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Diversity, role in decomposition, and succession of zoosporic fungi and straminipiles on submerged decaying leaves in a woodland stream

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Abstract Leaf litter is a very important primary source of energy in woodland streams. Decomposition of leaf litter is a process mediated by many groups of microorganisms which release extracellular enzymes for the degradation of complex macromolecules. In this process, true fungi and straminipiles are considered to be among the most active groups, more active than the bacteria, at least during the early stages of the process. Colonization increases the quality of the leaves as a food resource for detritivores. In this way, matter and energy enter detritus-based food chains. Previously, aquatic hyphomycetes were considered to be the major fungal group responsible for leaf litter decomposition. Although zoosporic fungi and straminipiles are known to colonize and decompose plant tissues in various environments, there is scant information on their roles in leaf decomposition. This study focuses on the communities of zoosporic fungi and straminipiles in a stream which are involved in the decomposition of leaves of two plant species, *Ligustrum lucidum* and *Pouteria salicifolia*, in the presence of other groups of fungi. A characteristic community dominated by *Nowakowskiella elegans*, *Phytophthora* sp., and *Pythium* sp. was found. Changes in the fungal community structure over time (succession) was observed: terrestrial mitosporic fungi appeared during the early stages, zoosporic fungi, straminipiles, and aquatic Hyphomycetes in early-to-intermediate stages, while representatives of the phylum Zygomycota were found at early and latest stages of the decomposition. These observations highlight the importance of zoosporic fungi and straminipiles in aquatic ecosystems.

Keywords Aquatic ecosystem · Diversity · Leaf decomposition · Straminipiles · Succession · Zoosporic fungi

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Introduction

Leaf litter is the major source of energy supporting food webs in small, forested streams (Wallace et al., 1999). The breakdown of leaf litter is the result of physical and chemical factors causing mechanical fragmentation and leaching, and the activities of shredders (invertebrates), aquatic fungi, and bacteria (Hieber & Gessner, 2002). Aquatic fungi comprise a diverse assemblage of true fungi and fungus-like organisms belonging to the Blastocladiomycota, Chytridiomycota, Zygomycota, Ascomycota, and Basidiomycota (Fungi), and Hyphochytriomycota, Labyrinthulomycota, and Peronosporomycota (Straminipila) (Shearer et al., 2004). Fungi can decompose complex carbon and nitrogen compounds such as cellulose, hemicellulose, lignin, pectins, proteins, humic acids, and many other substances, by releasing of extracellular enzymes (Jennings, 1989, 1995; Kjøller & Struwe, 1992; Moore-Landecker, 1996). Fungi have been considered the most active organisms in leaf decomposition, even more than bacteria during the early stages of the process (Bärlocher & Kendrick, 1974; Suberkropp & Klug, 1976). Nikolcheva & Bärlocher (2004) observed that the increase in fungal biomass and sporulation associated with leaf litter in streams is positively correlated with decomposition rates. This indicates that fungi have a significant role in the dynamics of the coarse particulate organic matter, while bacteria assume a greater role in the decomposition of fine particulate and dissolved organic matter. Moreover, fungal growth results in an increase in the content of organic nitrogen in leaf litter (Kaushik & Hynes, 1971), and, thus, makes leaves more palatable and nutritious for invertebrates (Bärlocher, 1985). Hence, fungi act as trophic intermediates of energy flow between the fallen leaves and higher trophic levels (Cummins & Klug, 1979; Bärlocher, 1985, 1992; Suberkropp, 2001).

The growth of many fungi is dependent on the chemical composition of leaves (Gessner & Chauvet, 1994). Plant species with a low content of structural macromolecules (cellulose, hemicellulose, and lignin) and defense compounds (e.g., phenols) are more prone to microbial colonization and decomposition at faster rates (Stout, 1989; Gessner & Chauvet, 1994; Hättenschwiler et al., 2005).

Chemical and physical changes in leaves during decomposition affect the structure of the microbial community (Das et al., 2008) and lead to the establishment of different fungal assemblages (Ingold, 1942; Pugh, 1958; Bärlocher & Kendrick, 1974; Bärlocher & Oertli, 1978; Bärlocher & Schweizer, 1983; Schoenlein-Crusius et al., 1990, 1998; Bärlocher, 1991; Nikolcheva et al., 2003; Nikolcheva & Bärlocher, 2005). Therefore, a succession of species through the different stages of decomposition would be expected (Bärlocher, 1992).

Fungal assemblages involved in leaf breakdown in aquatic environments include representatives of the Ascomycota (especially the Hyphomycetes), zoosporic fungi (Blastocladiomycota and Chytridiomycota), straminipiles (Peronosporomycota, Hyphochytriomycota, and Thraustochytriomycota), and to a lesser extent some representatives of Zygomycota and Basidiomycota (Dix & Webster, 1995; Gessner et al., 2007). However, zoosporic organisms, some aquatic Hyphomycetes and yeasts have been particularly recognized as "indigenous or native" decomposers in freshwaters (Park, 1972; Powell, 1993; Dix & Webster, 1995). The Hyphomycetes (also called mitosporic or anamorphic fungi) is an artificial group of fungi that are asexually reproducing members of the phyla Ascomycota and Basidiomycota (Webster, 1992).

Zoosporic organisms comprise phylogenetically unrelated groups of taxa belonging to kingdoms Fungi ("zoosporic fungi") and Straminipila ("straminipiles") that are grouped together primarily due to the presence of flagellated spores (zoospores) (Müeller et al., 2004). These organisms are ubiquitous in aquatic environments (Sparrow, 1960; Barr, 2001) and are largely known as decomposers of recalcitrant organic matter (including cellulose, chitin, and sporopollenin) (Powell, 1993; Kiziewicz, 2004), showing a great abundance and diversity on plant debris (Willoughby, 1974). Even so, the aquatic Hyphomycetes have traditionally been considered to be the dominant group in leaf decomposition (Bärlocher, 1992; Suberkropp, 1992, Baldy et al., 1995; Chauvet & Suberkropp, 1998; Nikolcheva & Bärlocher, 2004; Seena et al., 2008; Gulis et al., 2009), especially in temperate streams (Bärlocher, 1992).

