Research article

Assemblage of dematiaceous and Ingoldian fungi associated with leaf litter of decomposing *Typha latifolia* L. (Typhaceae) in riverine wetlands of the Pampean plain (Argentina) exposed to different water quality

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ABSTRACT

The purpose of this study was to analyze the rate of sporulation, richness, and spore diversity of dematiaceous and Ingoldian fungi colonizing *Typha latifolia* leaves during a 40-day period of decomposition, as well as the loss of mass in *Typha latifolia,* in four riverine wetlands of Pampean plain (Argentina) with different water quality. Higher sporulation rates, richness, and diversity of the fungi as well as loss of mass of the leaves that they colonized were associated with lower water quality. *Anguilospora longissima, Arthrinium* sp.*, Margaritispora aquatica,* and *Tricellula botryosa* were dominant taxa. Redundancy analysis showed two fungal assemblages related to different environmental conditions. One assemblage was related to higher nutrient levels and higher temperature, characterized mainly by dematiaceous fungi. The other assemblage was related to higher levels of pH and dissolved oxygen, which was mainly represented by Ingoldian fungi. The results obtained in our study demonstrated the link between these fungal assemblages and changes in water quality, revealing their potential as indicators of environmental changes in rivers exposed to different types of land use.

1. Introduction

The decomposition of organic matter in aquatic ecosystems is a complex process carried out mainly by bacteria and fungi together with fragmentation caused by different groups of invertebrates (Hieber and Gessner, 2002; Eloseguí, 2009). Bacteria and fungi are able to produce enzymes that transform complex molecules like cellulose into simple compounds (Romaní et al., 2009). Even though both participate in the process, fungi have a more important role than bacteria since fungi can recycle nitrogen and generally assimilate organic substrates more efficiently than bacteria (Hodge et al., 2000). Fungi are characterized by two main phases: one vegetative and the other reproductive. While the former is important in the colonization and decomposition of available organic substrate, the production of degrading enzymes, and the absorption of nutrients, the latter is characterized by the formation of spores with remarkable morphological diversity and is key to the dispersal and population dynamics of litter-degrading fungi (Roldan et al., 1988; Sigee, 2005).

The sporulation rate of fungi associated with decomposition is a broadly useful marker of their activity in freshwater ecosystems (Romani et al., 2009). The composition and structure of the mycobiota associated with decomposition are highly dependent on substrate composition, physical and chemical water conditions (water temperature, hydrological regime, and various other water quality attributes, including pH and redox condition), and other environmental factors such as the soil, riparian vegetation, and the climate (Pascoal, 2005; Kraus et al., 2011). Anthropic disturbance of streams may also affect the ecosystem structure, leading to the establishment of different fungal assemblages, whose dominant representatives may be potential indicators of ecological disturbance according to the stressor type and its

severity (Sole et al., 2008). Some data are available regarding fungal taxa as biomarkers of water quality, such as those in water polluted by industrial effluent, domestic waste, and other spills.

Different types of land use (e.g., agriculture, livestock farming, urban and industrial development) modify the physical and chemical characteristics of water and influence the biota and functioning of the fluvial systems (Pozo and Elosegui, 2009). Moreover, the nature and availability of substrates for decomposition can influence the frequency and abundance of fungi in aquatic ecosystems (Marano et al., 2011).

The rivers and streams of the Pampean plain (Argentina) are characterized by low flow rates due to the slope of the surrounding terrain, resulting in frequent formation of riverine wetlands (RWs) in the most depressed areas of these watercourses, which are characterized by abundant and diverse aquatic vegetation, a source of organic matter for decomposers (Ringuelet, 1966; Giorgi et al., 2005). *Typha latifolia* (L. 1753; Typhaceae) is one of the emergent macrophytes that frequently develop around these RWs. It is a plant species of world distribution that inhabits swampy soils and that can grow up to 3 m high, flowering mainly in summer (Lahitte et al., 1997). Its senescent leaves, which have low nitrogen content, are recalcitrant and so exhibit a slow decay rate (Alvarez and Becares, 2006). This plant constitutes one of the main sources of autochthonous input to the fraction of organic matter in these water systems.

Numerous RWs in the Pampean plain are threatened by the advance of urbanization and agriculture, affecting the quality of water and altering the natural habitat (Gómez et al., 2016). RWs contribute ecosystem services to the watercourses, such as the recycling of organic matter, thus contributing to the processes of self-depuration, and fungi are considered to be key actors in these processes (Liu, 2015). The objective of this study was to analyze the rate of sporulation and the richness and diversity of spores from the assemblage of dematiaceous (pigmented) and Ingoldian (hyaline) fungi that colonize *T. latifolia* leaves in a 40-day period of decomposition, during summer and early winter, in four RWs with varying water quality as a consequence of different types of land use.

2. Materials and methods

2.1. Study area

The study was carried out in RWs situated in four streams in the Pampean plain (Fig. 1). Two of these streams, the Cajaravillas and the Chubichaminí, are situated in a rural area with extensive livestock production. The other two streams, the del Gato and the Carnaval, are located in suburban areas where there is also agricultural activity, with an intense exploitation of groundwater that leads to the loss of water to these systems. In July the Carnaval RW was dry. During the study period the temperature fluctuated between 6.2 °C (June) and 38 °C (January) and rainfall ranged from 0.5 mm (February) and 112.53 mm (May).

