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Diversity of Cellulose-Degrading Microorganisms in Submerged Forest Litter Decomposition

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Abstract: Although reservoirs, floodplains and riparian backwaters are known to be significant sources of coarse particulate carbon, the microbial communities responsible for cellulose breakdown under long-term flooding are still poorly understood. The diversity, succession and enzymatic potential of cellulose-degrading microorganisms colonizing leaf litter in a reservoir-inundated riparian forest of southern Western Ghats, India were compared. Three sites at the littoral were deployed with litter bags containing leaves of Tectona grandis and Terminalia arjuna and the litter bags were retrieved at 0, 15, 30, 60, 90 and 120 days. The mass loss of the litter, water physiochemistry, and extracellular cellulase activities (CMCase, FPase and β-glucosidase) and microbial diversity of cellulolytic microorganisms were quantified using a combination of 16S rRNA and ITS2 amplicon sequencing and Congo red plate screening of cultivable microorganisms. The teak, arjuna and bamboo litter decay rates were 0.0118, 0.0096 and 0.0071 d-1, respectively, with the initial value of lignin:N ratio accounting for 81% of variance in the decay rate. In total, 142 cellulolytic isolates were found, with 41 bacterial and 27 fungal strains with high hydrolysis capacity. During the active cellulolytic phase, bacterial communities evolved from Proteobacteria-dominated early colonizers to Bacteroidota, Actinomycetota and Firmicutes which occurred at days 15 and 60, and aquatic hyphomycetes dominated the fungal communities from days 15 to 60. The highest activity of the CMCase was observed at day 60, and this activity was related to the fungal Shannon diversity ($r = 0.84$, $p < 0.001$). The results indicate that bacterial–fungal succession control cellulose turnover in submerged litter and cellulolytic diversity can be used as a functional indicator of the health of inundated forest ecosystems.

Keywords: Cellulolytic Microorganisms; Submerged Litter; Leaf Litter Decomposition; Aquatic Hyphomycetes; Cellulase Activity; Western Ghats; Reservoir Ecosystems.

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I. Introduction

One of the most important energy bases of aquatic food webs is the forest litter entering standing and flowing waters. Allochthonous leaves, twigs and bark often surpass the contribution of in-situ primary production to supply organic carbon in forested headwaters and inundated margins of reservoirs, and the rate at which they are processed dictates nutrient availability, oxygen demand and transfer of energy to invertebrates and fish (Tank et al., 2010). A massive amount of litter is submerged at one time, and when forest land is flooded, be it seasonally along floodplains or permanently behind dams, the decomposition of this litter will influence the water quality for many years after the flooding. The biological agents of this breakdown are central to understanding and assessing aquatic habitat from an ecosystem perspective and to managing the impacts of these agents. The process of leaf litter decomposition in water is a multi-stage process that includes leaching of soluble compounds, conditioning by microorganisms, fragmentation by shredding invertebrates and physical degradation by abrasion. Throughout these stages, it has been acknowledged that decomposer organism diversity plays a vital role in determining decomposition rate and stability; decomposers have complementary enzymatic and trophic functions (Gessner et al., 2010). Structural polysaccharides of plant cell walls (mainly cellulose and hemicellulose) make up 40-60% of the dry mass of leaves and their hydrolysis controls the release of most of the carbon from litter. The utilization of microbial cellulose is achieved by a synergistic action of a system of enzymes: endoglucanases, exoglucanases (cellobiohydrolases), and β-glucosidases Lynd et al., (2002) that break insoluble crystalline cellulose to soluble sugars. It is phylogenetically diverse (found in some, but not all, aerobic and anaerobic bacteria and in many filamentous fungi) (Wilson, 2011). Fungi, especially the aquatic hyphomycetes, have long been thought to be the primary colonizers and conditioners of submerged leaves in freshwater habitats.

