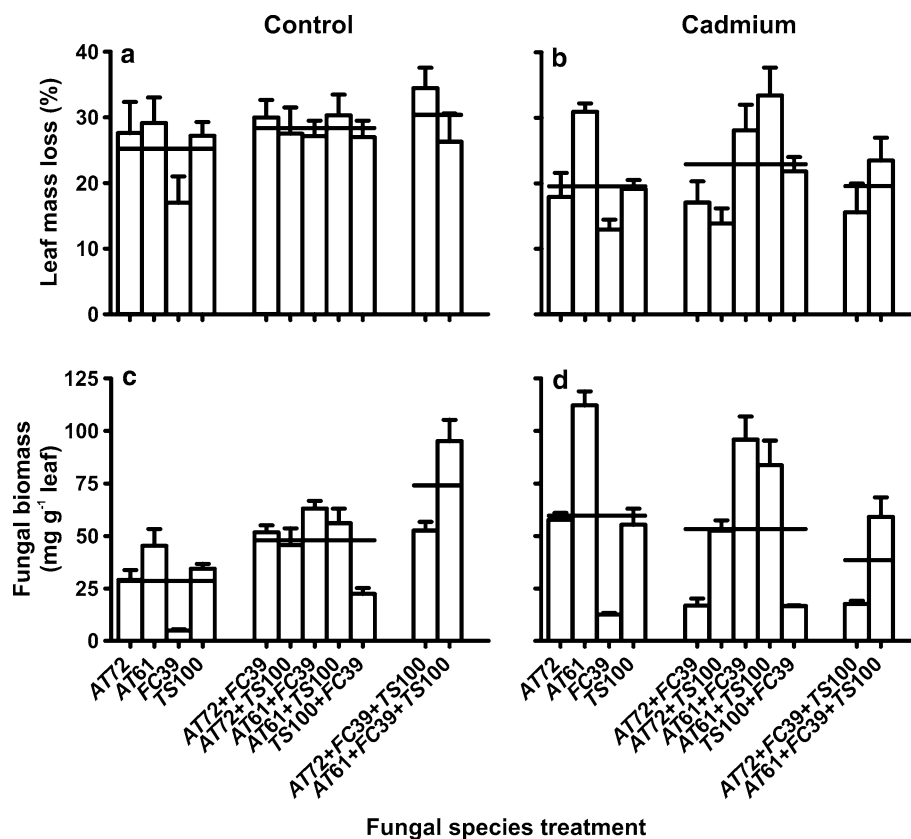
Cadmium affected leaf decomposition by fungi differently depending on the functional type present in the assemblage (Fig. 1a, b). In microcosms without Cd, the average leaf mass loss varied between 17% in the monoculture of *Flagellospora curta* (FC39) and 34% in multicultures with three species containing *Articulospora tetracladia* isolated from the unpolluted site (AT72) (Fig. 1a). Cadmium, species identity, and interaction between Cd and species identity significantly affected leaf mass loss (three-way nested ANOVA,  $P < 0.05$ ; Table 1). Leaf mass loss decreased after Cd addition in multicultures with three species containing AT72 (Tukey's test,  $P < 0.05$ ; Fig. 1b), but not in mixtures containing the strain of *A. tetracladia* isolated at the polluted site (AT61) (Tukey's test,  $P > 0.05$ ).

Fungal biomass increased with increasing species number ( $y = 22.2x + 5.2$ ,  $r^2 = 0.42$ ;  $P < 0.0001$ ), except in the presence of Cd (Fig. 1c, d). Without Cd addition, fungal biomass on leaves varied between 5 mg g<sup>-1</sup> leaf dry mass in monoculture of FC39 and 95 mg g<sup>-1</sup> leaf dry mass in the multicultures of three species containing AT61 (Fig. 1c). Species identity, species number, and interactions between Cd and the other factors significantly affected fungal biomass (three-way nested ANOVA,  $P < 0.05$ ; Table 1). Fungal biomass increased with Cd addition in monoculture containing AT61, and decreased in (1) duoculture containing AT72 and FC39 and (2) multiculture of three species containing AT72 or AT61 (Tukey's test,  $P < 0.05$  for all cases; Fig. 1d). The highest fungal biomasses corresponded to treatments with the strain AT61.