To date, biodiversity studies have been mostly focused on mitosporic fungal communities (e.g., Ingold, 1942; Shearer & Webster, 1991; Shearer, 1993; Laitung & Chauvet, 2005), and only a few have documented the presence of Chytridiomycota (Schoenlein-Crusius & Milanez, 1989; Schoenlein-Crusius et al., 1990;

Wellbaum et al., 1999; Nikolcheva & Bärlocher, 2004; Seena et al., 2008) and Peronosporomycota (Bärlocher & Kendrick, 1974; Nikolcheva & Bärlocher, 2004; Nechwatal et al., 2008). Very few studies have focused on the communities of zoosporic organisms during leaf decomposition in freshwaters (Schoenlein-Crusius & Milanez, 1989, 1998; Schoenlein-Crusius et al., 1990, 1992, 1998, 1999). Since attention has centered on aquatic hyphomycetes species, methods that specifically encourage sporulation (i.e., moist chamber technique) have been used, underestimating other groups such as Chytridiomycota and Peronosporomycota. Furthermore, Chytridiomycota are expected to be overwhelmed by mycelial forms under such experimental conditions (Cooke & Rayner, 1984). However, with the use of different techniques Schoenlein-Crusius et al. (1990) found a greater number of zoosporic organisms than Hyphomycetes on submerged leaves of *Ficus macrocarpa* L., *Quercus robur* L., and *Alchornea triplinervia* (Spreng.) Muell. Arg. Recently, Seena et al. (2008), using molecular techniques, estimated the fungal diversity in decaying leaves of *Quercus alba* L., *Acer rubrum* L., and *Tilia cordata* Mill, recording 15 sequences belonging to *Nowakowskiella* spp., one to *Nowakowskiella hemisphaerosphora* Shanor, and two unidentified species of Chytridiales. Nechwatal et al. (2008) documented the presence of *Pythium* spp. and *Phytophthora* spp. (Peronosporomycota) on declining stands of *Phragmites australis* (Cav.) Trin. ex Steud. in a German lake. It must be emphasized that little is known about interactions between aquatic Hyphomycetes and zoosporic fungi and straminipiles on decomposing leaves (Bärlocher, 1992). It is important to note that the methods for estimating overall rates of fungal production are mostly based on [^{14}C] acetate incorporation rates into ergosterol (Newell & Fallon, 1991), which only occurs in higher fungi, but ergosterol is absent in Chytridiomycota and Peronosporomycota (Gessner et al., 1997, 2003).

Since zoosporic fungi and straminipiles are frequently observed on vegetable debris from aquatic environments (Willoughby & Redhead, 1973), we propose that they have been overlooked by inappropriate methodologies for both identification and estimation of population sizes, and that these organisms play a more significant role in the decomposition of leaves in freshwaters than previously thought.

The objectives of this study were: (i) to assess the relative contribution of zoosporic fungi and straminipiles to the overall species composition, frequency, abundance, and diversity on submerged leaves in an Argentinean woodland stream, and (ii) to discuss the methods for analyzing the structure and function of fungal communities during leaf decomposition.

Materials and methods

Study area

The study was conducted at Las Cañas stream (34°47′58.5″ S–57°57′19.3″ W, 34°47′29.3″ S–57°59′49.2″ W) which is located in the "Selva Marginal Punta Lara" Natural Reserve, on the northeastern side of Ensenada and Berazategui districts (Buenos Aires province, Argentina). The stream runs through a riverine marginal forest of native species (*Blephalocalix tweedii* (Hook et Arn) Berg., *Ocotea acutifolia* (Nees.) Mez., and *Pouteria salicifolia* (Spreng.) Radlk.), which represent 82% of the biomass with the remaining 18% belonging to the exotic Chinese *Ligustrum lucidum* Ait. (Cabrera & Dawson, 1944; Cabrera, 1960; Dascanio et al., 1994).

Litterbag experiment

Senescent leaves (just before abscission) from several individuals of *L. lucidum* (Oleaceae) and *P. salicifolia* (Sapotaceae) growing along stream banks were collected on August 26, 2007. The leaves were air-dried in the laboratory for 96 h at room temperature (Moreira, 2006). The litterbag technique (Bocock & Gilbert, 1957) was employed using 25 × 20 cm, 1-mm mesh plastic bags, each one containing 15 g of *L. lucidum* or 10 g of *P. salicifolia* leaves (fresh weight). Three bags of each plant species were placed onto 45 × 20 cm expanded polystyrene supporting structures and distributed in the stream on August 30, 2007 following a randomized block design (Zar, 1996). Three bags of each species were retrieved at 5, 10, 14, 20, 30, 42, 57, 72, 90, and 120 days for *L. lucidum* and 5, 14, 30, 42, 72, 120, 180, 240, and 300 days for *P. salicifolia* and transported to the laboratory in a coolbox.

Laboratory analysis

Litterbags were processed within 1 h after collection. The material in each bag was placed in a 1000-μm mesh plastic sieve, washed repeatedly with sterile deionized water to remove undesirable sediments and, whenever possible (according to the consistency of the leaves), carefully cleaned with a wet brush.

The baiting technique (Sparrow, 1960; Stevens, 1974; Barr, 1987) was used for assessing species composition, frequency and abundance. Five leaf disks (5-mm diameter) were placed into Petri dishes flooded with 30 ml of deionized water. In each Petri dish, a rubber disk (7.5-cm diameter) with five 1-cm-diameter holes was placed floating on the water. A 5-mm-diameter sterile corn leaf (*Zea mays* L.) or a sterile sesame seed (*Sesamum indicum* L.) was added as bait in each hole of the rubber disk. Each dish was considered a sample unit. Five replicates were made for each of the baits and plant species and for all exposure times, including senescent leaves (i.e., T_0: 0 days of exposure time). In *L. lucidum* at 120 days, only the species composition could be characterized, due to the consistency of highly degradated leaves. Dishes were incubated at room temperature for 4–60 days.

The species composition of zoosporic fungi, straminipiles, and associated mycobiota (mitosporic fungi and Zygomycota) was determined by microscopic (Olympus BX 40 microscope) examination of baits after 4, 7, 14, 21, 30, 42, and 60 days. Taxonomic identifications were made according to Coker (1923), Sparrow (1960), Karling (1977), Rocha & Pires-Zottarelli (2002) for zoosporic fungi and straminipiles, and Ellis (1971, 1976) and Matsushima (1975) for mitosporic fungi.

Statistical analysis

A total of 210 Petri dishes and 1,050 baits were analyzed. The presence–absence (occurrence) of the taxa and number of isolations (N_i) were recorded in each sample unit. One isolation was considered to be as a colony which developed on a bait from one or several propagules. The frequency of colonization and abundance was calculated according to Marano et al. (2008): (i) Frequency of colonization (FC): (number of sample units colonized by a taxon/number of sample units examined) × 100 (Figuereda & Barata, 2007), and (ii) abundance (A): (number of isolations recorded for a taxon/number of baits employed) × 100. Species were assigned to five groups according to the frequency scale of Braun–Blanquet: ubiquitous (100–80.1% occurrence); common (80–60.1% occurrence); usually present (60–40.1% occurrence); scarce (40–20.1% occurrence), and rare (20–0.1% occurrence) (Kershaw, 1973; Letcher & Powell, 2001, 2002).