These fungi have a large number of extracellular enzymes and abundantly produce spores on dead leaves and make dead leaves more palatable to invertebrate shredders (Krauss et al., 2011). In streams with biomass partitioning, fungi normally constitute a much higher proportion of microbial production on the leaf surfaces than do bacteria, although the proportion of bacterial production increases with softening and fragmentation of the litter (Hieber & Gessner, 2002). Bacteria and fungi do not occur singly, but in interaction; the extracellular enzyme activities exhibited in decomposing litter are different and follow different patterns over time depending on the interactions among bacteria and fungi (Romaní et al., 2006). The effects of microbes are also modified by environmental factors, e.g., dissolved nutrient enrichment stimulates the growth of fungi and litter loss in experimental reaches in streams (Gulis & Suberkropp, 2003). The cellulolytic machinery of basidiomycetes and ascomycetes are very different, and these differences can account for the different cellulolytic efficiencies under various environmental conditions (Baldrian & Valášková, 2008). The majority of this knowledge is from fast-flowing streams with good oxygen content in the temperate zone. A different environment is found in submerged forest litter in reservoir margins and tropical backwaters, where the water is relatively slow flowing, the litter is deposited in thick layers, the litter–sediment interface may become hypoxic, and the high summer temperatures coupled with warm temperatures in winter create an environment conducive to high microbial activity. The relative abundance of fungi and bacteria, the predominant cellulolytic taxa and the season in which maximum cellulase activity may occur may differ from those in temperate streams under these conditions. The cellulolytic microbiota of submerged litter in the Western Ghats of peninsular India, a global biodiversity hotspot where many reservoirs have flooded riparian and moist deciduous forests, has been little investigated and previous studies have mostly been based on cultivation. The disparity restricts the predictability of the managers for the turnover of organic matter, depletion of oxygen and release of nutrients in newly impounded and seasonally flooded forest habitats. Comprehensive amplicon sequencing in high throughput is a tool to make culture-based screening more useful for taxonomic diversity–to-function connection. While plate assays reveal which organisms can break down cellulose, and to what extent, sequencing identifies much more of the community that can't be grown in the lab and records the changes in these organisms over time as the cellulose breaks down. Activities of extracellular cellulases are then measured directly on the litter itself, linking community structure to realized ecosystem function.

Objectives

In the present study, the following objectives are set to cope with this gap in the knowledge:

- To measure the decomposition rates of three major litter components under natural littoral conditions and to make a relationship with the initial litter quality.
- To describe the diversity and cellulolytic activity of cultivable bacteria and fungi colonizing submerged litter as well as the succession of total bacterial and fungal community using amplicon sequencing.
- To test the relationships between microbial diversity, extracellular cellulase activity and litter mass loss, and to examine the potential of cellulolytic diversity as a functional indicator of ecosystem health in inundated forest habitats.

In Section I, Introduction, the ecological background of submerged litter decomposition and the research gap were set. In the first part (Introduction), the ecological background of the decomposition processes of submerged litter and the research gap were set. In Section II, ‘Materials and Methods', the study area, the litter bag experiment, the physicochemical, microbiological, enzymatic and molecular procedures are described and the statistical framework is summarized. Section III, Results, includes a table and figure to illustrate decomposition dynamics, diversity of cellulolytic isolates and community succession and enzyme activity. In the Discussion (Section IV), these results are discussed in the context of the literature and what they mean for the management of aquatic ecosystems. The main results are summarized in the Conclusion (Section V), and directions for future research are indicated.

II. Materials and Methods

Study Area

The study was carried out in the littoral zone of a reservoir in the southern Western Ghats of Tamil Nadu, India, which has been permanently and seasonally flooded over a band of riparian moist deciduous forest along the reservoir bank. The climate is considered a tropical monsoon type, with the bulk of the rainfall coming from the south west monsoon (June–September) and a lesser amount from the north east monsoon (October–December). The flooded edge is occupied by dead standing trees, and an uninterrupted supply of leaf litter from the current riparian canopy of tall teak (*Tectona grandis*), arjuna (*Terminalia arjuna*) and thorny bamboo (*Bambusa bambos*). Three sampling locations (S1–S3) were located along the coast line, a minimum distance of 800 m from each other, with water depths of 1.0–1.5 m at the time of sampling and soft, organic rich substrate. Site S1 was situated near a forested inflow channel, S2 was in a sheltered embayment with build-up of thick litter and S3 was along the open shoreline exposed to wind and water action.