2.2. Litterbag experiment

Senescent leaves from *T. latifolia* were collected before the experiment, in June 2017, in the Cajaravillas RW (site with low anthropic impact). The leaves were processed according to the methodology proposed by Romaní et al. (2009). The litterbag technique (Bocock and Gilbert, 1957) was employed to estimate fungal sporulation rates and loss of litter mass. At each RW eight bags (20 × 25 cm, pore size 1 mm) were submerged and attached to stakes for 40 days, a time period that is considered to be adequate for fungal colonization according to Lee et al. (2002). Three bags (containing 2 g of leaves per unit) were employed for estimating sporulation rate and five (each with 5 g of leaves) were used to calculate the loss of leaf mass. This process was repeated on four consecutive occasions from summer until early winter 2018. Dissolved oxygen, oxygen saturation, pH, temperature, turbidity, conductivity,

and dissolved total solids were measured *in situ* using a HORIBA multiparameter meter. Aliquots were collected from each sample to analyze dissolved inorganic nutrients. They were filtered immediately through glass fiber filters (Whatman GF/C). Water samples to be analyzed for biochemical and chemical oxygen demand (BOD and COD) were also collected and stored at 4 °C until they reached the laboratory.

2.3. Laboratory analysis

The water samples were analyzed to measure soluble reactive phosphorus, nitrite, and ammonia nitrogen using colorimetric determination. Nitrate was reduced to nitrite before its determination. BOD was determined after 5 days incubation at 20 °C and COD by oxidation with potassium dichromate in acidic medium. All determinations were made according to Mackereth et al. (1978) and APHA (1998).

2.4. Rate of fungal sporulation, richness, and diversity

The study of sporulation rates was carried out by triplicate using leaf fragments following the method of Romaní et al. (2009). Spores were identified using specific mycological keys and quantified with a microscope (Olympus BX50). The result was expressed as the number of spores per mg of leaf mass per day. The relative abundance of each fungal taxon was obtained according to Zhang et al. (2004), then placed into one of four groups: dominant (> 50%), more common (> 30–50%), common (> 10–30%), and rare (≤10%) occurrence. Richness and Shannon-Wiener index of diversity (Shannon and Weaver, 1949) were estimated using sporulation data.

2.5. Loss of litter mass

To estimate the loss of mass of *T. latifolia* litter after 40 days, the material in each bag was processed and washed with sterile deionized water to remove any sediment that had accumulated on leaf fragments. The samples were then dried to constant mass at 70 °C and calcined at 500 °C to estimate the ash-free dry mass (Barlocher, 2005).

2.6. Data analysis

Biological data were explored by one and/or two-way Analysis of Variance (ANOVA) and means were compared by the Duncan and Tukey tests, respectively. Principal Component Analysis (PCA) was employed to explore the sampling sites based on environmental variables. These variables were transformed logarithmically $\log_{10} (x+1)$. Redundancy Analysis (RDA) was used to explore the relationship between species composition and the environmental variables. According to Ter Braak & Smilauer (1998) when the gradient length in standard deviation units, in a preliminary detrended correspondence analysis, exceeds two units, unimodal species response curves are to be expected and, therefore, ordination techniques based on weighted averaging are recommended. Species abundance data were $\log_{10} (x+1)$ transformed. Environmental data were standardized. Environmental data were standardized and the inflation factor was < 20 according to ter Braak (1986).

3. Results

3.1. Physical-chemical characteristics of water

An exploratory PCA, whose axes explained 63% of the variation, revealed that the Chubichaminí and Cajaravillas RWs showed higher levels of conductivity, total dissolved solids, and oxygen saturation percentage. On the other hand, the del Gato and Carnaval RWs showed higher nutrient concentrations and BOD_5 as well as turbidity level (Table 1; Fig. 2).
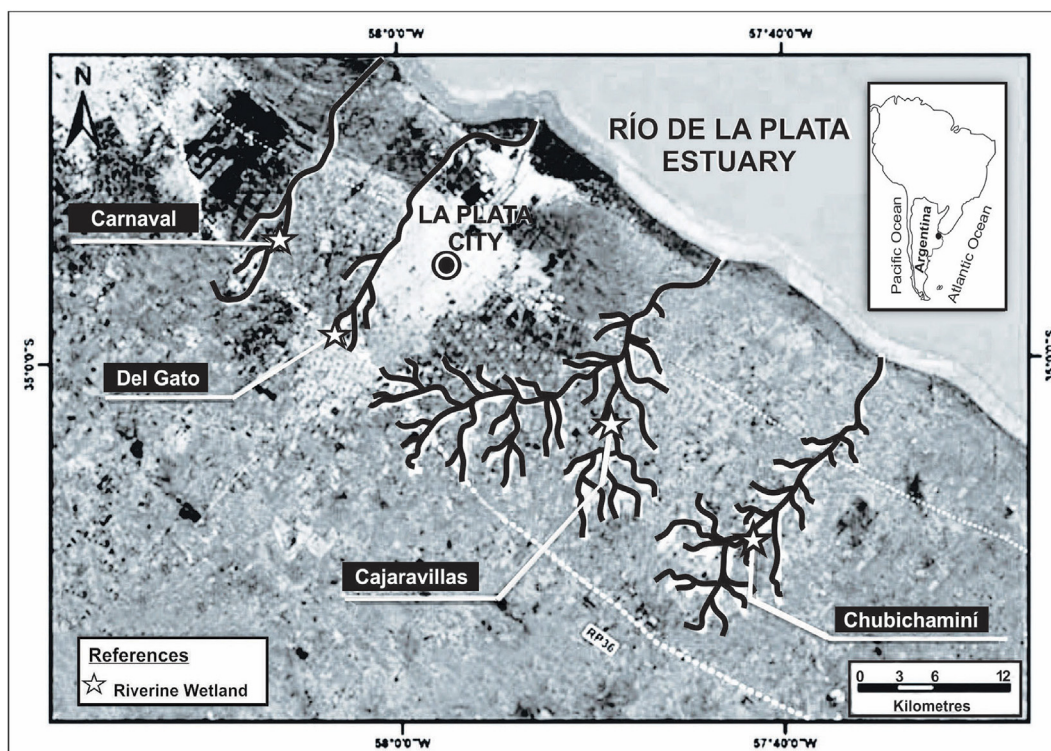
Fig. 1. Study area and location of the riverine wetlands.