Community structure was analyzed by: (i) species richness (S); (ii) Shannon's diversity index $H' = -\sum_{p_i}^{S} p_i \cdot \log_2(p_i)$, where p_i is the abundance of the species i that contributes to total diversity; (iii) Evenness $E = H'/H'_{\max}$, where $H'_{\max}$ is the maximum value of the diversity for the number of species that are present (Magurran, 1988); (iv) Simpson's dominance index $D = 1 - \sum_{i=1}^{S} (p_i)^2$, which was calculated for each time of exposure and for each plant species based on fungal abundance; and (v) Sorensen's similarity index $SI = 2j/(a + b)$, where a is the number of species in *L. lucidum*, b in *P. salicifolia*, and j the number of species common to both plant species. This index ranges between 1 (complete similarity) and 0 (no shared species).

Data were tested for normality before analysis. Student's t-test was employed to explore differences in: (i) the overall species richness between plant species; and (ii) the richness of zoosporic fungi and straminipiles with respect to other fungal groups in each plant species.

The Mann–Whitney test was used to compare differences in the number of isolations of zoosporic fungi and straminipiles between plant species. The Kruskall–Wallis test was employed to explore differences in frequency and abundance between times of exposure in each plant species (Zar, 1996). When differences were significant ($P < 0.05$), Dunn's test (performed with XLSTAT, version 2009; Dunn, 1961) was used to determine where the differences occurred.

Diversity indexes (H') were tested for significance with the H–t test using the software program Biodap® (Clay & Thomas, 1996).

Cluster analysis by unpaired group mean averages (UPGMA) algorithm using the Morisita–Horn index (Müeller et al., 2004) was performed using MVSP package (version 3.1) (Kovach, 1999) to classify the samples.

Results

Species composition and richness

A total of 80 taxa were identified (Table 1), 16 zoosporic fungi and straminipiles (20%) and 64 taxa belonging to other fungal groups: 62 mitosporic fungi (77.5%) and two Zygomycota (2.5%). Fifty-eight taxa were identified for *L. lucidum* (14 zoosporic fungi and straminipiles, 42 mitosporic fungi, and two Zygomycota) whereas 63 taxa were identified for *P. salicifolia* (ten zoosporic fungi and straminipiles, 51 mitosporic fungi, and two Zygomycota). Forty out of the 80 taxa were shared by both plant species, whereas 18 were exclusive to *L. lucidum* and 24 to *P. salicifolia* (SI: 0.63). Species richness was not significantly different between plant species ($P > 0.05$). The species richness of zoosporic fungi and straminipiles (S_L: 14; S_P: 10) was significantly lower ($P < 0.05$) than of other fungal groups (S_L: 44; S_P: 53).

The greatest number of taxa was recorded at 42 days for *L. lucidum* and at 14 and 72 days for *P. salicifolia*. Regarding zoosporic fungi and straminipiles, the greatest number was obtained at 10 days for *L. lucidum* (nine taxa) and at 42 days for *P. salicifolia* (six taxa) (Fig. 1).

Frequency and abundance

The frequency of colonization (FC) of the overall fungal community was 93% for *L. lucidum* and 90% for *P. salicifolia* whereas the abundance (A) was 94.2 and 96.5%, respectively. No differences of statistical significance ($P > 0.05$) in the total FC and A of whole the fungal community were found between plant species. *L. lucidum* had greater FC and A of zoosporic fungi and straminipiles (FC: 91.1%; A: 33%) than of mitosporic fungi (FC: 74.4%; A: 11.8%) ($P < 0.05$), whereas for *P. salicifolia* the FC and A of zoosporic fungi and straminipiles (FC: 60%; A: 22.6%) and of mitosporic fungi (FC: 81.1%; A: 31.3%) did not differ ($P > 0.05$). The FC and A of zoosporic fungi and straminipiles were greater in *L. lucidum* than in *P. salicifolia* ($P < 0.05$).

Phytophthora sp. (FC: 42.5%; A: 27.4%), *Nowakowskiella elegans* (FC: 35.5%; A: 13.9%), and *Pythium* sp. (FC: 19.5%; A: 11.9%) were dominant, and *Dimorphospora foliicola* (FC: 22.5%; A: 9%) and *Dictyuchus* sp. (FC: 12.5%; A: 6.3%) were well represented. The remaining taxa showed a FC < 10% and A < 5%. The FC and A of taxa at each time of exposure in both plant species are shown in Table 2.

For both plant species, differences of statistical significance were found in the A and the FC of the taxa at different times of exposure ($P < 0.05$, Fig. 2).

Some terrestrial mitosporic fungi (*Alternaria* sp. and *Cladosporium* sp.) were common before immersion (T_0) but until 10 days of exposure all the taxa were rare or scarce, except for *Nowakowskiella elegans* (that was common in *L. lucidum*). *Phytophthora* sp. was common to ubiquitous between 10 and 72 days in both plant species, *Pythium* sp. was usually present at 14 days in *L. lucidum* and 30 days in *P. salicifolia*, while *Dictyuchus* sp. was usually present at 42–57 and 90 days in *L. lucidum*.

Diversity

The diversity of the fungal community in *L. lucidum* (H': 3.92) was lower than that of *P. salicifolia* (H': 4.32) ($P < 0.05$). The evenness was 0.68 for *L. lucidum* and 0.74 for *P. salicifolia*, which coincides with the dominance index obtained: 0.13 and 0.11, respectively.

The diversity (Figs. 3, 4) was low at T_0 for both plants. In *L. lucidum*, it increased in the early stages (5–14 days), decreased at intermediate stages (20–30 days), and increased significantly at 42 days ($P < 0.05$). In *P. salicifolia*, the diversity increased up to 30 days remaining practically constant from 30 to 180 days ($P < 0.05$).

Succession

SI between times of exposure were low, ranging between < 0.01 and 0.4. The only exceptions were found between 5 and 10 days (SI: 0.6) in *L. lucidum* and 180 and 300 days (SI: 0.7) in *P. salicifolia*, which appeared to be the most similar times of exposure for its species composition.

The SI between T_0 and 5 days for both plants were similar (SI: 0.2 and SI: 0.1, respectively). In the case of *L. lucidum*, the phylloplane fungi (*Bipolaris* sp., *Cladosporium* sp., *Fusarium* sp., *Paecilomyces* sp., *Penicillium* sp., *Pestalotiopsis guepinii*, and *Trichoderma* sp.) persisted up to 5 days, while from 10 to 42 days, these taxa were absent, and at 57 days, some of them re-colonized the leaves.