Experimental Design and Litter Bag Deployment

The fresh leaves of the three species were picked from the forest floor next to the reservoir, dried to constant weight and stored in paper bags. Air-dried leaves were subdivided into portions and placed in nylon litter bags (20 × 20 cm) with a mesh size of 5 mm to enable the colonization by microorganisms and invertebrates, while minimizing the loss of fragments. Subsamples were oven dried at 60 °C to get an air-dry to oven-dry conversion factor for each species, and other subsamples were dried in the air to determine initial litter chemistry. A total of 162 litter bags were deployed (3 species x 3 sites x 6 retrieval dates x 3 replicates). The position of naturally submerged litter was reproduced by tying bags to nylon ropes attached to the substrate, which lay on the sediment surface. At 0, 15, 30, 60, 90 and 120 days 3 bags of each species were collected from each of the sites – handling control (collected after 24 hrs). On retrieval, each bag was put into a sterile polyethylene bag and stored in an insulated box with ice with a maximum of 4 h until the laboratory. Leaves were carefully washed with sterile reservoir water to wash away any sediment or invertebrates that may have stuck on the leaf surface. A total of 15 leaf discs (12 mm diameter) were cut from each bag using a sterile cork borer, five of which were used for the isolation of microorganisms, 5 used for the determination of enzymes and 5 frozen at −80 °C for DNA extraction. All the remaining material was oven-dried at 60 °C for 72 h, weighed and ashed at 550 °C for 4 h (ash free dry mass – AFDM). The mass of the discs removed was estimated by using paired discs and the total mass was subtracted from that.

Water Physicochemistry and Litter Chemistry

A calibrated multiparameter probe was used to measure water temperature, pH, and dissolved oxygen and electrical conductivity in situ at 0.2 m above the litter bags at each retrieval. The filters were glass-fibre filters and nitrate-nitrogen and soluble reactive phosphorus were analysed in the water samples using cadmium reduction and molybdenum blue methods respectively. C and N of initial litters were analysed using an elemental analyser and lignin and cellulose, gravimetrically using the acid detergent fibre method. The initial lignin: N and C:N ratios were determined with these variables.

Isolation and Screening of Cellulolytic Microorganisms

Five leaf discs were homogenized in each bag in 10 mL of sterile 0.85% saline and serial dilutions were spread on carboxymethyl cellulose (CMC) agar, which is the only carbon source provided. The medium for bacteria contained cycloheximide (50 μg mL^{-1}) and for the fungi contained chloramphenicol (100 μg mL^{-1}). In accordance with the standard procedure for this group, aquatic hyphomycetes were also isolated from single conidia obtained from the leaf discs that were aerated for 48 h in sterile water. The plates were incubated at 28 °C for 3-7 days and the morphologically different colonies were purified. To confirm cellulolytic ability, the plates were flooded with 0.1% Congo red (CR) for 15 min before destaining with 1

M NaCl, which is all due to the specific interaction of the dye to the intact β-1,4-glucans (Teather & Wood, 1982). Hydrolysis capacity (HC) index was determined by dividing the diameter of the clear zone by the diameter of the colony and all isolates that had HC > 2.0 were considered highly cellulolytic.

Cellulase Enzyme Assays

The cellulolytic activities of selected isolates were obtained from the culture filtrates obtained after 72 h. of growth of the bacteria in liquid mineral medium supplemented with 1% CMC and 7 days growth of fungi in medium with 1% microcrystalline cellulose. Endoglucanase (CMCase) and total cellulase (filter paper activity FPase) were measured as the amount of reducing sugars released using the dinitrosalicylic acid (DNS) reagent and reported as international units (IU) as per the International Union of Pure and Applied Chemistry (IUPAC) Ghose, (1987) standard recommendations. Five leaf discs were placed in 0.05 M acetate buffer (pH 5.0) and incubated at 30 °C for 24 hours to measure extracellular enzyme activities on the litter itself (μmol glucose released h^{-1} g^{-1} AFDM for the case of CMCase and FPase; and μmol p-nitrophenol h^{-1} g^{-1} AFDM for the case of β-glucosidase).

