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MICROBIOLOGY OF AQUATIC SYSTEMS

Combined Effects of Dissolved Nutrients and Oxygen on Plant Litter Decomposition and Associated Fungal Communities

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Abstract Aquatic ecosystems worldwide have been substantially altered by human activities, which often induce changes in multiple factors that can interact to produce complex effects. Here, we evaluated the combined effects of dissolved nutrients (nitrogen [N] and phosphorus [P]; three levels: concentration found in oligotrophic streams in the Cerrado biome, 10× and 100× enriched) and oxygen (O_2; three levels: hypoxic [4% O_2], depleted [55% O_2], and saturated [96% O_2]) on plant litter decomposition and associated fungal decomposers in laboratory microcosms simulating stream conditions under distinct scenarios of water quality deterioration. Senescent leaves of *Maprounea guianensis* were incubated for 10 days in an oligotrophic Cerrado stream to allow microbial colonization and subsequently incubated in microcosms for 21 days. Leaves lost 1.1–3.0% of their initial mass after 21 days, and this was not affected either by nutrients or oxygen levels. When considering simultaneous changes in nutrients and oxygen concentrations, simulating increased human pressure, fungal biomass accumulation, and sporulation rates were generally inhibited. Aquatic hyphomycete community structure was also affected by changes in nutrients and oxygen availability, with stronger effects found in hypoxic treatments than in depleted or saturated oxygen treatments. This study showed that the effects of simultaneous changes in the availability of dissolved nutrients and oxygen in aquatic environments can influence the activity and composition of fungal communities, although these effects were not translated into changes in litter decomposition rates.

Keywords Anthropogenic stress · Aquatic hyphomycetes · Headwater streams · Laboratory microcosms · Multiple stressors

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Introduction

Anthropogenic activities have substantially altered aquatic ecosystems worldwide [1, 2]. Among anthropogenic-induced impacts, eutrophication (i.e., increase in nutrient availability) is frequently found to affect aquatic ecosystems [3]. The concentrations of dissolved nutrients (especially nitrogen [N] and phosphorus [P]) in inland waters tend to increase with the use of the adjacent areas for agricultural practices and as a result of wastewater discharges [4, 5], and may be accompanied by concomitant changes in other environmental variables [6, 7]. The nutrient enrichment of fresh waters affects the composition, structure, stability, and productivity of food webs, leading to changes in ecosystem functioning (e.g., nutrient cycling and energy flow) [8, 9]. However, the response of aquatic communities and processes to changes in multiple stressors is less studied and more difficult to predict as multiple stressors often have contrasting individual effects.

Aquatic communities in small streams with closed canopy derive most of their energy and carbon from litter of terrestrial origin [10]. The decomposition of this litter is primarily mediated by microbes, especially aquatic

hyphomycetes [11, 12]. These fungi mineralize organic carbon and nutrients, convert them into biomass (somatic growth and reproductive structures) and promote the release of fine litter particles, causing litter mass loss [11–14]. The degradation of leaf tissue by enzymatic maceration and the accumulation of fungal biomass increase the nutritional value and the palatability of litter for invertebrate detritivores that promote further litter decomposition [15]. Microorganisms can obtain nutrients from both the organic substrate and the water column [11, 16]. Thus, increases in nutrients loads from the catchment can stimulate microbial activity and leaf decomposition [17–19], and also induce changes in the structure of fungal communities [16, 17, 20].

The potential stimulatory effects of nutrient enrichment on microbial activity may, nevertheless, be offset or even counteracted if there are concomitant changes in other environmental variables with potentially inhibitory effects on microbial activity, such as reductions in dissolved oxygen concentration [7]. Decreases in oxygen concentration in a laboratory experiment negatively affected fungal biomass, conidial production, and species richness of aquatic hyphomycetes associated with decomposing litter [21]. Fungal biomass decreased with decreases in oxygen concentration along a pollution gradient in northwestern Portugal [7]. Similarly, a laboratory experiment showed that in eutrophic environments the microbial degradation of leaf litter was slower under low oxygen conditions [22].

The combined effects of concomitant increases in nutrient concentrations and decreases in oxygen concentration in stream water, as expected from increases in anthropogenic pressures, on microbial activity, and litter decomposition are difficult to anticipate as these potentially have opposing individual effects. Here, we evaluated the combined effects of changes in dissolved nutrients and oxygen concentrations (three levels each) on plant litter decomposition and associated microbial decomposer (fungal biomass, reproductive activity and composition of aquatic hyphomycetes communities) in laboratory microcosms simulating stream conditions under distinct scenarios of water quality deterioration. We predicted a stimulation of fungal biomass, conidial production, and hence higher litter decomposition at higher nutrients and oxygen concentrations when considered individually [14, 21, 23]. In scenarios of water quality degradation (i.e., increased concentration of nutrients and decreased concentration of oxygen) the response of decomposers is less predictable, since increases in nutrient concentrations alone generally stimulate while decreases in oxygen concentrations alone generally inhibit microbial activity and litter decomposition. We also predict changes in aquatic hyphomycetes communities with changes in nutrients and oxygen levels.