3.2. Sporulation rates, richness and diversity of fungal assemblage, and mass loss in T. latifolia

Sporulation rates were higher in May, particularly in the Carnaval RW ($p < 0.05$, Fig. 3B). Significant differences in sporulation rates were found between the Ingoldian and dematiaceous fungal groups in the Chubichaminí, the Cajaravillas, and the Carnaval RWs ($p < 0.05$), being particularly high for the Ingoldian fungi in May in the latter RW (67.9 spore/mg/day). Sporulations rate of each fungus in the four RWs was variable (Supplementary material), though the highest ones were mainly found *Amniculicola longissima* (55.44 spore/mg/day) and *Flabellospora* sp. (3.81 spore/mg/day) in the Carnaval RW in May, *Margaritispora aquatica* (6.82 spore/mg/day) in the del Gato RW, *Zalerion* sp. 1 (4.24 spore/mg/day) in the Cajaravillas RW, and *Arthrinium* sp. (6.56 spore/mg/day) and *Fibulotaeniella* sp. (6.68 spore/mg/day) in the Chubichaminí RW. Seventy-six fungal taxa were identified, 23 being Ingoldian hyphomycetes and the others dematiaceous (Table 2).

Fibulotaeniella sp. was the only representative of the Basidiomycota and the others identified belonged to the Ascomycota. The richness of fungal taxa was significantly higher in the del Gato RW compared to that of the Chubichaminí and Carnaval RWs ($p < 0.05$; Fig. 3C). However, the abundance of fungal taxa was variable in each RW during the sampling period. While *Amniculicola longissima* (= *Anguilospora longissima* Ingold) was dominant in the Carnaval RW in May and in the Chubichaminí RW in July, *Arthrinium* sp. dominated in the Cajaravillas RW in March, *Margaritispora aquatica* was dominant in the del Gato RW in May and in the Chubichaminí RW in March, and *Tricellula botryosa* was dominant in the Chubichaminí RW in February (Table 2). A two-way ANOVA revealed that the diversity index of the fungal assemblages showed an interaction between RWs and sampling time ($p < 0.05$). H′ was significantly higher in the del Gato RW than in the Carnaval in May, than in the Cajaravillas RW in March, and than in the Chubichaminí RW in March and July. Only in the Carnaval and del Gato RWs H′ was significantly higher in February and March compared with

Table 1

Average values, in brackets maxima and minima, of the water physical and chemical parameters of the riverine wetlands. CHU: Chubichamini; CAJ: Cajaravillas; GAT: del Gato; CAR: Carnaval.

