Invertebrates and microbiota associated with aquatic macrophyte degradation in a shallow lake in southern Brazil

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Abstract

Aquatic macrophytes are the main producers of organic matter in shallow aquatic ecosystems. They are also food sources for many herbivores. When macrophytes die, they enter the debris chain, are conditioned by microbial action and colonized by benthic invertebrates which remobilize nutrients from their biomass. In subtropical aquatic systems, the participation of shredder invertebrates has been questioned, highlighting the participation of fungi and bacteria in the degradation of organic matter. This study evaluated the degradation of two submerged aquatic macrophytes, *Mayaca fluviatilis* and *Stuckenia pectinata*, determining the quality of debris and microbiota and invertebrate trophic group density throughout the degradation process. Our results indicated that plants with lower polyphenol concentrations had higher degradation speeds. The shredders invertebrates had reduced abundance in both macrophytes, emphasizing the importance of bacteria and fungi in the nutrient cycling process in subtropical shallow lakes.

Keywords: Bactéria, decomposition, detritivorous invertebrates, fungi, Mayaca fluviatilis, Stuckenia pectinata.

Invertebrados e microbiota associados à degradação de macrófitas aquáticas em um lago raso no sul do Brasil

Resumo

Macrófitas aquáticas são os principais produtores de matéria orgânica em ecossistemas aquáticos rasos, sendo fonte alimentar para uma série de herbívoros. Após sua morte, entram na cadeia de detritos, são condicionadas por ação microbiana e colonizados por invertebrados bentônicos, responsáveis pela remobilização dos nutrientes de sua biomassa. Em sistemas aquáticos subtropicais, a participação de invertebrados fragmentadores tem sido questionada, ressaltando a participação de fungos e bactérias na degradação da matéria orgânica. Este trabalho teve como objetivos avaliar a degradação de duas macrófitas aquáticas submersas, determinando a qualidade do detrito e a densidade da microbiota e de grupos tróficos de invertebrados ao longo do processo. Os resultados indicaram que a planta com menor concentração de polifenóis teve velocidade maior de degradação. Os invertebrados fragmentadores tiveram abundância reduzida em ambas macrófitas, ressaltando a importância de bactérias e fungos no processo de ciclagem de nutrientes em lagos rasos subtropicais.

Palavras-chave: Bactérias, decomposição, fungos, invertebrados detritívoros, Mayaca fluviatilis, Stuckenia pectinata.

Introduction

In the southern coastal plain of Brazil, approximately 40% of the land comprises shallow lakes and wetland ecosystems, with aquatic macrophytes present in high abundance and biodiversity (Rolon & Maltchick 2006). These plants are the main primary producers, with large biomass and high growth rates (Albertoni, Palma-Silva, Trindade, & Furlanetto, 2014). They are consumed by an extensive range of herbivores, including vertebrates and invertebrates (Lodge, 1991). After they die, they become debris and act as food resources and habitats for detritivores, mainly invertebrates, with fundamental roles in nutrient cycling and incorporation in primary production (Esteves & Gonçalves, 2011; Carvalho,

Hepp, Palma-Silva, & Albertoni, 2015).

Throughout the decomposition process, coarse particulate organic matter (CPOM), autochthonous or allochthonous, is transformed into fine particulate organic matter (FPOM) (Gonçalves, Martins, Ottoni, & Couceiro, 2014). This transformation occurs through basic mechanisms such as leaching, conditioning by microbial action and colonization by benthic invertebrates (Webster & Benfield, 1986; Graça, Bärlocher, & Gessner, 2005). Decomposition rates may be accelerated by several factors: the influence of environmental variables, nutrient levels (e.g. nitrogen and phosphorus) (Andersen, Grasset, Thormann, Rochefort, & Francez, 2010; Overbeek *et al.*, 2018), the presence of

chemical inhibitors (such as polyphenols and tannins), and the structure of microbial communities or aquatic invertebrates (Graça *et al.*, 2005; Gonçalves Jr *et al.*, 2014).