Table 1 Species composition of zoosporic fungi, straminipiles, mitosporic fungi and Zygomycota during leaf breakdown of *L. lucidum* and *P. salicifolia*

Taxa	*L. lucidum*	*P. salicifolia*
Zoosporic fungi and straminipiles		
Achlya aff. *rodrigueziana*		•
Aphanomycopsis saprophytica Karling	•	
Catenochytridium sp.	*	*
Cladochytrium replicatum Karling	*	*
Cylindrochytridium johnstonii Karling	*	*
Dictyuchus monosporus Leitgeb	•	
Dictyuchus sp.	*	*
Monoblepharella sp.		•
Monoblepharis hypogyna Perrot	•	
Monoblepharis polymorpha Cornu	•	
Nowakowskiella elegans (Nowak.) J. Schröt.	*	*
Nowakowskiella sp.	*	*
Phytophthora sp.	*	*
Pythium sp.	*	*
Rhizidiomyces hirsutus Karling	•	
Septochytrium variabile Berdan	•	
Mitosporic fungi		
Altenaria sp.	*	*
Arthrinium sp.	•	
Beverwykella pulmonaria (Beverw.) Tubaki	*	*
Bipolaris sp.# 1	•	
Bipolaris sp.# 2		•
Chaetomium globosum Kunze		•
Cladosporium sp.	*	*
Clavatospora stellata (Ingold & V. J. Cox) Sv. Nilsson	*	*
Clonostachys sp.		•
Coelomycete sp.# 1		•
Coelomycete sp.# 2		•
Coelomycete sp.# 3		•
Cylindrocarpon sp.	*	*
Cylindrocladium sp.	*	*
Dactylaria aff. *chysosperma*	*	*
Dactylaria aff. *mitrata*		•
Dactylaria aff. *mucronulata*		•
Dactylaria sp.# 1	*	*
Dactylaria sp.# 2	*	*
Dactylaria sp.# 3	*	*
Dactylella lysipaga Drechsler	•	
Dactylella sp.# 1	*	*
Dactylella sp.# 2	*	*
Dactylella sp.# 3	•	
Dactylella sp.# 4	•	
Dictiochaeta sp.		•
Dimorphospora foliicola Tubaki	*	*

Table 1 continued

Taxa	*L. lucidum*	*P. salicifolia*
Dorathomyces stemonitis (Pers.) Morton & Sm.		•
Endophragmia boeweii J. L. Crane	*	*
Endophragmiella socia (M. B. Ellis) S. Hughes		•
Epiccocum nigrum Link	*	*
Exophiala sp.	•	
Fusarium oxysporum E. F. Sm. & Swingle	*	*
Fusarium sp.# 1	*	*
Fusarium sp.# 2	*	*
Fusarium sp.# 3		•
Fusarium sp.# 4		•
Helicosporium sp.	•	
Heliscus submersus Hudson	*	*
Hyphomycete sp.# 1	*	*
Humicola sp.	*	*
Idriella sp.	*	*
Isthmolongispora minima Matsush.		•
Monacrosporium bembicodes (Drechsler) Subram.	•	
Myrothecium verrucaria (Alb. & Schwein.) Ditmar	•	
Paecilomyces sp.	*	*
Penicillium rubrum Sopp.		•
Penicillium sp.		•
Pestalotiopsis guepinii (Desm.) Steyaert		•
Phaeoisaria clematidis (Fuckel) S. Hughes		•
Phialophora sp.	•	
Pithomyces aff. *artro-olivaceus*	•	
Pithomyces chartarum (Berk & Curtis) Ellis	*	*
Porospora sp.		•
Ramichloridium sp.	*	*
Tetracladium setigerum (Grove) Ingold	*	*
Torula herbarum (Pers.) Link	*	*
Trichoderma sp.	•	
Tricladium anomalum Ingold	*	*
Triramulispora gracilis Matsush.	*	*
Volutella ciliata (Alb. & Schwein.) Fr.		•
Zalerion sp.	*	*
Zygomycota		
Mortierella sp.	*	*
Mucor sp.	*	*

(*) Taxa found on both plant species; (•) taxa exclusive to one plant species

For both plant species, cluster analysis (Fig. 5) showed that T_0 (Group 1) differed from the other times of exposure (Group 2). In group 2, we could distinguish two sub-groupings: (A) which included the early and intermediate stages of the decomposition; and (B) which included the later ones. The only exception was found for 240 days, which was related to the early and intermediate stages in *P. salicifolia*.

Dactylaria sp.# 1, *Tetracladium setigerum*, *Fusarium oxysporum*, *Pythium* sp., and *Phytophthora* sp. appeared to be early-to-intermediate colonizers (early species), while *Dictyuchus* sp., Hyphomycete sp.# 1,

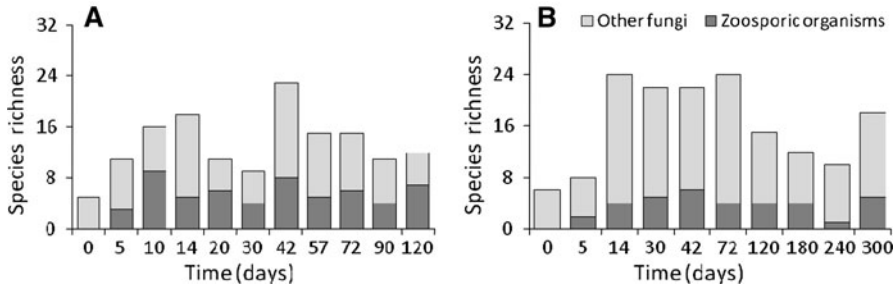

Fig. 1 Species richness of zoosporic organisms and of other fungal groups (mitosporic fungi and Zygomycota) at each time of exposure. **A** *L. lucidum*. **B** *P. salicifolia*

and *Pythomyces chartarum* appeared at the latest stages of the decomposition of *L. lucidum* (late colonizers) (Fig. 6). In the case of *P. salicifolia*, the early species appeared to be *T. setigerum, Fusarium* sp.# 1, *Beverwykella pulmonaria*, and *Phytophthora* sp., while Hyphomycete sp.# 1 and *Chaetomiun globosum* were the late colonizers (Fig. 7).

Discussion

Relative contribution of fungal groups to the overall species composition, richness, frequency, and abundance

Zoosporic fungi, straminipiles, and mitosporic fungi were well represented throughout the decomposition process of *L. lucidum* and *P. salicifolia* leaves. Many authors have also suggested that aquatic Hyphomycetes dominate the breakdown of leaves (e.g., Bärlocher & Kendrick, 1974; Suberkropp & Klug, 1976; Bärlocher, 1992). In contrast, our results showed that *Nowakowskiella elegans*, *Phytophthora* sp., and *Pythium* sp. were dominant in relation to their frequency and abundance in both plant species studied. Based on the analysis of intensity of the bands by DGGE, Nikolcheva & Bärlocher (2004) observed that freshwater fungal communities on decomposing leaves were dominated by Ascomycota and Basidiomycota, but they also found a high diversity of Chytridiomycota. The Peronosporomycota were only found in summer, and their contribution was less than that of the Basidiomycota and Chytridiomycota. The Zygomycota contributed to the lowest percentage of the total intensity of the bands. In this study, the Zygomycota were poorly represented, reinforcing the hypothesis that these organisms are transient (Park, 1972) and that involvement in the breakdown of leaves is questionable (Cooke, 1976). Gessner & Schwoerbel (1989) suggested that Peronosporomycota (*Phytophthora* and *Pythium*) were favored and Hyphomycetes delayed when fresh rather than pre-dried leaves were used. If it is so, then Peronosporomycota might play a larger role in decomposition than was previously thought.