### Fungal species dominance on leaves

The pattern of fungal species dominance on leaves changed in the presence of Cd (Fig. 2). Without Cd addition, *A. tetracladia* showed a clear dominance (>85% for both AT72 and AT61) in all multicultures containing this species (Fig. 2a–d, f, g). Cadmium exposure decreased at least 60% the contribution of AT72 in multicultures with either two or three species (Fig. 2a, c, f), while it increased the

**Fig. 1 a, b** Leaf mass loss and **c, d** fungal biomass on decomposing alder leaves after 35 days in microcosms, **a, c** non-exposed or **b, d** exposed to 1.5 mg L<sup>-1</sup> cadmium (mean + SEM,  $n = 4$ ). Horizontal lines indicate average values in treatments containing one, two or three fungal species. *Articulospora tetracladia* UMB-72.01 (AT72), *A. tetracladia* UMB-61.01 (AT61), *Flagellospora curta* UMB-39.01 (FC39), and *Tricladium splendens* UMB-100.01 (TS100). Ergosterol concentration was converted into fungal biomass using specific ergosterol-to-biomass conversion factors for each isolate and taking into account the percentage contribution of each species in mixtures, assessed by real-time PCR (see “Materials and methods”)



**Table 1** ANOVAs of the effects of cadmium (Cd), species number and species identity on leaf mass loss and fungal biomass

	Effect	df	SS	MS	F	P
Leaf mass loss	Identity (species number)	8	1,428.2	178.5	4.1	<0.01
	Species number	2	149.3	74.6	1.7	0.19
	Cd	1	951.1	951.1	22.0	<0.01
	Identity (species number) × Cd	8	874.1	109.3	2.5	0.02
	Species number × Cd	2	101.8	50.9	1.2	0.31
	Error	65	2,807.7	43.2		
Fungal biomass	Identity (species number)	8	46,165.4	5,770.7	37.5	<0.01
	Species number	2	1,714.4	857.2	5.6	<0.01
	Cd	1	120.2	120.2	0.8	0.38
	Identity (species number) × Cd	8	9,878.9	1,234.9	8.0	<0.01
	Species number × Cd	2	11,883.4	5,941.7	38.6	<0.01
	Error	66	10,162.0	154.0		

contribution of *FC39* to more than 70% (Fig. 2a, e, f). The strain *AT61* maintained its contribution above 90% in multicultures exposed or not to Cd (Fig. 2b, d, g). In the absence of Cd, *TS100* and *FC39* had similar contributions to the total fungal DNA in duoculture, but the exposure to Cd increased the contribution of *FC39* to more than 90% (Fig. 2e).

#### Partitioning biodiversity effects on leaf decomposition and fungal biomass

Net biodiversity effects and the underlying mechanisms changed under Cd exposure (Fig. 3). Without Cd addition, net biodiversity effects on leaf mass loss were positive for assemblages containing *A. tetracladia* from the unpolluted (*AT72*) or the polluted site (*AT61*) (*t* tests,  $P < 0.05$ ; Fig. 3a). These effects were mainly a result of trait-independent complementarity for assemblages with *AT72* and were a result of both trait-independent complementarity and dominance effects for assemblages with *AT61*. The positive net biodiversity effects due to trait-independent complementarity and dominance were kept under Cd exposure in assemblages with *AT61* (*t* test,  $P = 0.02$ ), but no significant net biodiversity effects were found in assemblages with *AT72* (*t* test,  $P = 0.79$ ; Fig. 3b).

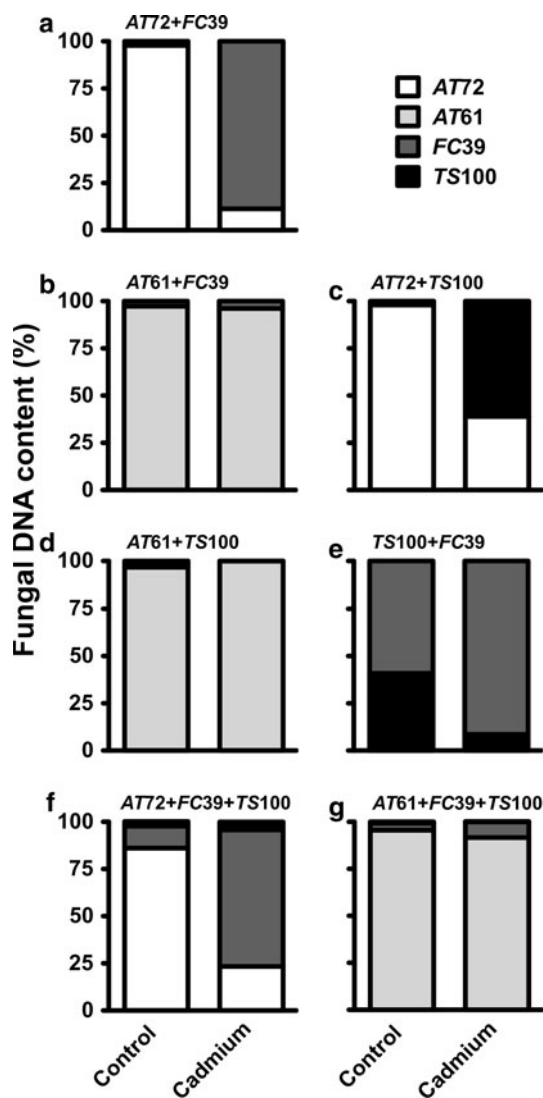
Net biodiversity effects on fungal biomass were also positive for assemblages non-exposed to Cd containing *AT72* or *AT61* (*t* tests,  $P < 0.05$ ) due to trait-independent complementarity between species (Fig. 3c). The exposure to Cd led to negative net biodiversity effects for assemblages containing *AT72* (*t* test,  $P = 0.01$ ) mainly as a result of dominance effects, and no significant net biodiversity effects were found in assemblages containing *AT61* (*t* test,  $P = 0.95$ ; Fig. 3d).