In temperate climates, shredder invertebrates are important for the decomposition of vegetal debris (Graça *et al.*, 2005; Gonçalves Jr, Graça, & Callisto, 2006). In tropical and subtropical environments, the virtual absence or low density of this group has been demonstrated for different debris, e.g. in aquatic macrophytes (Silva, Silveira, Palma-Silva, & Albertoni, 2010; Carvalho *et al.*, 2015; Albertoni, Hepp, Carvalho, & Palma-Silva, 2018) and tree species (Telöken, Albertoni, & Palma-Silva, 2011; Telöken, Hepp, Palma-Silva & Albertoni, 2014). In ecosystems with low shredder densities, microbial conditioning during the decomposition process is very important (Telöken *et al.*, 2014; Santschi, Gounand, Harvey, & Altermatt, 2017; Fogelman, Bilger, Holt, & Matlaga, 2018).

In this context, we sought to determine leaf degradation coefficients, and evaluate the presence and relative importance of microbiota associated with the decomposition of two aquatic macrophyte species, *M. fluviatilis* and *S. pectinata*. Our hypothesis proposed that plants with higher quality debris (low polyphenol and high nitrogen concentrations) would decompose faster, favoring a higher density of bacteria and fungi. We also investigated the abundance of invertebrate functional feeding groups associated with this detritus, anticipating low shredder densities, reinforcing the importance of microbiota in aquatic macrophyte degradation in shallow subtropical aquatic ecosystems.

Materials and Methods

Study Area

The study was performed in a small urban lake (32°01'40"S, 52°05'40"W) located in the Carreiros Campus of the Federal University of Rio Grande - FURG, Rio Grande, Rio Grande do Sul, Brazil. The lake has an approximate area of 1 ha and a maximum depth of 1.6 m, which fluctuates according to annual precipitation, averaging between 1200 and 1500 mm (Alvares, Stape, Watches, Gonçalves, & Sparovek, 2013). The climate of the region is subtropical humid (Cfa, according to the Köppen classification), with an average annual temperature of 13°C in winter and > 22°C in summer (Alvares *et al.*, 2013). The lake has oligo-mesotrophic characteristics, with low primary productivity and low nutrient concentrations (Palma-Silva *et al.*, 2013).

Field sampling and processing

The submerged aquatic macrophytes; *S. pectinata* (L.) Börner and *M. fluviatilis* Aubl., were collected and air dried in the laboratory for approximately two weeks. To determine degradation coefficients, we incubated materials in litter bags. Bag dimensions were 20 cm x 30 cm, with 1 cm² holes in the upper mesh and 1 mm² holes in the lower mesh. We used 20 litter bags for each plant species, and incubated 10 g of debris (initial dry mass). The bags remained in the lake at an average depth of 50 cm from the sediment in January 2016. On incubation days 1, 3, 7 and 18, we removed five sample replicates and measured limnological variables using a

multiparameter probe (Horiba®). During the study, we measured mean dissolved oxygen in the lake; 8.61 ± 0.37 mg.L⁻¹, electrical conductivity 71.2 ± 13.5 µS.cm⁻¹, temperature 25.02 ± 0.37 °C, turbidity of 53.8 ± 2.65 NTU, and pH of 6.37 ± 1.69 .

For each collection, one bag was used for microbial debris analysis, and four to determine dry mass and leaf degradation coefficient (k) (Graça *et al.*, 2005), and associated invertebrates. Dry mass was determined by oven drying at 35°C to a constant mass. Invertebrates were separated from the debris using a sieve (mesh 250 µm) and fixed in 80% Rose Bengal stained alcohol. They were screened using a stereomicroscope, and identified to the lowest possible taxonomic level (Merritt, Cummins, & Berg, 2008; Domínguez & Fernández, 2009). We also determined nitrogen concentrations, total phosphorus and organic carbon in the debris, according to Rice, Baird, Eaton, & Clesceri (2012), and polyphenol levels following the method of Graça *et al.* (2005).

For microbial analysis, we followed the methodology of Hickenbick, Ferro, and Abreu (2004). We fixed biological material in tubes, using 1 cm² of *S. pectinata* leaf and four leaves of *M. fluviatilis* in 2.5 mL 3.7% formaldehyde. Afterwards, the material was sonicated in an ultrasonic homogenizer (Qsonica®) adjusted to 25 W, with an amplitude of 60 A. Samples on ice, were sonicated over two 30-second pulses, with 10 second intervals.