The species composition and richness were found to be similar in the leaves of the plant species in this study, in contrast to what was observed by Das et al. (2008) in *Acer saccharum* Marsh. and *Quercus alba* L. However, the leaves of *L. lucidum* had a higher number of representatives of zoosporic fungi and straminipiles than of mitosporic fungi, whereas those of *P. salicifolia* had no differences. Also, zoosporic fungi and straminipiles were more frequent and abundant in the former species. Such differences could be attributed to the different degrees of sensitivity of fungi to the soluble antifungal substances present in green leaves (Bärlocher & Oertli, 1978) and to the fact that fungal growth rates seem to be directly related to the chemical composition of leaves (Gessner & Chauvet, 1994). On the contrary, zoosporic fungi and straminipiles are able to select the appropriate substrates, due to the mobility and chemotaxis of their zoospores (Dick, 1976; Mitchell & Deacon, 1986) that enable them to selectively accumulate at or avoid a substrate through the direction of swimming (Carlile, 1993). The attachment and germination of their zoospores appears to be related to the presence of substrates for growth in the leaves (mainly hemicellulose, cellulose, and lignin) and inhibited by antifungal substances such as phenolic compounds (Frankland, 1992). Thus, zoospores can swim toward suitable substrates whereas conidia of aquatic Hyphomycetes reach their substrates by chance even though they have morphological adaptations (e.g., tetraradiate, sigmoids or variously branched shapes) to optimize attachment (Dang et al., 2007; Kearns & Bärlocher, 2008).

Table 2 Frequency of colonization (FC) and abundance (A) of the taxa on the leaves of *L. lucidum* and *P. salicifolia*

Frequency of colonization	Days										
	T_0	5	10	14	20	30	42	72	90	120	Total
Ligustrum lucidum											
Nowakowskiella elegans	0	70	30	80	40	30	60	20	60	60	50
Phytophthora sp.	0	40	70	60	90	90	20	20	30	0	46.7
Pythium sp.	0	20	40	60	20	30	30	30	30	30	32.2
Dictyuchus sp.	0	0	20	0	0	10	60	60	20	50	24.4
Fusarium oxysporum	0	10	40	40	10	10	0	10	0	0	13.3
Alternaria sp.	30	30	10	0	0	0	0	10	0	0	8.9
Dactylaria sp.# 1	0	20	30	20	0	0	0	0	0	0	7.8
Dictyuchus monosporus	0	0	0	30	30	0	10	0	0	0	7.8
Hyphomycete sp.# 1	0	0	0	0	0	0	0	0	20	50	7.8
Catenochytridium sp.	0	0	0	10	0	0	30	0	10	10	6.7
Dimorphospora foliicola	0	0	0	10	0	20	20	0	10	0	6.7
Tetracladium setigerum	0	10	30	20	0	0	0	0	0	0	6.7
Cladochytrium replicatum	0	0	10	0	10	0	10	0	20	0	5.6
	T_0	5	14	30	42	72	120	180	240	300	Total
Pouteria salicifolia											
Phytophthora sp.	0	10	100	100	90	80	0	20	0	0	44.4
Dimorphospora foliicola	0	0	40	20	90	90	30	40	50	30	34.4
Nowakowskiella elegans	0	0	30	40	50	40	30	30	0	40	24.4
Hyphomycete sp.# 1	0	0	0	0	10	30	70	70	0	10	20
Fusarium sp.# 1	0	0	20	60	30	40	0	0	20	10	16.7
Alternaria sp.	70	0	10	20	0	10	0	0	20	0	12.2
Beverwykella pulmonaria	0	10	10	40	0	30	0	0	0	0	10
Cladosporium sp.	70	0	10	0	10	0	0	0	0	0	10
Cylindrocarpon sp.	0	0	30	30	0	10	0	0	10	0	7.8
Pythium sp.	0	10	10	10	10	10	0	10	0	40	6.7
Tricladium anomalum	0	0	20	30	0	10	0	0	0	0	6.7
Endophragmia boeweii	0	0	0	0	0	0	30	20	0	40	5.6
Heliscus submersus	0	0	0	40	0	0	10	0	0	0	5.6
Idriella sp.	0	0	0	0	0	20	0	30	0	0	5.6
Nowakowskiella sp.# 1	0	0	0	10	0	0	10	30	0	20	5.6
Pithomyces chartarum	0	0	10	0	0	0	40	0	30	0	5.6
Abundance	Days										
	T_0	5	10	14	20	30	42	72	90	120	Total
Ligustrum lucidum											
Phytophthora sp.	0	12	42	46	48	46	4	12	10	0	24.4
Pythium sp.	0	16	26	52	4	22	8	18	8	18	19.1
Nowakowskiella elegans	0	18	8	22	12	6	20	4	38	24	16.9
Dictyuchus sp.	0	0	12	0	0	10	16	30	10	26	11.6

Table 2 continued

	T_0	5	14	30	42	72	120	180	240	300	Total
Pouteria salicifolia											
Phytophthora sp.	0	6	80	80	44	44	0	12	8	0	30.4
Dimorphospora foliicola	0	0	12	4	42	20	8	10	44	10	16.7
Nowakowskiella elegans	0	0	10	12	18	22	16	8	0	12	10.9
Hyphomycete sp.# 1	0	0	0	0	2	8	20	16	0	0	5.1

Only the species with FC and A > 5% are showed

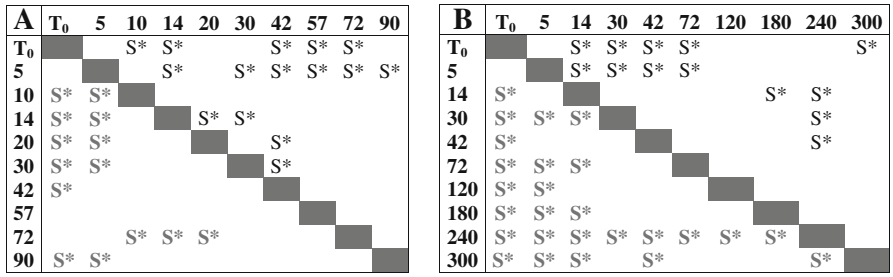

Fig. 2 Differences in the frequency (*left lower part*) and abundance (*right upper part*) between times of exposure. **A** *L. lucidum*. **B** *P. salicifolia*. T_0 leaves before immersion. *S** significant differences ($P < 0.05$)

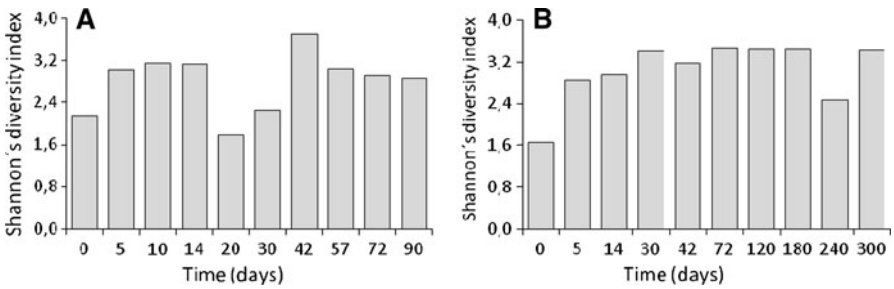

Fig. 3 Shannon's diversity index at each time of exposure. **A** *L. lucidum*. **B** *P. salicifolia*