|	CHU	CAJ	GAT	CAR
DO (mg/l)	5.13 (10.0–2.5)	7.23 (10.6–2.8)	3.3 (4.21–1.7)	4.7 (5.6–4)
Oxygen saturation (%)	52 (100–27)	70 (97–34)	33 (38–20)	45 (52–41)
pH	7.6 (8.3–7.2)	7.9 (8.6–7.2)	7.3 (8.2–6.8)	7.9 (9.2–6.6)
Temperature (°C)	15 (24–8)	15 (24–8)	15 (19–10)	17 (27–9)
Turbidity (UNT)	131 (273–29)	60 (83–24)	156 (403–51)	198 (429–79)
Conductivity (µS/cm)	828 (952–576)	772 (886–518)	396 (647–154)	433 (842–204)
TDS (mg/L)	531 (609–368)	495 (570–331)	273 (414–135)	280 (543–132)
PO_4– P (mg/L)	0.23 (0.26–0.14)	0.32 (0.61–0.09)	0.968 (1.202–0.772)	1.41 (1.416–1.07)
NO_3–N (mg/L)	0.04 (0.09–0.01)	0.06 (0.09–0.03)	0.05 (0.06–0.05)	0.06 (0.09–0.02)
NO_2–N (mg/L)	0.02 (0.04–0.00)	0.03 (0.05–0.02)	0.08 (0.21–0.03)	0.039 (0.74–0.02)
NH_4–N (mg/L)	0.09 (0.16–0.05)	0.08 (0.16–0.00)	0.50 (1.40–0.07)	0.04 (0.09–0.001)
Total P (mg/L)	0.27 (0.40–0.20)	0.43 (0.68–0.33)	1.02 (1.12–0.91)	1.76 (2.00–1.38)
Total N (mg/L)	2.11 (2.68–1.67)	2.44 (3.03–1.90)	11.48 (13.67–9.33)	10.01 (11.43–8.53)
BOD_5 (mg O_2/L)	7.5 (12–3)	7 (19–1)	9.75 (21–5)	17 (18–16)
COD (mg O_2/L)	59 (67–50)	53 (84–40)	56 (75–48)	130 (228–31)
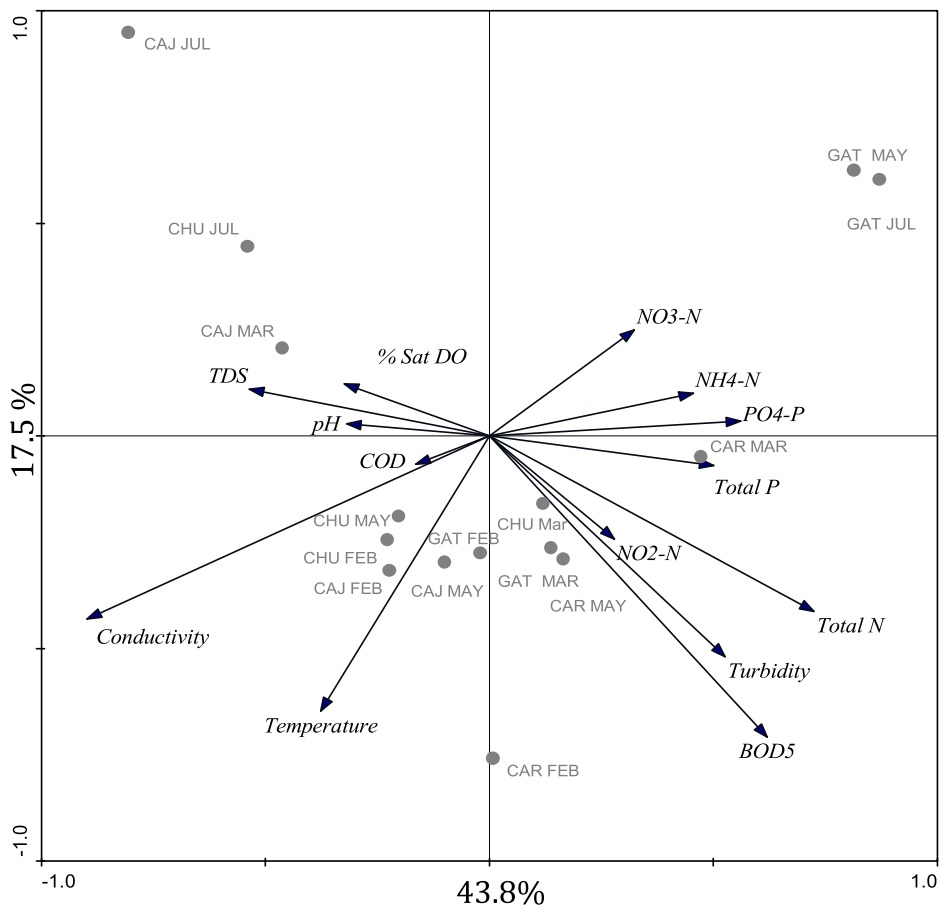
Fig. 2. PCA ordination of the sampling sites at each time according to the physicochemical variables of water. (CAJ: Cajaravillas; CAR: Carnaval; CHU: Chubichamini; GAT: del Gato. FEB: February; MAR: March; MAY: May; JUL: July).

that in May (see Fig. 3D).

In relation to the loss of mass of *T. latifolia* leaves, higher percentage values were observed during the summer period (February and March),

being significantly higher in the del Gato RW compared to the Carnaval RW ($p < 0.05$, Fig. 3A).

RDA showed that the first two axes explained 64.9% of the
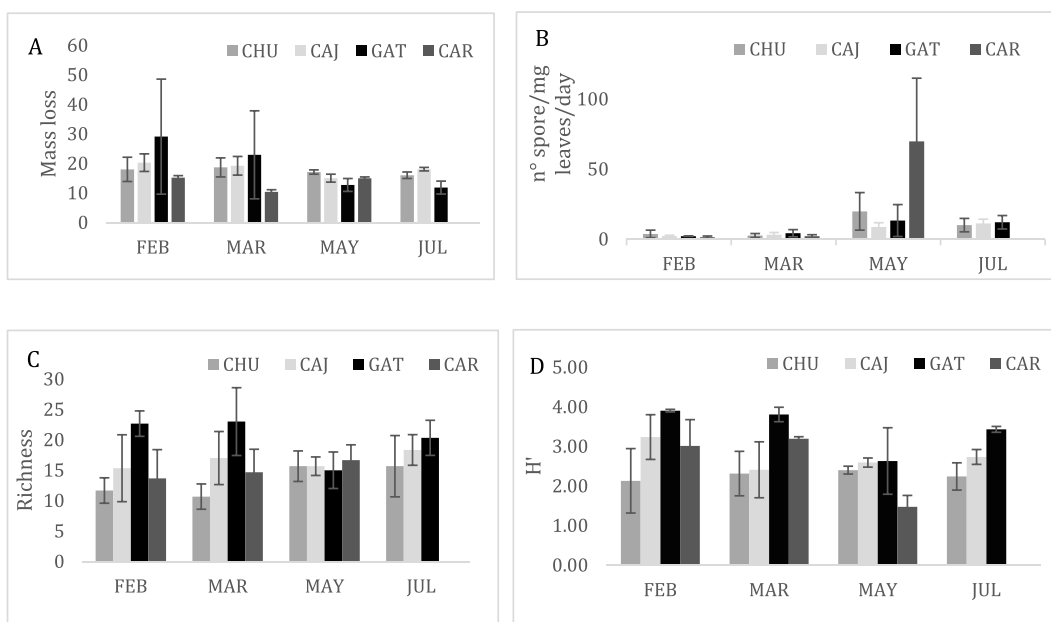