Samples were then vacuum filtered through a nucleopore membrane filter (0.2 μm pore) and rendered darker with "Irgalan Black". In a laminar flow hood, the filtered samples were stained with 10 drops of acridine orange fluorochrome for 10 min, and then filtered and washed twice in distilled water to remove excess dye. To generate triplicate semi-permanent slides, the dry filters containing samples were placed on a histological slide with a drop of mineral oil, and sealed with a coverslip and enamel. The samples were identified, and slides were stored at 5 °C for preservation until counts were made.

Slide samples were counted under a fluorescence light microscope (Olympus BX51), using a WB optical filter, DM500 dichroic mirror, BP450–480 nm excitation filter, and BA515 barrier filter. Thirty bacteria fields and 150 fields were photographed to assess the abundance of hyphae and fungal spores, respectively. All fields were randomly selected across slides. For counting, we used the ImageJ® program (Wayne Rasband, National Institutes of Health, USA, Version 1.48d).

Data Analysis

To determine the degradation coefficient (k), we used the exponential decay model, $M_t = M_0^{e^{-kt}}$, where M_t is the remaining mass at time t (days), M_0 is the initial mass, e is the basis of the Napierian logarithm, and k is the degradation coefficient (Graça *et al.*, 2005). We constructed a linear regression to fit the initial dry mass (air-dried) with the dry mass of those processed during incubation periods (ovendried). After test the data normality, the nitrogen, phosphorus, carbon and polyphenol concentrations between the two plants were compared using Student's t-tests, for

each day of debris removal.

Invertebrates associated with the debris were investigated using taxa richness, and the Shannon diversity index (H') (Magurran, 2004), and categorized according to functional feeding groups (FFG) (Tomanova, Goitia, & Heles, 2006; Merritt, Cummins, & Berg, 2008; Domínguez & Fernandez, 2009). Organism densities between the two plants for each period, were compared using one-way ANOVA, and diversity indices were assessed using Student's t-tests with Monte Carlo randomization. Microbiota densities over time between the two plants were compared using two-way ANOVA, where factor one was microbiota density (bacteria, hyphae and spores) and factor two was time (days 0, 1, 3, 7, 18). All data analyses were performed on free Past software for scientific data analysis (Hammer, Harper, & Ryan 2001).

Results and Discussion

The degradation rate was similar for the two plants until the fourth day, when *S. pectinata* demonstrated greater mass loss. By the 18th day of the degradation period, *M. fluviatilis* showed a 40% loss of dry mass, at a degradation rate of 0.114 d⁻¹. During this same period, the mass loss of *S. pectinata* was 67%, at a rate of 0.243 d⁻¹, indicating a decomposition process approximately twice as fast as *M. fluviatilis* (Figure 1).

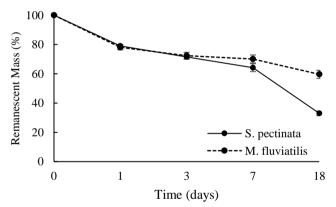


Figure 1. Dry mass decay (bar = standard error) of *S. pectinata* and *M. fluviatilis* debris over indicated decomposition times, in a shallow subtropical lake in southern Brazil (0 to 18 = incubation days).

In an extensive review of degradation rates in aquatic macrophytes in Brazil, Gonçalves-Jr. *et al.* (2014) did not mention either species used in their research. Other studies performed near the study site, cited the degradation rate of *S. pectinata* (syn. *Potamogeton pectinatus*) as $k = 0.019 \, d^{-1}$ (Carvalho, Hepp, Palma-Silva, & Albertoni, 2015).

Despite its abundance in lentic water bodies throughout Brazil, data on *M. fluviatilis* decomposition are outdated, been cited 80% of remained mass after 40 days decomposition, in a tropical reservoir in summer (Silva, Oliveira, Escarpinati, Fonseca-Gessner, & Paula, 2011)Several factors influence the rate of decomposition in aquatic systems; temperature (Song, Yan, Cai, & Jiang, 2013), nutrient concentrations (Webster & Benfield, 1986), and the composition of decomposing microbial communities (Shilla, Asaeda, Fugino, & Sanderson, 2006).

Nutrients

Debris nutrient concentrations differed between both macrophyte plants at each incubation period. Phosphorus levels were higher in *S. pectinata* (p = 0.048), and for both plants there was a decrease in phosphorus levels at experiment end. Phosphorus concentrations in *S. pectinata* ranged from 39.59 to 19.59 $\mu g.g^{-1}$, with maximum levels on the seventh day of incubation. Phosphorus concentrations in *M. fluviatilis* ranged from 27.53 to 12.30 $\mu g.g^{-1}$, peaking on the third day of incubation.