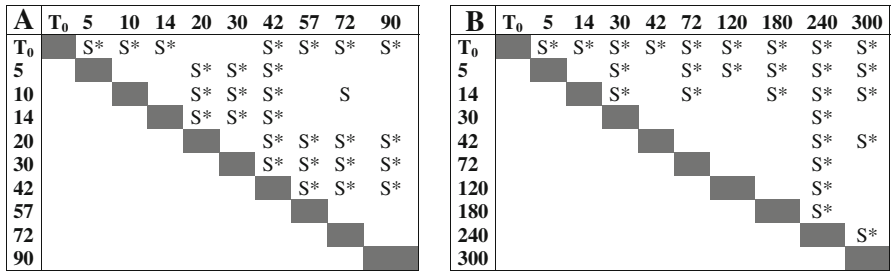

Fig. 4 Differences in the Shannon's diversity indexes between times of exposure. **A** *L. lucidum*. **B** *P. salicifolia*. *S** significant differences ($P < 0.05$)

Diversity and succession

The diversity increased at early stages of decomposition in both plant species. In *L. lucidum* at intermediate stages, it decreased and then reached its maximum, whereas in *P. salicifolia*, it remained practically constant at intermediate stages. After a certain period of immersion, the physical and chemical conditions of the leaves probably changed and allowed the establishment of a more diverse community (Schoenlein-Crusius & Milanez, 1998). The above results are in agreement with those of Dix & Webster (1995) who found that the structure of the fungal community varies both qualitatively and quantitatively during decomposition, with greater diversity in early stages, followed by a stage of

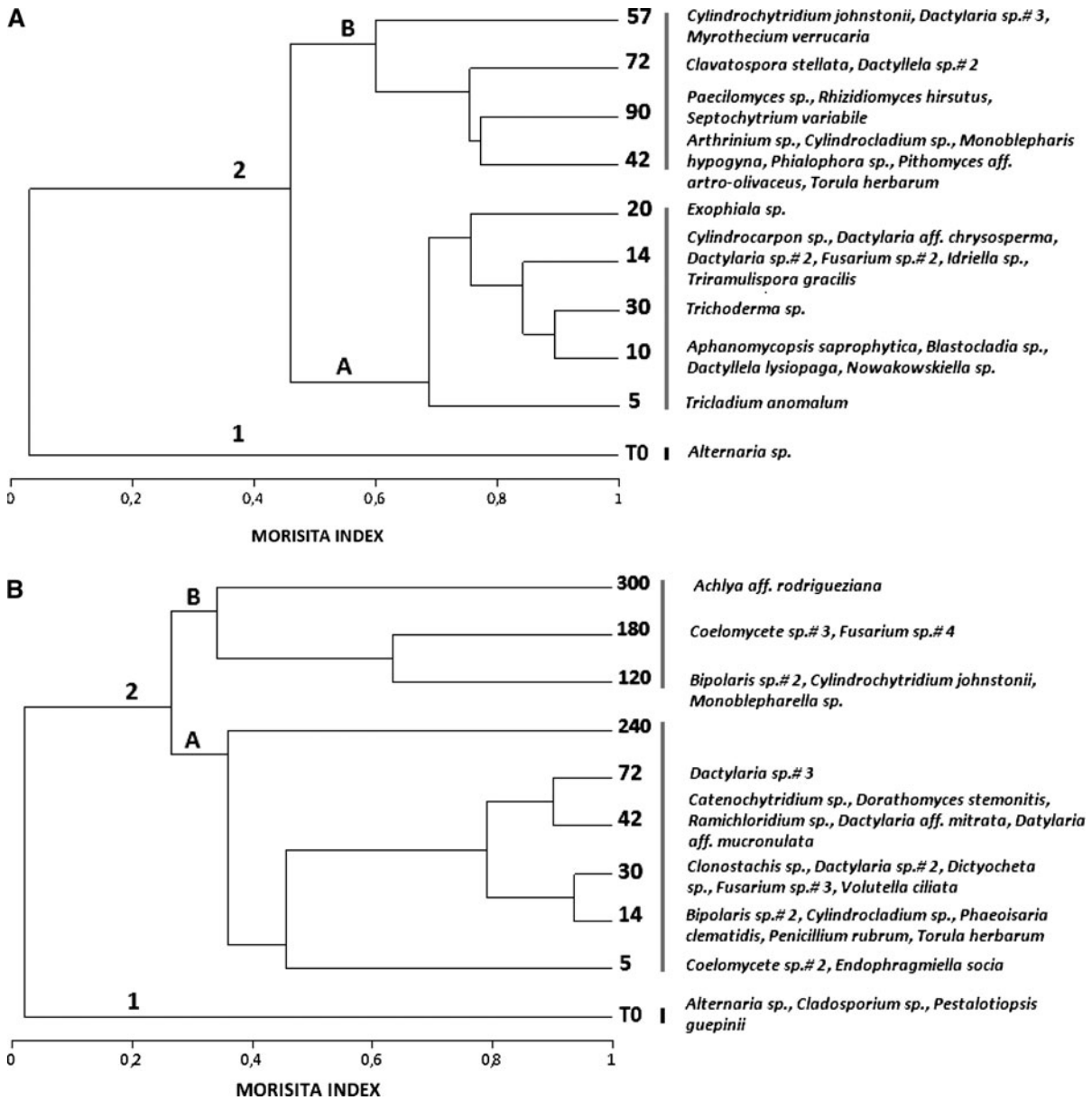

Fig. 5 Cluster analysis by UPGMA algorithm and Morisita index as a similarity measure, for the abundance of species at each time of exposure (days). **A** *L. lucidum*. **B** *P. salicifolia*

stability with the appearance of dominant species, and finally a decline in the diversity. The decrease in the diversity might be explained by their low tolerance to metabolites produced by other species, such as growth inhibitors, toxins, and antifungal substances (Park, 1972; Steciow, 1992).