Methods

Collection and Conditioning of Leaves

We collected senescent leaves of *Maprounea guianensis* Aubl., a tree species frequent in the riparian vegetation of Cerrado streams [24], with nets (1 m^2, 10 mm mesh) placed in the riparian zone of Cabeça de Veado stream, Botanical Garden, Brasilia, Brazil (15° 53′ 11.74″ S, 47° 50′ 33.27″ W; 1056 m a.s.l.). Leaves were air-dried, enclosed in mesh bags (20 × 20 cm, 0.5 mm mesh; ~ 1.5 g of leaves bag^{-1}) and incubated in a second-order reach of Cabeça de Veado stream for 10 days in January 2014, to allow microbial conditioning [25]. During the incubation period, stream water was 21.3 ± 0.2 °C (mean ± SD, n = 2), slightly acidic (pH 6.3 ± 0.5), well oxygenated (10.5 ± 0.7 mg O_2 L^{-1}) and had low conductivity (4.9 ± 0.4 μS cm^{-1}). Water nutrient concentrations were previously analyzed by Fonseca and Mendonça-Galvão [26] between September 2011–March 2012 and averaged 0.027 mg nitrate L^{-1}, 0.035 mg total nitrogen L^{-1}, and 0.008 mg soluble reactive phosphorus L^{-1}. After microbial conditioning, we transported the leaves to the laboratory, rinsed them with distilled water and cut leaf discs with a cork borer (12 mm diameter) avoiding main veins.

Experimental Setup

We assessed the individual and combined effects of variations in the concentration of dissolved nutrients and oxygen (three levels each), in a full factorial design, on leaf litter decomposition, fungal biomass and reproductive activity of aquatic hyphomycetes in laboratory microcosms. The microcosms were adapted from Medeiros et al. [21] and consisted of 250-mL flasks containing 50 mL of nutrient solution according to the following treatments: nutrient concentrations similar to those found in oligotrophic streams in the Cerrado biome (ambientNP, 1 mg $NaNO_3$ and 0.066 mg K_2HPO_4 per liter of distilled water; 0.165 mg N L^{-1} and 0.012 mg P L^{-1}), nutrient concentrations 10× higher (mediumNP, 10 mg $NaNO_3$ and 0.66 mg K_2HPO_4; 1.65 mg N L^{-1} and 0.12 mg P L^{-1}) and nutrient concentrations 100× higher (highNP, 100 mg $NaNO_3$ and 6.6 mg K_2HPO_4; 16.5 mg N L^{-1} and 1.2 mg P L^{-1}). We maintained the Redfield ratio in all treatments. Although the NO_3-N concentration in the ambientNP microcosms is much higher (ca. 6 times) than that reported for the stream where the bags were incubated for microbial leaf conditioning, it is similar to dissolved inorganic N (DIN) concentration found in non-impacted streams in the Cerrado (average DIN for 60 reaches within 7 non-impacted streams = 0.219 mg L^{-1}; [27]). We supplemented all treatments with 75.5 mg $CaCl_2$ and 10 mg $MgSO_4.7H_2O$ per liter of nutrient solution to supply microcosms with salts generally found in stream water (in addition to Na and K already present in $NaNO_3$ and K_2HPO_4;

nutrient solutions were adapted from Ferreira and Chauvet [14] and Dang et al. [28]. We controlled the levels of dissolved oxygen (hypoxic, 4%; depleted, 55%; saturated 96%) in the microcosms by adding nitrogen (N_2) gas and air using plastic tubes inserted through the lid of the microcosms and connected respectively to a N_2 bottle (White Martins Gases Industriais Ltda, São Paulo, Brazil) and to an aquarium pump, which promoted turbulence and kept discs in constant motion (Fig. 1a). Valves placed on each microcosm individually controlled the diffusion rate of both gases (Fig. 1b). We measured the dissolved oxygen concentration twice a day in each microcosm using an oximeter (JENWAY–970, Gransmore Green, England) and adjusted the diffusion rates when necessary. The selected nutrients and oxygen levels intended to simulate different scenarios, e.g., a reference condition typical of non-impacted oligotrophic streams of the Cerrado biome (ambientNP—saturated), a moderate impact (e.g., mediumNP—depleted), and a strong impact (highNP—hypoxic) resulting from increased human pressure.

We randomly distributed sets of 10 leaf discs into 36 microcosms (3 nutrient levels × 3 dissolved oxygen levels × 4 replicates) and incubated them at 20 °C for 21 days. We replaced the nutrient solutions of each microcosm every 3 days and the conidial suspensions were preserved with formalin (4%) for subsequent identification of aquatic hyphomycetes conidia (see below).

Litter Decomposition

At the end of the experiment (day 21), we used five randomly chosen discs of each microcosm to determine the remaining dry mass (DM) after drying at 60 °C for 72 h. We then ignited DM at 550 °C for 4 h for determination of ash mass. We estimated remaining ash-free dry mass (AFDM) as DM–ash mass. We estimated initial AFDM from a set of five discs removed from the leaves prior to the incubation in the microcosms. We expressed litter decomposition as the relative litter mass loss (LML), LML = (initial AFDM − final AFDM) initial AFDM.