Molecular Identification and Amplicon Sequencing

Pure isolates were used in order to obtain genomic DNA for use in a commercial kit. The 16S rRNA gene primers 27F and 1492R were used to amplify and sequence the bacterial isolates, while fungal isolates were amplified and sequenced using the internal transcribed spacer (ITS) region with the primers ITS1 and ITS4. Sequences were compared with NCBI GenBank database by using BLASTn, and the best hit with percentage identity was noted. DNA was extracted from frozen leaf discs and replicate extracts from the same species, site and date were combined to provide 54 composite samples for community analysis. The V3-V4 of bacterial 16S rRNA gene (341F/805R) and ITS2 of fungi (ITS3/ITS4) were sequenced using Illumina MiSeq sequencer (2 × 300 bp). The reads were quality-filtered, denoised, and clustered into amplicon sequence variants (ASVs) using the DADA2 pipeline Callahan et al., (2016) and chimeras were filtered out. The bacterial ASVs were classified with the SILVA database and fungal ASVs with the UNITE database. Bacterial and fungal reads were rarefied to 18,000 and 12,000, respectively, prior to diversity analysis.

III. Results

Water Physicochemistry and Litter Quality

The two factors most closely associated with the quality of the litter are water chemistry and water temperature. The water quality in the littoral zone during the 120 days' experimental period was more or less uniform. Temperature ranged from 24.6 to 28.9 °C, pH from 6.8 to 7.4, and electrical conductivity from 62 to 118 μS cm^{-1}. Dissolved oxygen fluctuated between 3.1 and 7.8 mg L^{-1} at sites S1 and S3 while the site S2 (which was protected from wind) recorded 3.1 to 4.6 mg L^{-1} after day 60 when fine particulate organic matter was increasing. The highest nitrate-N concentrations (0.18–0.42 mg L^{-1}) and soluble reactive phosphorus (0.021–0.064 mg L^{-1}) were found in the inflow at S1, close to the forest. The initial quality of the three litter types was significantly different. The ratio of lignin:N was lowest for teak leaves (14.2); arjuna leaves and bamboo leaves have ratios of 19.6 and 31.4, respectively, and the cellulose content of the leaves was highest in bamboo (34.6% of dry mass).

Litter Decomposition Dynamics

All three types of litter underwent a negative exponential decrease in mass (R^2 = 0.93–0.98). The initial leaching loss was 8-14% during the first 15 days, followed by a steady loss of mass. The mean amount of mass remaining after 120 days of submergence were 24.3% of teak, 31.6% of arjuna and 42.7% of bamboo. The decomposition rate coefficients were 0.0118, 0.0096 and 0.0071 d^{-1}, indicating teak as a fast, arjuna as fast to medium and bamboo as medium decomposer. The results of the two-way ANOVA indicated that k was significantly influenced by litter species ($F = 46.8$, $p < 0.001$) and site ($F = 5.9$, $p = 0.01$), but there was

no significant interaction effect. The decomposition was fastest at S1 and slowest at the hypoxic site S2. For the nine species–site combinations, the first lignin:N ratio accounted for 81% of the variation in k ($R^2 = 0.81$, $p < 0.001$).

Diversity of Cultivable Cellulolytic Microorganisms

The diversity of microorganisms possessing cellulolytic activity. The diversity of cultivable cellulolytic microorganisms. A total of 142 cellulolytic isolates were recovered from the submerged litter with 89 bacteria and 53 fungi. Of the bacterial and fungal isolates 41 and 27 were highly cellulolytic (HC > 2.0). Bacterial isolates were derived from four different phyla with 34% of the Firmicutes, 29% Proteobacteria, 22% Actinomycetota and 15% Bacteroidota. The isolate fungi were mostly Ascomycota (87%), comprising both aquatic hyphomycetes and moulds from terrestrial sources. Table 1 shows the top ten isolates that were the most active and recovered isolates.