As observed in this study, and as reported by Newell (1976) and Gessner et al. (1993), a succession of species (mycosere) occurred during leaf decay, implying the existence of distinct niches and/or different strategies of colonization. Successional trends could be recognized from our results: (i) terrestrial mitosporic fungi (*Alternaria* sp., *Cladosporium* sp., and *Pestalotiopsis guepinii*) appeared during the early stages of the decomposition up to 14 days of exposure; (ii) zoosporic fungi, straminipiles, and aquatic Hyphomycetes showed a tendency to colonize the leaves in early-to-intermediate stages,

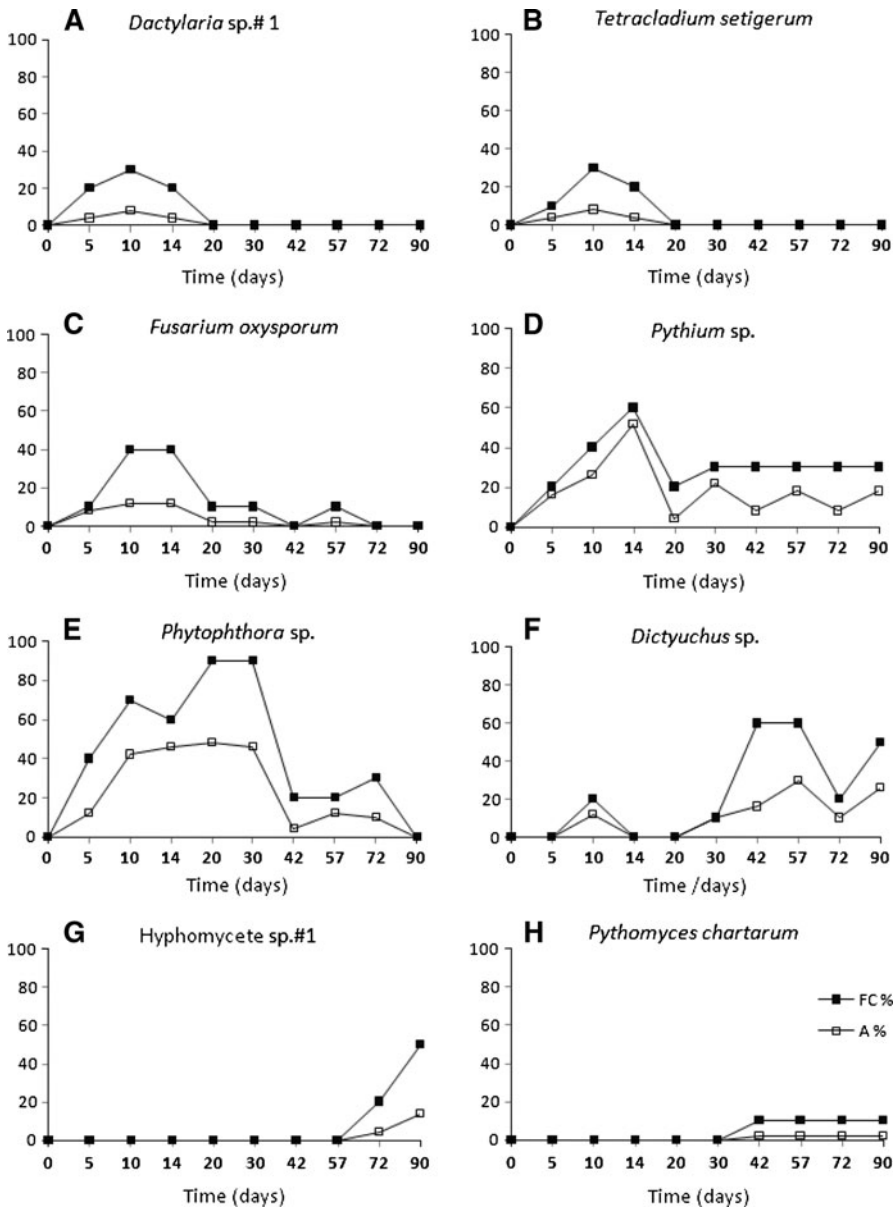

Fig. 6 Frequency of colonization (FC %) and abundance (A %) on *L. lucidum* leaves. **A–D** Early colonizers. **E** Intermediate colonizers. **F–H** Late colonizers

appearing with a low abundance after 5 days of immersion and increasing their abundance after 10 days; and (iii) representatives of the phylum Zygomycota (*Mortierella* sp. and *Mucor* sp.) were observed during the early and latest stages of the decomposition. These results were in agreement with the findings of Kaushik & Hynes (1971), Bärlocher & Kendrick (1974), and Ananda et al. (2008) who observed that some terrestrial Hyphomycetes, such as *Alternaria* and *Cladosporium* (in our case also *Pestalotiopsis guepinii*) were common in senescent and recently submerged leaves. After the immersion of leaves, the number of terrestrial taxa decreased (Bärlocher & Kendrick, 1974; Suberkropp & Klug, 1980) and persisted up to 5–14 days, when they were replaced by aquatic Hyphomycetes. Bärlocher & Kendrick (1974) and Chergui & Pattee (1988), however, observed that terrestrial fungi are normally replaced by aquatic Hyphomycetes within 24 h of immersion. In agreement with our results, Moreira (2006) studying the leaves of *Tibouchina pulchra* (Cham.) Cogn. noted that at first there is a predominance of terrestrial fungi followed by the presence of zoosporic fungi and straminipiles and aquatic

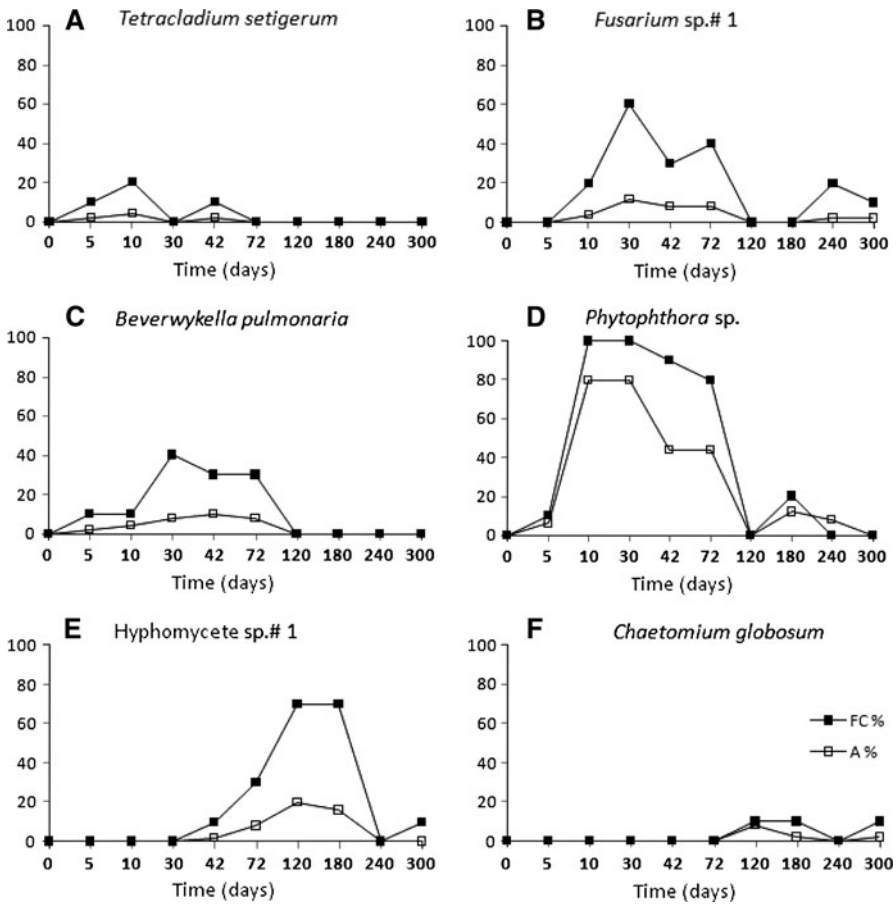

Fig. 7 Frequency (FC %) and abundance (A %) on *P. salicifolia* leaves. **A** Early colonizer. **B–D** Intermediate colonizers. **E–F** Late colonizers