Fungal Biomass

We froze (− 20 °C) the other set of five discs until ergosterol extraction, used as a measure of fungal biomass [29]. We immersed the leaf discs in 8 mg KOH L^{-1} methanol and extracted the lipids at 80 °C for 30 min followed by purification

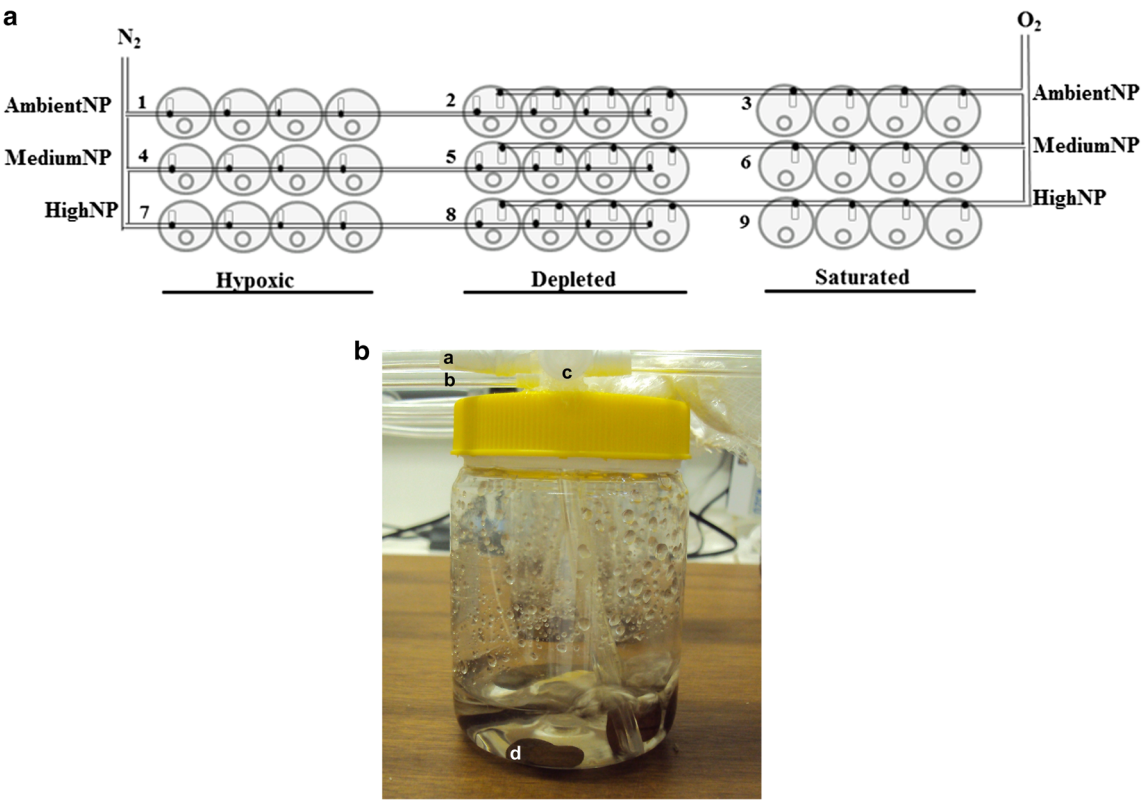

Fig. 1 Experimental setup (**a**) with the microcosms being supplied with O_2 and N_2 to maintain distinct oxygen concentrations 4% O_2 (hypoxic), 55% O_2 (depleted), and 96% O_2 (saturated). Microcosms with nutrient concentrations representative of those found in oligotrophic streams in the Cerrado Biome (ambientNP) are included on lines 1–3; those with nutrient concentrations 10× higher (mediumNP) are included on lines 4–6; and those with nutrient concentrations 100× higher (highNP) are included on lines 7–9. Details of one microcosm (**b**) are N_2 input (a), O_2 input (b), valve (c), and discs (d)

of the crude extract by solid-phase extraction (Vac RC tC18 cartridges, 500 mg; Waters Sep-Pak®, Waters Corp., Milford, MA, USA) using a vacuum system. We eluted ergosterol with isopropanol and quantified it by high-performance liquid chromatography (HPLC, Dionex ICS Series PDA, Sunnyvale, CA, USA; detection wavelength, 282 nm; flow rate, 1.5 mL s^{-1}; column temperature, 33 °C; injection volume, 20 μL). We expressed the results as microgram ergosterol per gram AFDM.

Conidia Production by Aquatic Hyphomycetes

We filtered aliquots of conidial suspension through 5-μm pore-size membranes (Millipore Corporation, Bedford, MA, USA). We stained the filters with 0.1% lactophenol cotton blue (NewPROV, Pinhais, Brazil) and examined them under an optical microscope at 400× (Metrimpex, Labimex Modelo Studarlab®, Budapest, Hungary) for the identification and counting of aquatic hyphomycetes conidia. We expressed species richness as the number of species per sample and sporulation rates on day 21 as the number of conidia released per milligram litter AFDM per day (no. conidia mg^{-1} AFDM day^{-1}; [30]).

Data Analysis

We used linear models to test the effects of nutrients and oxygen levels (both categorical factors) and their interaction on leaf mass loss, fungal biomass, and sporulation rate, and species richness of aquatic hyphomycetes. Multi-panel boxplots for each response variable versus nutrients and oxygen levels showed similar variance among treatments for all variables but sporulation rates and species richness. To take this difference into account we used a particular variance structure ("VarIdent" function of "nlme" R package; [31]) for both sporulation rates and species richness models, which allowed residual spread to vary in relation to nutrients and oxygen levels. As species richness was examined on multiple sampling dates, we used an auto-regressive model of order 1 (function "corAR1" of "nlme" R package) to consider temporal autocorrelation between subsequent measures [32]. The optimal variance structure and correlation component were defined by model comparison using Akaike's information criterion. We fitted linear models using the functions "lm" or "gls" (generalized least squares and restricted maximum likelihood method in the case of species richness) of "base" and "lme" packages, respectively, in R software (version 3.2.2; [33]). We addressed pairwise multiple comparisons with Tukey tests using "glht" function of the "multcomp" R package [34].