Table 1: Representative Cellulolytic Isolates Recovered from Submerged Forest Litter, Their Molecular Identity and Cellulolytic Performance

Isolate Code	Closest Match (Identity %)	Phylum	HC Index	CMCase (IU mL^{-1})	FPase (IU mL^{-1})	Frequency (%)
SFB-07	*Bacillus subtilis* (99.6)	Firmicutes	3.4	0.86	0.21	38.9
SFB-12	*Cellulomonas flavigena* (99.2)	Actinomycetota	3.9	1.12	0.34	27.8
SFB-21	*Streptomyces griseorubens* (98.9)	Actinomycetota	3.1	0.74	0.19	22.2
SFB-26	*Cytophaga hutchinsonii* (98.7)	Bacteroidota	4.2	1.04	0.29	16.7
SFB-33	*Pseudomonas mendocina* (99.4)	Proteobacteria	2.3	0.48	0.11	44.4
SFB-41	*Paenibacillus polymyxa* (99.1)	Firmicutes	2.8	0.67	0.15	20.4
SFF-03	*Lunulospora curvula* (98.8)	Ascomycota	3.6	1.21	0.38	50.0
SFF-09	*Anguillospora longissima* (99.0)	Ascomycota	3.3	1.08	0.31	33.3
SFF-14	*Trichoderma harzianum* (99.8)	Ascomycota	4.6	1.46	0.52	29.6
SFF-22	*Aspergillus niger* (99.5)	Ascomycota	3.0	0.92	0.27	24.1

Note: HC index = ratio of clear zone diameter to colony diameter on CMC–Congo red agar; enzyme activities measured in culture filtrates; frequency = percentage of the 54 composite litter samples from which the isolate was recovered.

The representative isolates of table 1 revealed that the fungus *Trichoderma harzianum* and the gliding bacterium *Cytophaga hutchinsonii* exhibited the greatest hydrolysis capacity and enzyme activity, while the aquatic hyphomycete *Lunulospora curvula* and *Pseudomonas mendocina* were most commonly recovered. From the bacteria, *Cellulomonas flavigena* was the most common, but the most active bacteria in producing CMCase and FPase activities was C. flavigena. The activity of FPase was generally found to be higher for fungi than for bacteria, suggesting that they had more capability to attack crystalline cellulose. The 54 composite litter samples were used to determine the frequency of occurrence.

Succession of Bacterial and Fungal Communities

The 2,846 bacterial and 612 fungal ASVs were obtained after quality filtering and rarefaction of amplicon sequencing. During the experiment, bacterial richness and diversity increased from day 0 to day 90 and remained high at day 120 with the Shannon index value from 3.12 to 5.46. The diversity of fungi was found to have a hump-shaped curve: 1.84 on day 0, 3.71 on day 60, and 2.96 on day 120. PERMANOVA showed that time since submergence was the strongest driver of community composition (bacteria $R^2 = 0.41$; fungi $R^2 = 0.38$; both $p = 0.001$), followed by litter species (bacteria $R^2 = 0.17$; fungi $R^2 = 0.21$; $p = 0.001$) and site ($R^2 = 0.06$ and 0.05; $p < 0.05$). In the early stage (days 0–15), Proteobacteria were the most abundant bacterial group (58% of reads) with the genera Pseudomonas, Aeromonas and Acinetobacter, which are common opportunists in degradation of leached soluble compounds, being the most abundant. In the active cellulolytic phase (days 30–90) the relative abundance of Proteobacteria decreased to 31%, whereas *Bacteroidota* (24%, mainly *Flavobacterium* and *Cytophaga*), *Actinomycetota* (17%, mainly Cellulomonas and Streptomyces) and Firmicutes (12%, mainly *Bacillus* and *Paenibacillus*) increased. By day 120, the total percentage of reads assigned to obligately anaerobic cellulolytic genera (*Clostridium* and *Ruminiclostridium*) increased to 9% at the hypoxic site S2, but remained below 2% at S1 and S3. On days 15-60 aquatic hyphomycetes were dominant fungi, with up to 62% of fungal reads from the fungi *Lunulospora curvula*, *Anguillospora longissima*, *Flagellospora curvula* and *Triscelophorus monosporus*. By day 120, their percentage had dropped to 23%, as genera from the terrestrial origin, like Trichoderma, Penicillium, Aspergillus and unclassified Chytridiomycota, gained more importance.