Fig. 3. Means ± SD of mass loss of *T. latifolia* litter (A), sporulation rate (B), richness (C) and diversity index (H'; D) of the fungi in riverine wetlands. CHU: Chubichamini; CAJ: Cajaravillas; GAT: del Gato; CAR: Carnaval.

Table 2
List of taxa, acronyms and relative abundance of Ingoldian fungi ([i]) and dematiaceous fungi ([d]) found in each riverine wetland at a time ([a]). CHU: Chubichamini; CAJ: Cajaravillas; GAT: del Gato; CAR: Carnaval. Four asterisks, dominant (> 50%); three asterisks, more common (> 30–50%); C, two asterisks common (> 10–30%); one asterisks, rare (≤10%) occurrence.

Fungal Taxa	Acronyms	CHU	CAJ	GAT	CAR
Amniculicola longissima[i] *(Sacc. & P. Syd.) Nadeeshan & K.D. Hyde*	AMLG	∗∗∗∗Jul,a	∗∗∗Jul	∗∗Jul	∗∗∗∗May
Gemmulina botryosa[i] *(Descals) Descals & Marvanová*	GEBT	∗∗∗∗Feb	∗Feb	∗Jul	∗May
Margaritispora aquatica[i] *Ingold*	MGAQ	∗∗∗∗Mar	∗∗Mar	∗∗∗∗May	∗May
Arthrinium[d] sp.	ARTM	∗∗∗May	∗∗∗∗Mar	∗∗Feb	∗∗Feb
Zalerion[d] *sp. 1*	ZAL1	∗∗Mar	∗∗∗May	∗Feb	∗Feb
Fibulotaeniella[i] *sp.*	FIBU	∗∗∗May	∗∗Jul	∗∗Jul	∗May
Torula[d] sp.	TORL	∗Jul	∗May	∗∗Mar	∗∗Feb
Cordana[d] sp.	CORD	∗Mar	∗Mar	∗Feb	∗∗Mar
Diplococcium[d] *sp. 2*	DIP2	∗Mar	∗Mar	∗Mar	∗∗Feb
Diplococcium[d] *sp. 1*	DIP1	∗Feb	∗Feb	∗Feb	∗Mar
Curvularia[d] sp.	CURV	∗Feb	∗Feb	∗Feb	∗Mar
Tetraploa aristata[d] *Berk. & Broome*	TEAR	∗Jul	∗Feb	∗Mar	∗Feb
Stemphylium[d] sp.	STEM	∗Jul	∗Feb	∗Mar	∗Feb
Bipolaris[d] sp.	BIPO	∗Feb	∗Feb	∗Feb	∗Mar
Alternaria[d] sp.	ALTR	∗Jul	∗Jul	∗Mar	∗Mar
Alatospora acuminata[i] *Ingold*	ALAC	∗May	∗Jul	∗Jul	∗Jul
Endophragmiella biseptata[d] *(Peck) S. Hughes*	ENBI	∗Mar	∗May	∗Mar	∗Mar
Sigmoidea[i] *sp.*	SIGM	∗Mar	∗Feb	∗Feb	∗Feb
Brachysporium[d] sp.	BRAC	∗May	∗Jul	∗Feb	∗Feb
Truncatella[d] sp.	TRUN	∗Mar	∗Jul	∗May	∗Jul
Endophragmiella globulosa[d] *(B. Sutton) S. Hughes*	ENGL	∗Mar	∗Mar	∗Feb	∗Feb
Sporidesmium socium[d] *M.B. Ellis*	SPSO	∗Jul	∗May	∗Mar	∗Feb
Fusarium[i] sp.	FUSA	∗Feb	∗Feb	∗Feb	∗May
Beltrania[d] sp.	BELT	∗Feb	∗Feb	∗Feb	∗Feb
Dictyosporium[d] *sp.1*	DIC1	∗May	∗Jul	∗May	∗Mar
Oncopodium[d] sp.	ONCO	∗Feb	∗Feb	∗Mar	∗Mar
Tetraploa ellisii[d] *Cooke*	TEEL	∗May	∗May	∗Feb	
Lemonniera[i] *sp. 1*	LEM1	∗May	∗Jul		∗Mar
Tetracladium marchalianum[i] *De Wild*	TEMA	∗Jul	∗Jul		∗May
Tricladium[i] *sp.*	TRIC	∗Feb		∗Jul	∗May
Endophragmiella boothii[d] *(M.B. Ellis) S. Hughes*	ENBO	∗Mar		∗Mar	∗Mar
Brachysporium britannicum[d] *S. Hughes*	BRAB	∗May		∗Mar	∗Mar
Pseudotetraploa[d] sp.	PSEU		∗May	∗May	∗May
Sporidesmium[d] *sp. 3*	SPO3		∗Jul	∗May	∗May
Dictyoarthrinium sacchari[d] *(J.A. Stev.)*	DISA		∗Feb	∗Mar	∗Mar
Sporidesmium[d] *sp.4*	SPO4		∗Feb	∗Mar	∗May
Dictyoarthrinium africanum[d] *S. Hughes*	DIAF		∗May	∗Mar	∗Mar
Spegazzinia[d] sp.	SPEG		∗May	∗Mar	∗Mar
Sporidesmium[d] *sp.1*	SPO1	∗Mar	∗Feb		
Clavatospora[i] *sp.*	CLAV	∗Mar	∗Mar		
Tricladium anomalum[i] *Ingold*	TRAN	∗May	∗May		
Sporidesmium[d] *sp. 2*	SPO2	∗Mar	∗Feb		
Lemonniera[i] *sp 4*	LEM4		∗Jul		∗May
Brachysporium obovatum[d] *(Berk.) Sacc*	BROB			∗Feb	∗Mar
Tridentaria[i] *sp.*	TRID			∗Jul	∗May
Dictyosporium elegans[d] *Corda*	DIEL		∗Feb	∗Feb	
Diplococcium[d] *sp. 3*	DIP3		∗May	∗Mar	
Endophragmiella cambrensis[d] *M.B. Ellis*	ENCA		∗Mar	∗Feb	
Zalerion[d] *sp. 2*	ZAL2		∗May	∗Jul	
Heliscella stellata[i] *Ingold & V.J. Cox*	HEST	∗Jul		∗Mar	
Triscelosphorus[i] *sp.*	TRIU	∗Jul		∗Jul	
Beltraniella[d] *sp. 2*	BEL2	∗Mar		∗Feb	
Tetraploa abortiva[d] *Aramb. & Cabello*	TEAB	∗Jul		∗Feb	
Lemonniera[i] *sp.2*	LEM2	∗Jul			∗May
Sporidesmium bicolor[d] *(Peck) Pound & Clem*	SPBI		∗Feb		∗Feb
Diplocladiella[d] sp.	DIPL		∗Feb		
Cumulospora[i] sp.	CUMA		∗Feb		
Clathroconium[d] sp.	CLAT		∗Jul		
Beltraniella[d] *sp. 1*	BEL1		∗Mar		
Dictyosporium digitatum[d] *J.L. Che*	DIDI		∗Feb		
Dictyosporium zeylanicum[d] *Petch*	DIZE		∗May		
Dictyosporium tetraseriale[d] *Goh, Yanna & K.D. Hyde*	DITE		∗Mar		
Lemonniera centrosphaera[i] *Marvanová*	LECE		∗Jul		
Brachisporium dinglayae[d] *s. Hughes*	BRAD			∗Jul	
Bispora[d] sp.	BISP			∗Feb	
Pseudobeltrania[d] *sp.*	SEBE			∗Feb	
Sporidesmium cookei[d] *(S. Hughes) M.B. Ellis*	SPOC			∗Feb	
Sporidesmium coffeicola[d] *M.B. Ellis*	SPOF			∗Feb	
Sporidesmium leptosporum[d] *(Sacc. & Roum.) S. Hughes*	SPOL			∗Feb	
Dictyosporium triseriale[d] *Matsush*	DITR			∗Jul	
Dictyosporium[d] *sp. 2*	DIC2			∗Feb	