Aquatic hyphomycetes communities were ordinated by nonmetric multidimensional scaling (NMDS) based on a Bray-Curtis similarity matrix of transformed ($\log[x + 1]$) conidial production data. We compared the aquatic hyphomycetes communities among nutrient and oxygen levels using an analysis of similarity (ANOSIM) (Primer 6 v6.1.11 and Permanova + v1.0.1; Primer-E Ltd., Plymouth, UK; [35]).

Results

Litter Decomposition

Litter mass loss varied between 1.1–3.0% after 21 days of incubation and was not affected either by nutrient or oxygen levels (Table 1).

Table 1 Results of linear models to test the effect of nutrient (three levels: ambientNP, mediumNP, and highNP) and oxygen levels (three levels: hypoxic, depleted, and saturated) on litter mass loss, fungal biomass, and sporulation rate and species richness of aquatic hyphomycetes. Note that litter mass loss, fungal biomass, and sporulation rate were assessed at the completion of the microcosm experiment (after 21 days of incubation), while species richness was examined on multiple sampling dates (seven sampling dates corresponding to the microcosm medium change)

Source of variation	df	MS	*F*	*p*
Litter mass loss				
Intercept	1		265206.3	< 0.001
Nutrients	2	1.783	1.4	0.270
Oxygen	2	1.041	0.8	0.458
Nutrients × oxygen	4	1.451	1.1	0.367
Residuals	27	1.295		
Fungal biomass				
Intercept	1		212.6	< 0.001
Nutrients	2	85387	7.9	0.002
Oxygen	2	31237	2.9	0.073
Nutrients × Oxygen	4	35442	3.3	0.026
Residuals	27	10825		
Sporulation rate				
Intercept	1	–	17.0	< 0.001
Nutrients	2	–	3.7	0.039
Oxygen	2	–	27.6	< 0.001
Nutrients × oxygen	4	–	9.2	< 0.001
Residuals	27	–		
Species richness				
Intercept	1	–	1819.6	< 0.001
Nutrients	2	–	162.0	< 0.001
Oxygen	2	–	7.9	< 0.001
Nutrients × oxygen	4	–	0.3	0.877
Residuals	242	–		

Fungal Biomass

Fungal biomass at day 21 varied across treatments (153–478 μg ergosterol g^{-1} AFDM; Fig. 2a). The interaction between nutrients and oxygen levels influenced fungal biomass on litter (Table 1): in hypoxic and saturated oxygen conditions, fungal biomass did not significantly differ between nutrient concentrations (Tukey test, $p > 0.611$), while in depleted oxygen conditions, fungal biomass was significantly higher at high than at ambient nutrient concentrations (Tukey test, $p = 0.025$). When considering simultaneous changes in nutrients and oxygen concentrations simulating increased human pressure, fungal biomass accumulation was generally inhibited (Fig. 2a).

Conidia Production by Aquatic Hyphomycetes

Sporulation rates at day 21 varied between 3 and 337 conidia mg^{-1}AFDM day^{-1} across treatments (Fig. 2b). There was a significant interaction effect of nutrient and oxygen concentrations on sporulation rates (Table 1): in saturated oxygen conditions, sporulation rates were higher at ambient and medium than at high nutrient concentrations (Tukey test, $p < 0.008$), but were similar between ambient and medium nutrient concentrations (Tukey test, $p = 0.450$), while in hypoxic and depleted oxygen conditions, sporulation rates did not significantly differ among nutrient concentrations (Tukey test, $p > 0.232$). When considering scenarios simulating increased human pressure, sporulation rates were generally inhibited (Fig. 2b).

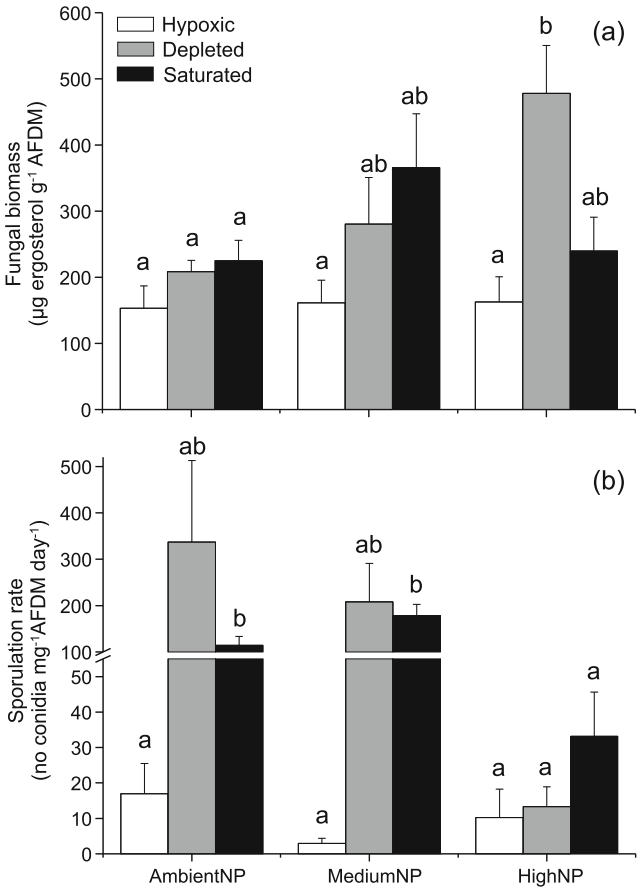

Fig. 2 Fungal biomass (**a**) and sporulation rate by aquatic hyphomycetes (**b**) after 21 days of litter incubation in laboratory microcosms at three nutrients (ambientNP, mediumNP, and highNP) and three oxygen levels (hypoxic, depleted, and saturated). Values are means ± 1SE ($n = 4$). Different letters indicate statistically significant differences ($p < 0.05$)