However, nitrogen concentrations in debris showed contrary behaviors between the two plants, with M. fluviatilis showing debris enrichment throughout decomposition, only decreasing on the 18th day. As with phosphorus, there were significant differences between nitrogen concentrations in the two plants (p = 0.044). In M. fluviatilis, nitrogen concentrations ranged from 1.67 (initial concentration) to 2.78 mg.g⁻¹ (third day of incubation). In S. pectinata, values ranged from 1.51 (first day) to 2.56 mg.g⁻¹ (18th day).

Organic carbon also showed significant differences between the two plants (p=0.008). Levels ranged from 7.42% to 2.91% in *S. pectinata*, and from 5.19% to 2.04% in *M. fluviatilis*. Unlike *M. fluviatilis*, *S. pectinata* detritus showed a tendency to increase organic carbon concentrations, which may reflect increases in fungal hyphae densities, contributing to higher concentrations of carbon and other nutrients, incorporating the biomass of these microorganisms.

Polyphenol levels were lower in M. fluviatilis at experiment end (1,567 UDO g- 1 Dry Mass) when compared to S. pectinata (2,967 UDO g- 1 DM) (p = 0.036). Polyphenol concentrations are indicators of debris palatability, as these compounds act as plant defenses (Lodge, 1991).

Invertebrates

We identified 25 invertebrate taxa associated with debris of the two plants. Nineteen were found in M. fluviatilis and 14 in S. pectinata (Table 1). The taxa found in M. fluviatilis had a higher diversity index (H'=2.01). The dominant organism in M. fluviatilis came from the Chironomidae (50%), predominantly Chironominae (collector), with 28% relative abundance, followed by Tanypodinae (predator, 22%) and copepod Cyclopoida (collector, 13%) (Table 2). In contrast, S. pectinata had a 14 taxa richness and a lower diversity index (H=1.84, p<0.010). The predominant groups were Tanypodinae (32%), Chironominae (20%) and $Cypridopsis\ vidua$ (Ostracoda, 15%), although these levels were only recorded at the beginning of the degrading process (Table 2). Diversity indices showed significant differences (p=0.010) between debris from the two plants.

The functional feeding groups found were predator (P), collector (C), scraper (S), and shredder (Sr). Predators were predominant in both macrophytes, with 49% and 44% in *M. fluviatilis* and *S. pectinata*, respectively. Collectors represented 42% and 39%, and shredders represented 2% and 0.4%, respectively in *M. fluviatilis* and *S. pectinata*. Scrapers represented 11% of organisms in both debris. This relative proportion of FFGs in debris was similar to other foliar degradation studies conducted in the same lake

(Telöken *et al.*, 2011). The low abundance of shredders participating in foliar degradation processes confirmed the results of previous studies in subtropical lakes (Silva *et al.*, 2010; Telöken *et al.*, 2011; Carvalho *et al.*, 2015; Albertoni *et al.*, 2018). Several authors have shown that the detritivorous invertebrate communities that colonizes debris, are poorly represented by shredders in tropical and subtropical regions (Capello, Marchese & Ezcurra de Drago, 2004; Gonçalves Jr. *et al.*, 2006; Carvalho & Uieda, 2009). Decomposition at low latitudes means increased participation of microbiota (bacteria and fungi) as decomposers, with little influence from detritivorous fragmentation (Irons, Oswood, Stout, & Pringle, 1994).

Table 1. Presence (+) of detrivorous invertebrates associated with *Mayaca fluviatilis* and *Stuckenia pectinata* debris in a shallow subtropical lake in southern Brazil. FFG: Functional feeding groups, predator (P), collector (C), scraper (S), shredder (Sr)