Table 2 (*continued*)

Fungal Taxa	Acronyms	CHU	CAJ	GAT	CAR
Lemonniera[i] sp.3	LEM3			*Jul	
Flabellospora[i] sp.	FLAB				*May
Melanocephala[d] sp.	MELA				*Mar
Helicosporium[i] sp.	HELI				*May
Tricladium patulum[i] Marvanová & Marvan	TRIP				*May

cumulative variance of the relationship between fungi and environmental variables, with the first axis (41.1%) linked to higher nutrient levels (total N and total P), higher organic matter content (COD), and higher temperature. In relation to this axis an assemblage of fungi was found, which, according to the concentration gradient, was formed by *Endophragmiella globulosa, Dictioartrynium africanum, Tricladium* sp., *Stemphylium* sp., *Brachysporium obovatum, Sporidesmium leptosporum, Lemonniera centrosphaera, Dictyosporium digitatum, Sporidesmium coffeicola, Dictyosporium zeylanicum, Tricladium patulum, Tricelophorus* sp., *Sigmoidea* sp., *Beltrania* sp., *Dictyosporium elegans, Brachisporium britanicum, Tricladium anomalum, Lemonniera* sp. 4, *Pseudotetraploa* sp., *Dictyosporium tetraseriale, Diplocladiella* sp., *Tetraploa abortiva, Brachysporium* sp., *Beltraniella* sp. 2, *Helicosporium* sp., *Diplococcium* sp. 3, *Bispora* sp. 1, *Dictyosporium* sp. 2, *Lemonniera* sp. 3, *Tridentaria* sp., *Endophragmiella boothii, Spegazzinia* sp., *Cumulospora* sp., *Clathroconium* sp., *Sporidesmium cookei, Clavatospora* sp., *Lemonniera* sp. 1, *Endophragmiella cambrensis, Beltraniella* sp. 1, *Pseudobeltrania* sp., *Dictyosporium triseriale, Endophragmiella biseptata, Melanocephala* sp., *Sporidesmium* sp. 2, *Heliscella stellata, Sporidesmium* sp. 3, *Sporidesmium bicolor, Tetraploa ellisii, Lemonniera* sp. 2, *Tetracladium marchalianum, Brachisporium dinglayae, Curvularia* sp., *Dictioartynium sacchari, Bipolaris* sp., *Diplococcium* sp. 2, *Cordana* sp., *Pseudobeltrania* sp., *and Fusarium* sp. At the other extreme, *Zalerion* sp. 1 was associated with lower COD concentrations, total N, and total P. The second axis of the RDA plot, which explained 23.8% of the accumulated variance, was linked mainly to higher levels of pH and dissolved oxygen. Fungal taxa such as *Amniculicola longissima, Alatospora acuminata, Zalerion* sp. 2, *Fibulotaeniella* sp., *Sporidesmium socium, Flabellospora* sp., *Dictyosporium* sp. 1 and *Margaritispora aquatica* were associated with higher concentration of these variables Fig. 4A.