Aquatic Hyphomycetes Communities

Six species of aquatic hyphomycetes were identified on decomposing litter: *Anguillospora filiformis* Greath., *Culicidospora gravida* Petersen, *Heliscus submersus* Hudson, *Lunulospora curvula* Ingold, *Mycocentrospora acerina* (Hartig) Deighton, and *Tricladium chaetocladium* Ingold (Fig. 3). *A. filiformis* was the dominant species in all treatments (relative abundance, 78% in highNP—depleted to 98% in highNP–hypoxic). Species richness of aquatic hyphomycetes was reduced under hypoxic conditions (mean of 1.5 species vs. 3.1 under depleted and saturated oxygen conditions) and at high nutrient concentrations (mean of 2.2 species vs. 2.8 at ambientNP and mediumNP) (Table 1; Tukey test, $p < 0.004$ for all significant comparisons).

Aquatic hyphomycetes communities differed among nutrient and oxygen concentrations (Fig. 4, Table 2). Changes in oxygen concentration significantly affected communities'

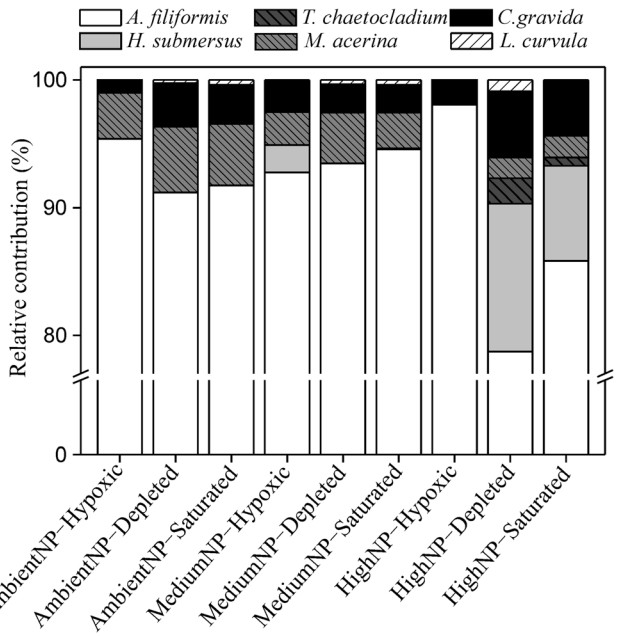

Fig. 3 Relative contribution of aquatic hyphomycetes species to the total conidial production after 21 days of litter incubation in laboratory microcosms at three nutrients (ambientNP, mediumNP, and highNP) and three oxygen levels (hypoxic, depleted, and saturated)

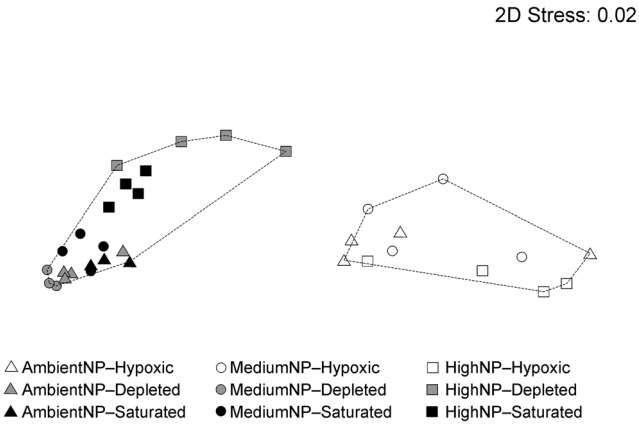

Fig. 4 Nonmetric multidimensional scaling (NMDS) ordination of aquatic hyphomycete communities after 21 days of litter incubation in laboratory microcosms at three nutrients (ambientNP, mediumNP, and highNP) and three oxygen levels (hypoxic, depleted, and saturated). The polygons group samples according with oxygen concentration: group on the left = saturated + depleted; group on the right = hypoxic

structure within each nutrient concentration, except at ambient nutrient concentrations where there were no significant differences between depleted and saturated oxygen conditions (ANOSIM, $R = 0.229$, $p = 0.143$; Fig. 4). However, nutrient concentrations did not significantly affect community structure in hypoxic conditions (ANOSIM, $R = -0.094$–0.094, $p = 0.257$–0.571) and no significant difference was found between ambient and medium nutrient concentrations in saturated oxygen conditions (ANOSIM, $R = 0.208$, $p = 0.057$). Conversely, changes in nutrient concentrations significantly affected community structure in depleted oxygen conditions (ANOSIM, $R = 0.365$–0.958, $p = 0.029$ for all three comparisons) and differences were also found between ambient and high and between medium and high nutrient concentrations in saturated oxygen conditions (ANOSIM, $R = 0.990$, $p = 0.029$ for both comparisons) (Fig. 4).