Taxa	M. fluviatilis	S. pectinata	FFG
Nematoda	+		P
Hirudinea Glossiphoniidae	•	+	P
Oligochaeta	+	+	С
Hydracarina	+	+	P
Platyhelminthes Turbellaria	+		P
Mollusca Planorbidae	+		S
Mollusca Ampullaridae		+	S
Cladocera Daphnidae		+	C
Copepoda Cyclopoida	+	+	P
Ostracoda <i>Cipridopsis</i> vidua		+	C
Ostracoda Cytheridella islovayi	+		Sr
Ostracoda <i>Strandesia</i> cuspida	+		Sr
Ephemeroptera Caenidae	+	+	S
Ephemeroptera Baetidae	+		S
Trichoptera Leptoceridae	+		C
Trichoptera Hidroptilidae	+	+	C
Odonata Coenagrionidae	+		P
Odonata Aeshnidae		+	P
Hemiptera Cicadellidae	+		P
Hemiptera Saldidae		+	P
Coleoptera Hydrophilidae	+		P
Diptera Dolichopodidae	+		P
Diptera Culicidade		+	Sr
Diptera Chironominae	+	+	C
Diptera Tanypodinae	+	+	P

Due to the phenolic toxicity of some plants, mainly from tannins, these compounds inhibit herbivore consumption, as in high concentrations the plants are unpalatable to phytophages, inhibiting the action of digestive enzymes and leaf consumption (Monteiro, Allbuquerque, & Araújo 2005). The great richness and diversity of macrophytes with lower polyphenol concentrations, reinforces the role of these compounds in herbivory resistance. However, this richness and diversity is not reflected in decomposition rates, as they are influenced by higher numbers of shredder invertebrates, which use leaf debris as food (Carvalho & Uieda, 2009). Our FFG data showed a virtual absence of this invertebrate group, which may be related to their influence on decomposition speeds. As no herbivory occurred directly, other groups (collectors, scrapers and predators) were favored by debris, using it primarily as a habitat, and the macrophyte with lower decomposition speed favors the establishment of a more diverse community.

Table 2. Succession of invertebrates in detritus from *Mayaca fluviatilis* (M) and *Stuckenia pectinata* (S) over decomposition times in a shallow subtropical lake in southern Brazil (1 to 4 = 0 to 18 incubation days).

Taxa	Stuckenia pectinata				Mayaca fluviatilis			
Taxa	S 1	S2	S 3	S4	M1	M2	M3	M4
Oligochaeta	0	0	1	3	2	5	6	58
Ostracoda	35	3	0	1	2	1	4	0
Copepoda	1	22	6	3	11	49	8	9
Caenidae	0	0	15	13	1	6	18	36
Chironominae	5	5	3	38	6	3	53	101
Tanypodinae	0	6	52	25	14	23	35	53
Others	0	8	3	5	30	2	9	30

Microbiota

Microbiota densities (bacteria, hyphae and spores) were higher in *M. fluviatilis* macrophyte (Table 3). Our results showed that microbial colonization behaviors in debris was different between the two macrophytes. Although a significant difference was observed between plants in terms of hyphae density (*M. fluviatilis* had the greater density, *p* = 0.005), bacterial colonization was higher at the beginning of the decomposition process in *S. pectinata*, whereas for *M. fluviatilis*, the highest density was fungal hyphae (Table 3). When we evaluated the relative abundance of microbiota, it was noted that, independent of the density, fungi (hyphae and spores) were highly important for the degradation process of *S. pectinata*. On the other hand, for *M. fluviatilis*, bacterial colonization tended to increase in importance relative to the end of the degradation process of this plant.

Although it is accepted that fungi dominate microbial communities in riverbeds and streams at decomposition onset (Suberkroop & Klug, 1976), several studies of lakes have shown that bacteria are dominant in early decomposition periods (Shilla, Asaeda, Fujino, & Sanderson, 2006). Several studies have shown that microbial colonization of macrophyte detritus contrasts between streams and lakes and wetlands, where fungi are the main decomposers in streams, whereas bacteria are in lentic systems (Gaur, Singhal, & Hasija, 1992). Romaní, Fisher, Mille-Lindblom, & Tranvik (2006) demonstrated that complex interactions between fungi and bacteria occurred in

aquatic habitats.

The qualitative structure of debris (cell wall composition, polysaccharides and supporting tissues) could cause alterations between higher bacterial activity as decomposers of more fragile supporting structures (Schlickeisen, Tietjen, & Arsuffi, 2003). Aquatic hyphomycetes (fungi) dominate vegetal decomposition in streams, because their hyphae penetrates leaf surfaces to enzymatically degrade complex carbon structures (Gessner & Chauvet, 1994). These authors also state that bacteria colonize the debris later, due to poor invasive capacity, as they are more associated with debris surfaces. Our results showed evidence of increased bacterial colonization at the beginning of *S. pectinata* degradation processes, and increased bacterial colonization importance at the end of *M. fluviatilis* degradation processes.