Extracellular Cellulase Activity on Litter

There was a significant change in cellulase activities during litter decomposition. Pooled CMCase activity was also low at day 0 (2.1 µmol glucose h-1 g-1 AFDM), rose rapidly to 18.6 at day 30, reached a maximum of 26.4 at day 60 and decreased to 15.7 at day 120. FPase activity was similar, with an increase to a maximum at day 60, while β-glucosidase peaked later, at day 90, indicating a buildup of cellobiose and short oligosaccharides resulting from the earlier action of endo- and exoglucanases. The enzyme dynamics and mass loss are presented in figure 1 together.

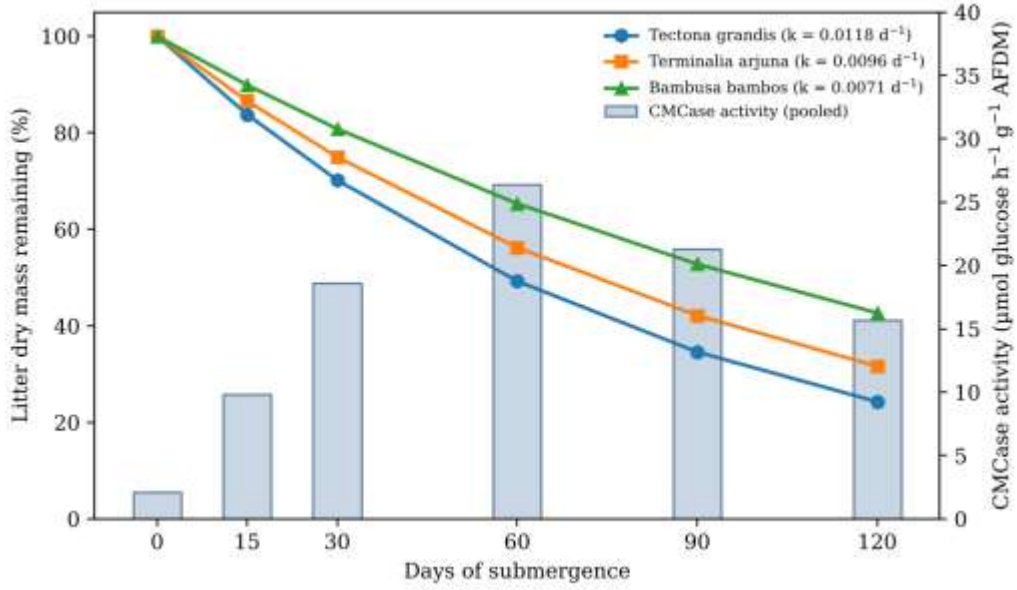


Figure 1: Litter Mass Remaining and Pooled CMCase Activity During 120 Days of Submergence

Litter dry mass remaining (lines on left axis) decreased with exponential decay for all three types of litter and the peaks of litter type activity of pooled CMCase (bars on right axis) occurred at day 60 with

decay from that point. The peak period of enzyme activity occurred during the greatest absolute mass loss of litter between days 30 and 60, suggesting that the active cellulolytic phase is the most significant on the total C release from submerged litter. Relating diversity to function.

Relationships Between Diversity and Function

Overall, fungal Shannon diversity ($r = 0.84$, $p < 0.001$, $n = 54$) was significantly and positively associated with CMCase activity, while bacterial Shannon diversity ($r = 0.52$, $p = 0.01$) was significantly, but less strongly, associated with CMCase activity. FPase activity had the highest correlation with relative abundance of aquatic hyphomycetes ($r = 0.71$, $p < 0.001$) and β-glucosidase activity had the highest correlation with the combined relative abundance of *Bacteroidota* and *Actinomycetota* ($r = 0.66$, $p < 0.001$). The decomposition rate between species and sites was highly related to cumulative CMCase activity over the 120-day period ($R^2 = 0.77$, $p < 0.01$). The average enzyme activity at S2 was 28% less than at S1, and the decrease was related to a decrease in diversity of fungi and an increase in the proportion of anaerobic bacterial taxa.