Table 2 Results of ANOSIM pairwise tests performed on aquatic hyphomycete communities after 21 days of litter incubation in laboratory microcosms at three nutrients (ambientNP, mediumNP, and highNP) and three oxygen levels (hypoxic, depleted, and saturated). To facilitate interpretation, significant p values are shown in bold

Treatments	R	p
Nutrients treatments		
AmbientNP × mediumNP	0.160	0.056
AmbientNP × highNP	0.663	**0.003**
MediumNP × highNP	0.681	**0.002**
Oxygen tratments		
Hypoxic × depleted	0.972	**0.001**
Hypoxic × saturated	0.986	**0.001**
Depleted × saturated	0.441	**0.003**

Discussion

The individual effects of nutrient enrichment on litter decomposition and associated decomposers are well studied (reviewed by Ferreira et al. [9]). However, increases in nutrient concentrations, as a result of agricultural activity or urban wastewater discharge, may be accompanied by decreases in oxygen concentrations [7]. The simultaneous variation in nutrient and oxygen concentrations can also occur in a scenario of global warming since temperature rise stimulates microbial activity, enhances the mineralization of nutrients and decreases oxygen solubility [6, 3]. This study showed that the effects of simultaneous changes in nutrient and oxygen concentrations in aquatic environments can influence the activity and composition of fungal communities, although these effects were not translated into changes in litter mass loss during the early stage of decomposition addressed here. We also found that the effects of changes in one factor on microbial variables depended on the level of the other factor, suggesting interactions between factors. For instance, microbial variables were less sensitive to changes in nutrient concentration when oxygen concentration was suboptimal (i.e., hypoxic conditions) than when oxygen concentration was higher. This agrees with the suggestion that when one factor is suboptimal, changes in another factor may not affect biological activities [36].

Litter mass loss after 21 days of incubation in laboratory microcosms was extremely low (< 3%) irrespective of the treatment, and may be attributed to *M. guianensis* litter being exposed in a stream for 10 days prior to being incubated in the microcosms. During stream exposure, leaching of soluble compounds likely caused a rapid initial mass loss, after which mass loss declined as recalcitrant compounds were left behind. Gomes et al. [25] found 17.5% of mass loss for *M. guianensis* litter after 10 days incubation in laboratory microcosms and Moretti et al. [37], Alvim et al. [38], and Medeiros et al. [39] found 5–15% of leaf mass loss for several Cerrado plant species after 1 week of field incubation and a maximum of 20% mass loss after 30 days, which supports the contention that a rapid initial mass loss followed by a strong decrease in the rate of litter decomposition over at least 1 month is common in leaves from the Cerrado biome. Moreover, we should also consider that microbial degradation of leaf litter might be low or inefficient in the initial period of decomposition (< 30 days) in tropical savanna streams because of the low water nutrient concentration and the low quality of leaves, which retard microbial colonization [27, 40, 41]. Litter mass loss could have been higher if litter had been less recalcitrant and nutrient-rich and if the experiment had been carried out for a longer period [37, 38]. Extending the duration of the experiment was, however, not possible in the present study due to the high cost of continuously supplying the microcosms with N_2. Nevertheless, the low litter mass

loss may have rendered it insensitive to changes in nutrient and oxygen concentrations.

Fungal biomass and sporulation rates were low in hypoxic conditions, as expected [7, 21]. This agrees with previous studies where there was a reduction in fungal biomass or reproductive activity of aquatic hyphomycetes when dissolved oxygen availability was low [21]. At intermediate oxygen levels, fungal biomass increased with increases in nutrient concentrations. Previous studies have also found an increase in biomass accumulation with increases in nutrient availability [11, 16, 17]. The unexpected decrease in fungal biomass when nutrient concentrations raised from intermediate to high in oxygen-saturated conditions (mediumNP—saturated vs. highNP—saturated) could be explained by different dynamics in biomass accumulation among treatments through time. For instance, the fungal biomass peak might have occurred earlier in high nutrient than in intermediate nutrient treatment [14], and be already declining by the sampling date (day 21).

Sporulation rates on day 21 did not significantly differ between low and intermediate nutrient conditions, which could also be due to differences in conidial production dynamics as for fungal biomass. However, sporulation rates were lower in high than in intermediate or ambient nutrient conditions, especially at depleted oxygen conditions, and this reflects the lower conidial production in high nutrient conditions over time (data not shown). In general, high nutrient concentrations (especially N and P) stimulate microbial reproductive activity, which results in a more efficient colonization of the substrate [19, 20]. The contrasting results observed for fungal biomass and sporulation rates (e.g., higher biomass was not reflected in higher sporulation rates) regarding nutrient and oxygen concentrations might be due to different strategies of aquatic hyphomycetes species, which can invest their energy in hyphae biomass (and thus, higher biomass) or spores (and thus, higher sporulation rates). In this case, not all fungal species may have had the ideal conditions for reproduction because of the low quality of leaf litter [38, 40, 41].

Changes in nutrient and oxygen concentrations simulating increased human pressure significantly affected aquatic hyphomycete community structure. Species richness of sporulating aquatic hyphomycetes was highest in microcosms with low and intermediate nutrient concentrations in depleted and saturated oxygen conditions, reflecting the antagonistic effects of these two factors on fungal interactions. This suggests that the number of species was reduced by deleterious or adverse effects on some species that are not adapted to the harsh conditions (highNP—hypoxic). These results show that the different interactions among these environmental conditions would promote changes in the structure of aquatic hyphomycetes communities. *A. filiformis* dominated the fungal conidia production, even in hypoxic conditions, which corroborates previous reports showing *A. filiformis* to be tolerant to low oxygen availability [42, 43]. *H. submersus* was observed only in microcosms with intermediate and high nutrient concentrations, although it was absent in hypoxic conditions regardless of the nutrient concentration. This species was exclusively associated with polluted environments in a previous study [43], suggesting that it benefits from increase in nutrient availability when enough oxygen is available. Thus, changes in aquatic hyphomycete communities' structure promoted by changes in nutrient and oxygen concentrations, as expected from global warming, intensification of agricultural activities and increases in wastewater discharges, is expected to propagate through aquatic food webs, considering that consumer feeding activity is affected by fungal biomass, identity and species richness [15, 44].