Hyphomycetes. According to Willoughby (1974), both groups are considered early colonizers. Three ecological strategies are recognized by Pugh (1980), Cooke & Rayner (1984), and Dix & Webster (1995): (i) competitive (C-selected); (ii) stress-tolerant (S-selected); and (iii) ruderal (R-selected). Our results suggested a predominantly ruderal life history strategy in aquatic Hyphomycetes, zoosporic fungi, and straminipiles as observed by Newton (1971), Bärlocher & Kendrick (1974), Pugh (1980), Manerkar et al. (2008), and Bärlocher (2009). Both groups are characterized by a short growth phase with high reproductive potential that enable them to quickly colonize ephemeral substrates (such as leaf litter in streams) and complete their life cycle in a short period of time (Gessner et al., 2007). Reproductive structures in ruderals appear to be relatively short-lived, thus the fungi do not survive for long or at high densities in stable, low nutrient, highly competitive substrates, because they must capture the readily available resources quickly. In some zoosporic fungi and straminipiles, when conditions are appropriate for growth, the asexual life cycle is completed rapidly resulting in the release of a large number of zoospores into water (Sparrow, 1960). Since zoospores have finite endogenous energy reserves, they would have to either encyst or attach to an appropriate substrate quickly (Gleason & Lilje, 2009). Lee (1997) found that Chytridiomycota are early colonizers of pollen grains, avoiding thereby competition or competing as ruderals (Pugh, 1980).

Almost all of the Peronosporomycota found in this study (*Dictyuchus* sp., *Pythium* sp., and *Phytophthora* sp.) were present throughout the decomposition process, whereas within the Chytridiomycota, only *Nowakowskiella elegans* was present in both plant species at all of the exposure times. Moreira (2006) also recorded *Pythium* at all times of exposure in the leaves of *Tibouchina pulchra*. However, in this study, some species appeared to be more linked to a particular successional stage (e.g., early, intermediate or late species) by adopting different ecological

strategies. *Pythium* sp. and *Phytophthora* sp. were observed in the initial stages as components of the early–intermediate community (probably R-selected species) in agreement with Moreira (2006), but *Dictyuchus* sp. was recorded with greater frequency and abundance in later stages of the decomposition. *Achlya* aff. *rodrigueziana* was recorded only at 300 days of exposure on the leaves of *P. salicifolia*. However, *Achlya* has been considered by Park (1972) as an early colonizer of substrates. This presence of certain species of Peronosporomycota in later successional stages might be related to their inability to compete with Hyphomycetes at the beginning of the decomposition, appearing later on, when sugar products of the degradation of cellulose and hemicellulose are available (Frankland, 1992). It is known that most species of Peronosporomycota have a lower nutritional complexity than Hyphomycetes, and probably exhibited an S-selected strategy (Cooke & Rayner, 1984; Schoenlein-Crusius et al., 1998; Pires-Zottarelli, 1999). The presence of *Nowakowskiella* sp.# 1 was characteristic of the later stages in *P. salicifolia*. Late species appeared in advanced stages of succession and are characterized by their ability of being adapted to the decrease in nutrients (reduced availability of resources) and possessing enzymes for breaking down complex substances, such as lignin (Frankland, 1992), exhibiting a C-selected strategy. *Catenochytridium* sp., *Cladochytrium replicatum* Karling, and *Cylindrochytridium johnstonii* Karling colonized the leaves after 10 days of exposure, with sporadic occurrences of low abundance at other stages. Newell et al. (1987) and Raghukumar et al. (1995) found that the Chytridiomycota have a low diversity in the decomposed leaves of *Rhizophora mucronata* Lam. and *R. mangle* L. in which the Peronosporomycota (*Halophytophthora* spp.) were dominant. Thus, the three types of life-history strategies defined by Pugh (1980), Cooke & Rayner (1984), and Dix & Webster (1995) are also exhibited by zoosporic fungi and straminipiles during leaf breakdown.

Methodology

The methodology applied to most studies of the fungal diversity on decaying leaves include moist chamber and particle plating, leading to identification of species by means of morphological characterization with conventional microscopy. However, while those techniques might be appropriate for detecting some of the components of the fungal community (such as terrestrial fungi and aquatic Hyphomycetes), they underestimate other groups that the techniques do not detect. In this study, baiting probably underestimated mitosporic fungi and overestimated the presence of zoosporic fungi and straminipiles. Furthermore, most of the fungal biomass on decaying leaves consists of vegetative hyphae and oospores that cannot be identified through microscopy. Molecular methods (DGGE, T-RFLP, Q-RT-PCR) have recently been applied to the analysis of aquatic fungi on leaves and are providing new detailed insights into their diversity. New techniques need to be developed to accurately estimate population sizes of all microbial groups in freshwater ecosystems.

Conclusions

In this study a characteristic fungal community dominated by *Nowakowskiella elegans*, *Phytophthora* sp., and *Pythium* sp. was found on decomposing leaves. The mitosporic fungi showed a greater richness, even though zoosporic fungi and straminipiles were ubiquitous while the mitosporic fungi were less frequent and abundant on both plant species. Early-to-intermediate stages of decomposition had higher species richness, abundance, and diversity than later stages did. Typically terrestrial mitosporic fungi remained until 5–14 days of immersion, being replaced by aquatic Hyphomycetes in the early–intermediate stages. Some of these terrestrial taxa recolonized the leaves in late stages. Differences in frequency and abundance of zoosporic fungi and straminipiles seem to indicate that they have a tendency to colonize the leaves at early-to-intermediate stages of degradation. The phylum Zygomycota was scarcely represented, appearing only at early and late stages, and their role in leaf decomposition, if any, is doubtful.

A combination of various conventional (e.g., baiting, moist chamber, particle plating) and molecular techniques appears to be the more promising approach for characterizing the fungal community structure and for filling the gaps in our knowledge of the contribution of fungal groups to the decomposition of leaves in freshwater streams. Considerable further research is needed to elucidate the importance of zoosporic fungi and straminipiles on leaf litter in streams. Moreover,

since fungal biomass is underestimated because of the lack of ergosterol in Chytridiomycota, Peronosporomycota, and other straminipiles, new methods for estimating the biomass during leaf breakdown are required.

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