While *S. pectinata* displayed higher polyphenol concentrations, it generated lower densities of microbiota organisms. Although this macrophyte had higher nitrogen concentrations initially, this suggested that debris conditioning was primarily influenced by polyphenol concentrations. Fungal colonization predominates in plants with higher nitrogen concentrations (Cummins, Petersen, Howard, Wuycheck, & Holt, 1973).

The release of carbon and nutrients through the decomposition of submerged aquatic macrophyte debris is a critical process in shallow lake ecosystems, as these plants represent one of the largest components of organic matter in these ecosystems (Song, Yan, Cai, & Jiang, 2013).

Table 3. Density (ind.cm⁻² x 10⁶) and relative abundance (%) of bacteria, hyphae, and spores during decomposition of *Stuckenia pectinata* (S) and *Mayaca fluviatilis* (M) in a shallow subtropical lake, in Brazil.

Microbiota	Stuckenia pectinata				Mayaca fluviatilis					
	S0	S1	S2	S3	S4	M0	M1	M2	M3	M4
Density (ind.cm ⁻² x 10 ⁶)										
Bacteria	6.02	2.35	1.12	5.97	0.54	2.71	3.57	3.25	4.71	6.89
Hyphae	2.34	1.77	1.63	0.68	0.36	6.55	9.11	7.29	1.64	5.63
Spores	0.94	0.10	2.37	4.06	0.18	4.65	1.94	4.53	1.25	0.65
Relative Importance (%)										
Bacteria	64.8	55.6	21.9	55.8	50.5	19.5	24.5	20.7	62.0	52.1
Hyphae	25.1	42.0	31.8	6.4	33.1	47.1	62.4	46.5	21.6	42.6
Spores	10.1	2.3	46.3	37.9	16.5	33.4	13.3	28.8	16.5	5.0

We showed that macrophytes displayed differences in nutritional content, and in the richness and diversity of detritivore invertebrates. These differences did not corroborate previous studies conducted in larger numbers in streams, especially for direct relationships between higher degradation rates, lower polyphenol concentrations and higher nitrogen concentrations, which increases debris palatability (Biasi et al., 2013). In their study, Rezende et al. (2019) found a positive correlation between hyphomycete colonization invertebrate richness, as a function of increased debris palatability, and a high density of shredder invertebrates as influencers of degradation velocity. Some factors may account for these differences: e.g. the low density of shredder invertebrates in tropical and subtropical lentic systems (Gonçalves, Graça & Callisto 2007; Albertoni, Hepp, Carvalho & Palma-Silva 2018). Longer degrading macrophytes may favor the permanence of a more structured debris-associated community (Slight, Moretti, Gonçalves, & Callisto 2010) and may explain the higher density and richness of invertebrates in M. fluviatilis debris. Differential microbiota colonization during the degradation process may also be an influencing factor, as both plants showed distinct behaviors in relation to the main microbiota groups: bacteria levels tended to increase in M. fluviatilis, while fungi levels increased in density in S. pectinata. Fungi and bacteria act together in the decomposition and mineralization of debris in aquatic environments, with complex interactions between degradation involving decomposers, which may be antagonistic (competitive) or synergistic (facilitative) (Mille-Lindblom & Tranvik, 2003). Future studies should quantify the growth rates of different microbiota groups, evaluating interactions between the different decomposers.

Conclusion

The role of different functional feeding groups of invertebrates and microbiota in the degradation of organic matter may be related to the characteristics of debris material. Our hypothesis was partially confirmed as nutrient concentration was less influential on invertebrate colonization and conditioning by microorganisms. Our results also showed a low abundance of shredder invertebrates in subtropical shallow lake ecosystems, emphasizing the importance of fungi and bacteria in degrading organic matter. Although our work has spatial and temporal limitations (performed in one lake and over a specific period), we believe our data indicates patterns of microbiota succession in aquatic macrophyte detritus, in shallow aquatic ecosystems, such as shallow lakes and wetlands. These data relate the abundance of microbiota in aquatic macrophyte debris in subtropical environments, and while preliminary, our data indicates that more research on ecological processes could elucidate the roles of invertebrates and microorganisms in nutrient cycling and energy flow in these ecosystems.

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