Conclusions

We demonstrated that simultaneous changes in nutrient and oxygen concentrations affected fungal growth, and reproduction and community structure of aquatic hyphomycetes associated with decomposing leaf litter to different extents, but not leaf litter mass loss during the early stage of decomposition. The individual effects of one factor on microbes depended on the level of the other factor. Although changes in nutrient and oxygen concentrations did not significantly affect microbial-induced litter decomposition, likely due to the poor litter quality or to functional redundancy among aquatic hyphomycete communities [37, 42], they may still affect litter decomposition where invertebrate detritivores are present since these consumers preferentially feed on microbial conditioned litter and are able to distinguish aquatic hyphomycetes species composition [15, 44]. The effects of changes in environmental variables on litter decomposition and associated biota may also depend on litter identity as litter with better carbon quality may be more sensitive to changes in nutrient availability compared with the litter used here [23]. Future research should consider the presence of consumers, differences in the quality of basal resources and long-term experiment duration when evaluating the effects of multiple environmental stressors on litter decomposition.

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References

1. Rockström J, Steffen W, Noone K, Persson A, Chapin FS, Lambin EF, Lenton TM, Scheffer M, Folke C, Schellnhuber HJ, Nykvist B, de Wit CA, Hughes T, van der Leeuw S, Rodhe H, Sörlin S, Snyder PK, Costanza R, Svedin U, Falkenmark M, Karlberg L, Correl RW, Fabry VJ, Hansen J, Walker B, Liverman D, Richardson K, Crutzen P, Foley JA (2009) A safe operating space for humanity. Nature 461:472–475
2. Woodward G, Gessner MO, Giller PS, Gulis V, Hladyz S, Lecerf A, Malmqvist B, Mckie BG, Tiegs SD, Cariss H, Dobson M, Elosegi A, Ferreira V, Graça MAS, Fleituch T, Lacoursière JO, Nistorescu M, Pozo J, Risnoveanu G, Schindler M, Vadineanu A, Vought LB, Chauvet E (2012) Continental-scale effects of nutrient pollution on stream ecosystem functioning. Science 336:1438–1440
3. Smith VH, Schindler DW (2009) Eutrophication science: where do we go from here? Trends Ecol Evol 24:201–207
4. Pascoal C, Cássio F, Gomes P (2001) Leaf breakdown rates: a measure of water quality? Int Rev Hydrobiol 86:407–416
5. Galloway JN, Townsend AR, Erisman JW, Bekunda M, Cai Z, Freney JR, Martinelli LA, Seitzinger SP, Sutton MA (2008) Transformation of the nitrogen cycle: recent trends, questions, and potential solutions. Science 320:889–892
6. Murdoch PS, Baron JS, Miller TL (2000) Potential effects of climate change on surface-water quality in North America. J Am Water Resour Assoc 36:347–366
7. Pascoal C, Cássio F (2004) Contribution of fungi and bacteria to leaf litter decomposition in a polluted river. Appl Environ Microbiol 70:5266–5273
8. Kuijper LJ, Berg MP, Morrien E, Kooi BW, Verhoef HA (2005) Global change effects on a mechanistic decomposer food web model. Glob Chang Biol 11:249–265
9. Ferreira V, Castagneyrol B, Koricheva J, Gulis V, Chauvet E, Graça MAS (2015) A meta-analysis of the effects of nutrient enrichment on litter decomposition in streams. Biol Rev 90:669–688
10. Wallace JB, Eggert SL, Meyer JL, Webster JR (1999) Effects of resource limitation on a detrital-based ecosystem. Ecol Monogr 69: 409–442
11. Gulis V, Suberkropp K (2003) Leaf litter decomposition and microbial activity in nutrient-enriched and unaltered reaches of a headwater stream. Freshw Biol 48:123–134
12. Pascoal C, Cássio F, Marcotegui A, Sanz B, Gomes P (2005) Role of fungi, bacteria, and invertebrates in leaf litter breakdown in a polluted river. J N Am Benthol Soc 24:784–797
13. Cornut J, Elger A, Lambrigot D, Marmonier P, Chauvet E (2010) Early stages of leaf decomposition are mediated by aquatic fungi in the hyporheic zone of woodland streams. Freshw Biol 55:2541–2556
14. Ferreira V, Chauvet E (2011) Synergistic effects of water temperature and dissolved nutrients on litter decomposition and associated fungi. Glob Chang Biol 17:551–564
15. Bärlocher F, Sridhar KR (2014) Association of animals and fungi in leaf decomposition. In: Jones G, Hyde K, Pang K-L (eds) Freshwater Fungi and Fungus-like Organisms. De Gruyter Series: Marine and Freshwater Botany. De Gruyter, Berlin, pp 413–441
16. Gulis V, Ferreira V, Graça MAS (2006) Stimulation of leaf litter decomposition and associated fungi and invertebrates by moderate eutrophication: implications for stream assessment. Freshw Biol 51: 1655–1669
17. Ferreira V, Gulis V, Graça MAS (2006) Whole-stream nitrate addition affects litter decomposition and associated fungi but not invertebrates. Oecologia 149:718–729