4. Discussion

According to these results, the Chubichamini and Cajaravillas RWs gathered a better quality of water, with higher concentrations of dissolved oxygen and lower concentrations of BOD, nitrogen and phosphorus and higher conductivity values due to the contribution of more mineralized groundwater. In the other RWs, Del Gato and Carnaval, worse water quality was observed as a consequence of enrichment with organic matter and nutrients.

The loss of mass of *T. latifolia* leaves exposed to the four RWs over a 40-day period, as well as sporulation rates of dematiaceous and Ingoldian fungi and their associated richness and diversity, were high in water with low quality, such as observed in the del Gato and the Carnaval RWs, with higher nutrient levels and biological oxygen demand and low levels of dissolved oxygen.

It is known that anthropic impact can modify the diversity, composition, and activity of aquatic fungal assemblages (Pascoal, 2005). Pollution in freshwater environments usually produces lower taxa richness and diversity, especially in aquatic fungi, though it is dependent on the type of pollution to which they are exposed. While exposure to heavy metals has a negative effect, higher concentrations of other nutrients can have a positive effect (Lecerf and Chauvet, 2008).

The structure of the fungal assemblage in the del Gato RW, showed higher values of richness and diversity, due to high concentrations of nutrients, particularly ammonium, which was found between 6 and 12 times higher than what was observed in the other RWs, These observations are compatible with those found by Gulis and Suberkropp (2004) and Noel et al. (2016), who pointed out that the enrichment of a watercourse with nutrients such as nitrogen and phosphorus causes an increase in the richness and diversity of aquatic hyphomycetes. However even today there are controversies about this trend, such as Sridhar et al. (2009) that found higher diversity of these fungi in a stream exposed to agricultural runoff with lower concentration of nutrients when compared to another with higher content.

Analyzing the fungal assemblage colonizing *T. latifolia*, we found more dematiaceous fungi (70%) than Ingoldian (30%) ones in the water. These results are in agreement with results reported by Marano et al. (2011) for the Cañas stream, located in the Pampean plain. The dematiaceous fungi are considered typical of the colonization of the leaf litter prior to their submersion in the water. Tthey can also come from other sources such as water, soil particles among others. While the Ingoldian fungi are mainly aquatic forms associated with the water column and the substrate, they can also exist as endophytic asymptotic forms of different plants, including non-aquatic species (Chauvet et al., 2016).

The decay rate of litter is a parameter usually considered a key indicator for assessing functional stream integrity (Gessner et al., 2002), the percent of litter mass remaining after a defined period can also be used in the same way (Maltbu et al., 1995; Jonson et al., 2001). In our study, the mass loss of *T. latifolia* leaf fragments was significantly higher in summer in the del Gato RW, a stream that showed the highest amounts of total nitrogen, particularly ammonium. It is known from the literature that leaf material from different species of *Typha* is recalcitrant to degradation and low in nitrogen content (Alvarez and Becares, 2006). Therefore, nitrogen compounds available in water, such as those found in the del Gato RW, might be a key chemical factor that modulates the decomposition processes of *T. latifolia* material together with temperature. In agreement with our results, temperature has been recognized in other studies as a determining factor in decomposition (Graca et al., 2015).