IV. Discussion

The decomposition rates found in this study were similar to decomposition rates of tropical and subtropical leaf litter in standing water and slow-flowing waters, and the close correlation of decomposition rates with initial lignin:N ratio corroborated the general understanding that litter quality is a major factor controlling organic matter processing in aquatic systems (Tank et al., 2010). The comparatively high nitrogen content and low lignin content in teak leaves allowed for rapid processing, while the high cellulose content and high lignin and silica content of bamboo leaves caused them to remain longer. This difference is ecologically significant for inundated forests: The composition of litter in the flooded canopy will affect the rate of mineralization of organic matter following impoundment, and thus the length of time for which the oxygen demand will occur. Dominated by recalcitrant litter, the reservoir margins can be long-term carbon stores, however, dominated by labile litter may lead to high but brief oxygen depletion. A distinct functional sequence was found in the changes in the microbial communities on submerged litter. The dominance of Proteobacteria in the first 2 weeks suggests that the initial colonizers made use of the dissolved organic matter leached from the leaf structure instead of the structural polysaccharides of the leaf. The following rise in the abundance of *Bacteroidota*, *Actinomycetota* and *Firmicutes* was followed by the rise in cellulase activity, and it is known that genera like Cytophaga, *Cellulomonas, Streptomyces* and *Bacillus* have cellulolytic abilities (Lynd et al., 2002; Wilson, 2011).

The strategies used are different, *Cytophaga* cells degrade cellulose by cell surface associated processes, which require at least surface to surface contact with the cellulose, while Cellulomonas and Streptomyces produce free extracellular enzymes. Co-occurrence of these strategies on the same substrate indicates functional complementarity and potentially will help to stabilize cellulose degradation in changing environments. During the major decomposition period, the fungal community was dominated by aquatic hyphomycetes, as found previously for submerged leaves Krauss et al., (2011) which are known as major decomposers. The species recorded, *notably Lunulospora curvula, Flagellospora curvula* and *Triscelophorus monosporus*, are typical of the warm tropical waters, and their abundance in collection and their ability to grow effectively with cellulose suggest that they are important in the role of decomposing cellulose in reservoir-inundated forests. Fungal diversity showed high degree of correlation with the CMCase activity, which suggested that fungal diversity increases the decomposition with complementary resource utilization (Gessner et al., 2010).

This high fungus dominance is also supported by the biomass-based estimates that fungi usually dominate the microbial production of decomposing leaves, with the contribution of bacteria becoming more important if the litter is degraded. In this study, the diversity of bacteria had not yet reached a maximum but continued to increase when fungal diversity had reached a maximum, while the activity of β-glucosidase reached a peak later than those of the other enzymes, CMCase and FPase. This temporal offset indicates

that bacteria were increasingly taking advantage of the cellobiose and the oligosaccharides generated by the action of the fungal enzymes on the litter, which has been previously suggested by the differential enzyme activities of bacteria and fungi on litter (Romaní et al., 2006). It is worth noting that the terrestrial moulds, such as *Trichoderma*, *Penicillium* and *Aspergillus* were raised in the later stages. The fungi were efficient producers of complete cellulase system and exhibited the highest hydrolysis capacity and FPase activity compared to the other isolates. Their relative abundance from day 60 could be because of their tolerance for lower levels of O2, and their ability to utilize the more persistent, lignified substrate after the more palatable polysaccharides have been used by the aquatic hyphomycetes. Such shifts in dominance with the decreasing quality of the substrate may be attributed to differences in cellulolytic machinery among the fungal lineages, especially in the ability to attack crystalline cellulose with *cellobiohydrolases*. Community structure and function were affected by site conditions. A decrease in the diversity of fungi and enzyme activities was observed with a decrease in dissolved oxygen levels to below 5 mg L^{-1} found at the sheltered site S2, which resulted in a slower rate of decomposition and an increase in anaerobic cellulolytic bacteria (*Clostridium* and *Ruminiclostridium*).