#### Discussion

Our study clearly shows that intraspecific traits altered biodiversity effects under metal stress: the positive effects of fungal diversity on leaf decomposition and fungal biomass production were kept under Cd stress only when the assemblage had a Cd-resistant functional type. Positive relationships between aquatic hyphomycete diversity and leaf decomposition (Bärlocher and Corkum 2003; Treton et al. 2004; Duarte et al. 2006; Raviraja et al. 2006; Pascoal et al. 2010) or fungal biomass production (Duarte et al. 2006; Raviraja et al. 2006; Pascoal et al. 2010) have been reported, but these relationships are likely to change under stress (Pascoal et al. 2010).

In this study, fungal diversity effects appeared to be stronger on fungal biomass production than on leaf decomposition as found by others (Duarte et al. 2006; Pascoal et al. 2010). If increased diversity had favored higher growth efficiencies [i.e., production/(production + respiration)], this mechanism would explain the less pronounced diversity effects on leaf decomposition. However, Cd appeared to decrease growth efficiency of the assemblages because fungal biomass decreased with the increase of species number, particularly in multicultures with three species. It is conceivable that growth efficiency and resource depletion may vary in different assemblages or under different environmental conditions, supporting the need of considering several response variables when examining biodiversity effects (Reiss et al. 2009).

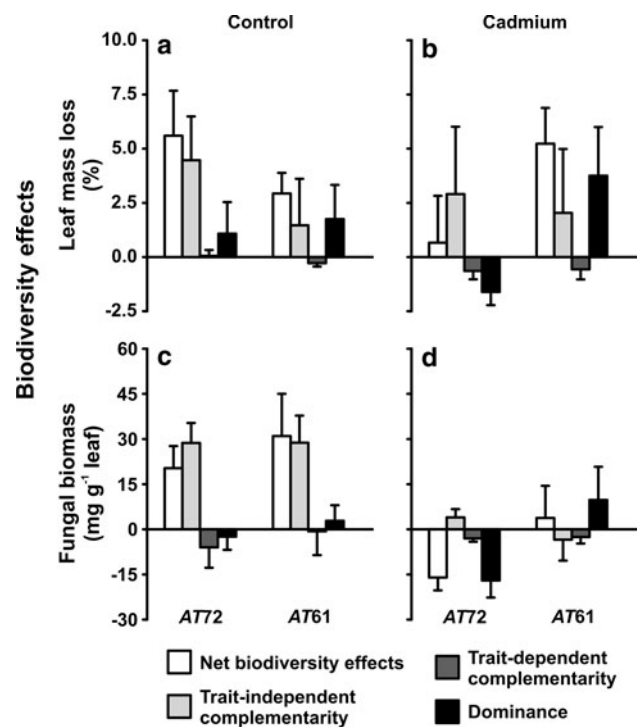
We used real-time PCR to track each species within fungal assemblages; this allowed us to discriminate the contribution of trait-independent complementarity, trait-dependent complementarity and dominance to net biodiversity effects (Fox 2005) in microbial assemblages. In the absence of Cd, trait-independent complementarity



**Fig. 2** Percentage contribution of each fungal isolate to the total DNA content in multicultures with **a–e** two and **f, g** three fungal species in control and cadmium-exposed microcosms, assessed by real-time PCR. At each treatment, DNA was extracted from four pooled replicates. Fungal species abbreviations as in Fig. 1

explained most of the positive net biodiversity effects, suggesting that increased leaf mass loss and fungal production resulted from the better performance of all fungi in mixtures rather than the supremacy of a dominant species. This finding agrees with the evidence that aquatic hyphomycete species preferentially colonize different patches of leaves (Bermingham et al. 1996). Moreover, different species have variable patterns of exoenzymes active against a range of plant polymers (Suberkropp et al. 1983), probably leading to a better resource use in multicultures.