In relation to fungal colonization, Gessner (2005) has proposed a sequence of phases that includes adhesion of spores to the substrate followed by their germination and the subsequent differentiation of hyphae that penetrate and so colonize the substrate. As a consequence of this process, it is common to find a peak of fungal activity on submerged litter after four weeks of incubation (Barlocher, 2012). It is also known that transformation of the substrate or changes in the environment can affect the activity of the fungi colonizing the substrate, as well as the activation of sporulation processes mainly associated with the characteristic conidiophores of each fungal taxa. Our results have shown that fragments of *T. latifolia* leaf submerged in water at phosphorus-rich sites (> 1 mg/L) for 40 days, with average temperatures of 15 °C, were able to generate *in vitro* the highest concentrations of conidia, such as those recorded in the Carnaval RW. Our results were coincident with observations of Suberkropp (1991) and Gulis and Suberkropp (2003) who also reported an increase in the production of conidia in streams with higher phosphorus concentrations (between 0.034 and 0.084 mg/L). However, Abelho and Graça (2006) observed that the addition of phosphate at 0.029 mg/L did not stimulate the sporulation rates of aquatic hyphomycetes, though its combination with nitrogen was effective.
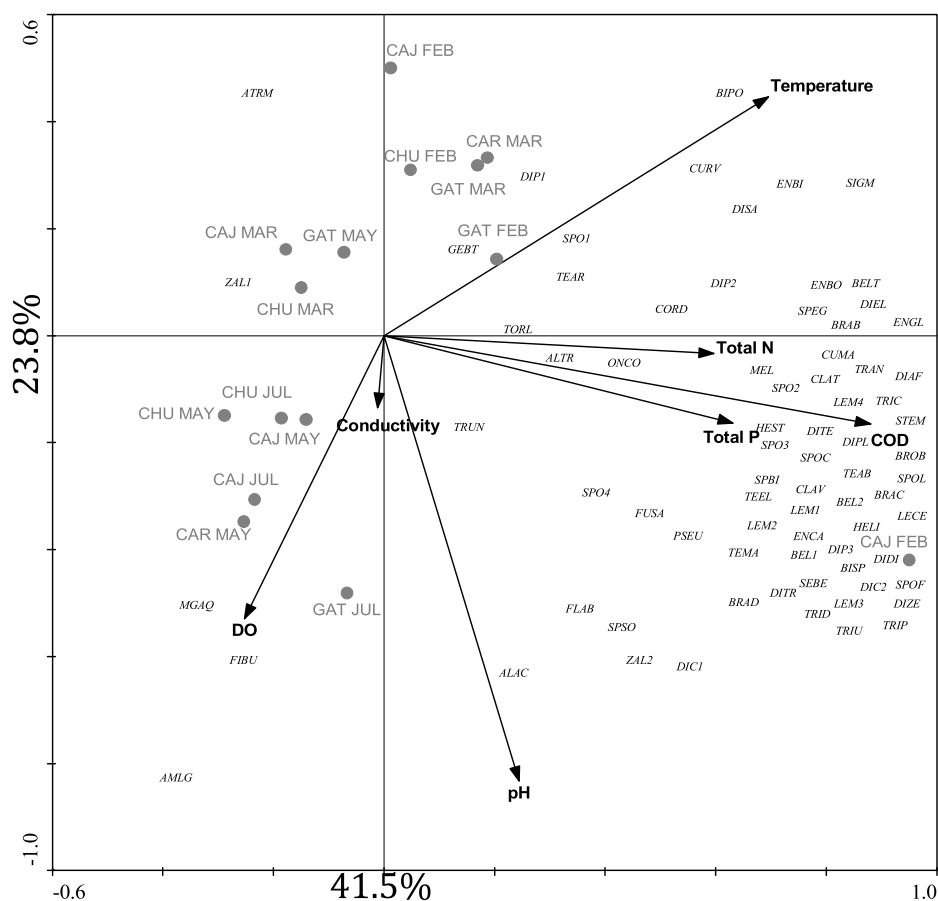
Fig. 4. Ordination diagram displaying the first two axes of the RDA with environmental variables and percentage of abundance of taxa. For acronyms of the taxa, see Table 2; CHU: Chubichamini; CAJ: Cajaravillas; GAT: del Gato; CAR: Carnaval . FEB: February; MAR: March; MAY: May; JUL: July.

The effect of seasonality on the production of conidia (Thomas et al., 1996) and on the decomposition of leaves in aquatic ecosystems (Jaques and Pinto, 1997) are also recognized in literature. In our study, decomposition was greater during the summer and these results are coincident with those reported by Alvarez and Becares (2006).

Conversely, sporulation rates were higher in autumn and early winter. During this period we recorded taxa such as *A. loggisima* and *A. acuminata*. These taxa were reported by Suberkropp in 1984 as occurring in a winter assembly in an annual study with leaves introduced every four weeks into a small hard water stream, Augusta Creek in Michigan.

RDA delineated two fungal assemblages according to the physico-chemical parameters and abundance of taxa found in the RWs. Assemblages linked to higher concentrations of nutrients and organic matter were represented by tolerant species such as *L. centrosphaera* and *T. marchalianum* (Sole et al., 2008). *T. marchalianum* has been found in conditions of severe contamination in rivers in Germany (Sidrhar et al., 2000). The other assemblage, related to lower concentrations of organic matter and nutrients, was represented by less tolerant species such as *A. longissima*, recognized by Solé et al. (2008) as an indicator of low concentrations of dissolved and total organic carbon (DOC and TOC) and chlorides.

Previous studies of the mycobiota composition of the streams and other fresh water ecosystems in the Pampean plain have demonstrated the existence of numerous dematiaceous fungi, however they provided little data about Ingoldian fungi (Cabello et al., 1990; Marano et al., 2011, Kravetz et al., 2018). Nevertheless, for the first time we report here the occurrence of *G. botryosa, L. centrosphaera, M. aquatica,* and *T. patulum* in a freshwater ecosystem in Argentina.

5. Conclusion

The results obtained in our study demonstrate the link between the fungal assemblages, diversity, richness, total and partial sporulation rates of the specific taxa, with changes in water quality. These observations suggest the potential use of Ingoldian and dematiaceous fungi assemblages as indicators of environmental changes in RWs exposed to different types of water quality. However, it is necessary to know more about the ecology of these fungi to understand their role in the decomposition processes of *T. latifolia* leaves and its relationship with environmental conditions.

Acknowledgments

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jenvman.2019.109409.

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