18. Rosemond AD, Benstead JP, Bumpers PM, Gulis V, Kominoski JS, Manning DWP, Suberkropp K, Wallace JB (2015) Experimental nutrient additions accelerate terrestrial carbon loss from stream ecosystems. Science 347:1142–1145
19. Suberkropp K, Gulis V, Rosemond AD, Benstead JP (2010) Ecosystem and physiological scales of microbial responses to nutrients in a detritus-based stream: results of a 5-year continuous enrichment. Limnol Oceanogr 55:149–160
20. Gulis V, Suberkropp K (2004) Effects of whole-stream nutrient enrichment on the concentration and abundance of aquatic hyphomycete conidia in transport. Mycologia 96:57–65
21. Medeiros AO, Pascoal C, Graça MAS (2009) Diversity and activity of aquatic fungi under low oxygen conditions. Freshw Biol 54:142–149
22. Tonin AM, Hepp LU (2011) Effects of nitrate enrichment on leaf litter decomposition. Acta Limnol Bras 23:86–94
23. Fernandes I, Seena S, Pascoal C, Cássio F (2014) Elevated temperature may intensify the positive effects of nutrients on microbial decomposition in streams. Freshw Biol 59:2390–2399
24. Silva-Júnior MC, Felfili JM, Walter BMT, Nogueira PE, Rezende AV, Morais RO, Nóbrega MGG (2001) Análise da flora arbórea de Matas de Galeria no Distrito Federal: 21 levantamentos. In: Ribeiro JF, Fonseca CEL, Souza-Silva JC (eds) Cerrado: caracterização e recuperação das Matas de Galeria1st edn. Embrapa Cerrados, Planaltina, pp 143–191
25. Gomes PP, Medeiros AO, Gonçalves Jr JF (2016) The replacement of native plants by exotic species may affect the colonization and reproduction of aquatic hyphomycetes. Limnologica 59:124–130
26. Fonseca BM, Mendonça-Galvão L (2014) Pristine aquatic systems in a long term ecological research (LTER) site of the Brazilian Cerrado. Environ Monit Assess 186:8683–8695
27. Fonseca BM, Mendonça-Galvão L, Padovesi-Fonseca C, Abreu LM, Fernandes AC (2014) Nutrient baselines of Cerrado low-order streams: comparing natural and impacted sites in Central Brazil. Environ Monit Assess 186:19–33
28. Dang CK, Chauvet E, Gessner MO (2005) Magnitude and variability of process rates in fungal diversity-litter decomposition relationships. Ecol Lett 8:1129–1137
29. Gessner MO (2005) Ergosterol as a measure of fungal biomass. In: Graça MAS, Bärlocher F, Gessner MO (eds) Methods to study litter decomposition. A practical guide. Springer, Dordrecht, pp 189–195
30. Bärlocher F (2005) Sporulation of aquatic hyphomycetes. In: Graça MAS, Bärlocher F, Gessner MO (eds) Methods to study litter decomposition. A practical guide. Springer, Dordrecht, pp 231–236
31. Pinheiro J, Bates D, Debroy S, Sarkar D, Team RDC (2013) nlme: linear and nonlinear mixed effects models. R package Version 3.1–109
32. Zuur A, Ieno EN, Walker N, Saveliev AA, Smith GM (2009) Mixed effects models and extensions in ecology with R. Springer Science & Business Media, Berlin
33. R Core Team (2013) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna URL http://www.R-project.org/
34. Hothorn T, Bretz F, Westfall P (2008) Simultaneous inference in general parametric models. Biom J 50:346–363
35. Clarke KR, Gorley RN (2001) Primer v5: user manual/tutorial. Primer-E Ltd., Plymouth
36. Prescott CE (2010) Litter decomposition: what controls it and how can we alter it to sequester more carbon in forest soils? Biogeochemistry 101:133–149
37. Moretti MS, Gonçalves Jr JF, Ligeiro R, Callisto M (2007) Invertebrates colonization on native trees leaves in a neotropical stream (Brazil). Int Rev Hydrobiol 92:199–210
38. Alvim EACC, Medeiros AO, Rezende RS, Gonçalves Jr JF (2015) Leaf breakdown in a natural open tropical stream. J Limnol 74:248–260
39. Medeiros AO, Callisto M, Graça MAS, Ferreira V, Rosa CA, França JS, Eller AP, Rezende RS, Gonçalves Jr JF (2015)

Microbial colonization and litter decomposition in a Cerrado stream are limited by low dissolved nutrient concentrations. Limnetica 34: 283–292

40. Gonçalves Jr JF, Graça MAS, Callisto M (2007) Litter decomposition in a Cerrado savannah stream is retarded by leaf toughness, low dissolved nutrients and a low density of shredders. Freshw Biol 52: 1440–1451
41. Alvim EACC, Medeiros AO, Rezende RS, Gonçalves Jr JF (2015) Small leaf breakdown in a Savannah headwater stream. Limnologica 51:131–138
42. Sridhar KR, Duarte S, Cássio F, Pascoal C (2009) The role of early fungal colonizers in leaf-litter decomposition in Portuguese streams impacted by agricultural runoff. Int Rev Hydrobiol 94:399–409
43. Pascoal C, Marvanová L, Cássio F (2005) Aquatic hyphomycete diversity in streams of northwest Portugal. Fungal Divers 19:109–128
44. Lecerf A, Dobson M, Dang CK, Chauvet E (2005) Riparian plant species loss alters trophic dynamics in detritus-based stream ecosystems. Oecologia 146:432–442