Net biodiversity effects changed under Cd exposure with shifts from trait-independent complementarity to dominance effects. The exposure to Cd led to a decrease of net biodiversity effects on leaf decomposition (no



**Fig. 3** Mechanisms contributing to the net biodiversity effects on **a, b** leaf mass loss and **c, d** fungal biomass in **a, c** control or **b, d** cadmium-exposed microcosms. Data presented are average values for all multicultures containing the functional type *AT72* or *AT61*, isolated from the unpolluted and polluted stream, respectively; the contribution of each fungal species was estimated from the relative DNA content of each fungus in multicultures (Fig. 2). Trait-independent complementarity, trait-dependent complementarity and dominance effects were estimated according to the tripartite partition model (Fox 2005). Fungal species abbreviations as in Fig. 1

significant effects) and fungal production (negative effects) for assemblages containing *A. tetracladia* isolated from the unpolluted site (*AT72*). Negative dominance effects caused this result, which can be interpreted as the most productive species in monoculture performing worse in multicultures, and suggests competitive interactions among fungal species under Cd stress. Also, assemblages with *A. tetracladia* from the polluted site (*AT61*) showed a decrease in net biodiversity effects (lack of significant effect) on biomass production, but less pronounced than those found in assemblages with *AT72*. Interestingly, the exposure to Cd of assemblages containing *A. tetracladia* from the metal-polluted site (*AT61*) led to positive net biodiversity effects on leaf decomposition. This finding suggests that *AT61* functional type has specific traits that confer on it not only high decomposer ability but also high tolerance to Cd. According to the insurance hypothesis, environmental fluctuations may bring out species traits that are redundant under constant conditions; but under stress, those traits may become important helping to maintain processes even if others fail (Yachi and Loreau 1999). Even though this

hypothesis has been proposed based on species diversity, our study provides evidence that intraspecific traits should be incorporated when examining biodiversity effects. Indeed, if only the Cd-sensitive functional type had been examined in this study, we would have failed to detect positive net biodiversity effects on leaf litter decomposition under metal stress.

Our study highlights the importance of environmental context to explain the variability of results when examining biodiversity–ecosystem functioning relationships as found previously by others (Mckie et al. 2009; Pascoal et al. 2010). Manipulating 1–4 aquatic hyphomycete species, Pascoal et al. (2010) showed that the exposure to zinc attenuated the positive effects of species richness on leaf decomposition. Also, the stress imposed by a cold perturbation on algal assemblages weakened the positive diversity–biomass relationship, because assemblages with more species had greater biomass reduction than the ones with fewer species (Zhang and Zhang 2006). In contrast, the exposure to stressors (e.g., drought, Mulder et al. 2001; abiotic stress due to geographic elevation, Callaway et al. 2002) led to an increase of positive interactions between plant species within assemblages.

We show that intraspecific traits, more than species number or identity, were important in maintaining processes under metal stress. Therefore, the incorporation of trait-based diversity measures into biodiversity and ecosystem functioning research may help to reconcile some apparent contradictory results on the functional role of biodiversity. Some studies have emphasized the positive effects of genetic diversity on ecosystem resistance to disturbance (Hughes and Stachowicz 2004) or resilience after climatic extremes (Reusch et al. 2005), and on the predictability in amphipod population survival in face of perturbations (Gamfeldt and Källström 2007). As recently reviewed by Hughes et al. (2008), genetic diversity has significant effects on different ecological processes with consequences at the population, community and ecosystem levels, being in some cases comparable in magnitude to the effects of species diversity. For microbial assemblages, it might not be imperative to conserve specific species, but rather to conserve specific metabolic pathways, which may be the result of millions of years of evolution and might be lost due to chance conditions (Falkowski et al. 2008).

In conclusion, fungal performances (biomass production and litter decomposition) were generally higher in multi-cultures than those expected from the weighted sum of monocultures due to complementarity between species. However, under metal stress, diversity effects on leaf decomposition decreased in assemblages containing the Cd-sensitive functional type, but were maintained in assemblages containing the Cd-resistant functional type

with evidence of dominance effects. This finding means that environmental changes and the presence or absence of certain traits altered diversity effects and the underlying mechanisms (from complementarity to dominance). Although the number of species tested in this study (three species) corresponds to the maximum number of dominant species commonly found on decomposing leaves in the field (Pascoal et al. 2005b; Duarte et al. 2009; Fernandes et al. 2009a), higher diversity is generally found in streams (e.g., 8–28 species; Duarte et al. 2009). Thus, we must exercise caution when generalizing our conclusions to the real environment. Even so, our study provides a proof of concept that the presence of intraspecific traits can be more important than species richness to maintain ecosystem functioning under changing environmental conditions. Therefore, we encourage further research on the functional role of intraspecific diversity using higher number of species and functional types to mirror the complexity of assemblages and events in nature.

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