The presence of hypoxia at the litter–sediment interface seems to switch cellulose degradation from a fungal-dominated, aerobic pathway to a slower, bacterial-dominated anaerobic pathway. The higher nitrate and phosphate concentrations in the forested inflow and the resulting faster decomposition at site S1, on the other hand, agree with experimental work indicating a positive effect of moderate nutrient levels on fungal activity and litter decomposition (Gulis & Suberkropp, 2003). The results suggest that the borders of reservoirs are environmentally heterogeneous, resulting in a mosaic of decomposition rates, from which direct implications for the local oxygen and nutrient budgets could be drawn. Sequencing and cultivation were found to be complementary from a methodological point of view. Screening the organisms by plate method with Congo red Teather & Wood, (1982) revealed only a proportion of the community with demonstrable cellulolytic activity and the amount of enzyme produced could be determined under standard conditions (Ghose, 1987). However, when the diversity of the litter was compared to the same by amplicon sequencing with exact sequence variants Callahan et al., (2016) it was found that the diversity present on the litter was much larger and that there was a significant temporal turnover of taxa including anaerobic and uncultured taxa that were not part of the culture collection. The similarities in the dominant cultivable taxa and the dominant sequence-based taxa during the active phase of the period increase confidence in the isolates in table 1 as representative of the functionally important ones.

The results have a number of implications for the management of aquatic habitats based on ecosystem services. First, the cellulolytic microbiota associated with submerged litter is a key, but frequently ignored, aspect of aquatic biodiversity, directly involved in carbon cycling and energy transfer up trophic levels. Second, fungal diversity, the relative abundance of aquatic hyphomycetes and of cellulase activity in association with litter, are sensitive to oxygen and nutrient conditions and have potential to be included in bio-assessment programmes as functional measures of ecosystem health, alongside the traditional physicochemical monitoring. Third, management of reservoir margins, such as retention of riparian vegetation and control of excessive organic loading within sheltered embayments and maintenance of water circulation, can help to maintain aerobic, diverse decomposer communities and prevent prolonged hypoxia. Finally, the isolates recovered here, especially *Trichoderma harzianum, Cellulomonas flavigena* and *Cytophaga hutchinsonii*, are highly cellulolytic and can be utilized to bioremediate organic rich waters and for biotechnological utilization of lignocellulosic residues. There are a few restrictions to be noted.

The study was conducted over only one 120-day period of one season and decomposition and community succession may vary during the monsoon when water levels, turbidity and flow fluctuate rapidly. The results are relative but not absolute abundances and do not directly indicate the expression of the genes, so further work integrating quantitative PCR, metagenomics and *metatranscriptomics* of genes coding for GHs would enable cellulolytic activity to be assigned more accurately to individual taxa. Additionally, the contribution of invertebrate shredders, which was not distinguished from microbial decomposition in the 5 mm mesh bags, should also be estimated with paired fine- and coarse-mesh bags.

V. Conclusion

The present study shows that cellulose degradation of forest litter in a tropical reservoir-inundated forest is a result of a diverse and dynamic community of cellulose-degrading microorganisms in which the succession closely follows the development of the forest litter degradation process. The degradation rates varied greatly with litter type but were mostly attributed to the quality of the litter, especially the lignin:N ratio. Fourteen two cellulolytic isolates were obtained, with the most active isolates being of the genera *Trichoderma*, *Cellulomonas*, *Cytophaga* and the aquatic *hyphomycete Lunulospora*. The order of functional sequence of Proteobacteria, aquatic hyphomycetes, *Bacteroidota, Actinomycetota* and *Firmicutes*, in ascending order, was confirmed by amplicon sequencing, which showed Proteobacteria colonizing the litter first, followed by a dominance of the aquatic hyphomycetes during the active cellulolytic phase, and by an increase of *Bacteroidota, Actinomycetota* and *Firmicutes* as cellulolytic activity progressed. Cellulase activity was highest after 60 days of submergence and was highly correlated with the fungal diversity, supporting the notion that decomposer diversity is linked with carbon turnover in inundated forest habitats. The fungi diversity, enzyme activity and decomposition rate were less at sheltered sites, and anaerobic cellulolytic bacteria were promoted under hypoxic conditions. The present results suggest that fungal diversity, aquatic hyphomycete abundance, and litter-associated cellulase activity are potential functional measures of ecosystem health in reservoirs, and in floodplain forests. Sheltered embayments should be maintained with minimum organic build-up, and adequate circulation of water to ensure an aerobic and diverse decomposer community is maintained; riparian vegetation should be protected; and management should focus on retaining riparian vegetation. Future studies should include observations during both monsoon and dry seasons, use *metagenomic*/*metatranscriptomic* techniques to correlate active taxa with specific cellulase genes and assess the bioremediation potential of the highly cellulolytic isolates for organic rich aquatic systems.

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