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Relationship of Nutrient molar ratio of microbial attached communities in organic matter utilization of Pachamalai forested stream

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Keywords

Biofilm,
Polysaccharide,
Koraiyar,
Mayiluthu,
Pachamalai hills.

Abstract

In the Mayiluthu and Koraiyar forested streams of Pachamalai hills, relationships between microbial decomposition activities at different stream substrata (leaves, branches, sand and gravel) and total carbon (C), nitrogen (N) and phosphorus (P) content of attached microbial communities and stream water matter fractions (particulate and dissolved nutrient concentrations) were examined. According to the results obtained, microbial communities associated to leaves and branches showed higher C: N and lower N: P molar ratios and higher polysaccharide degrading activity. Instead, biofilms on sand and gravel, where algae accumulated and fine detritic materials were more available, showed lower C: N and higher N: P molar ratios, as well as higher ligninolytic and peptidase activity, ligninolytic and peptidase activities. The obtained results showed that the enzyme activities of microbial attached communities are linked to their nutrient molar ratios.

Introduction

The soil matrix as well as chemical and physical properties of soils, like quality and amount of soil organic matter, pH, and redox conditions, have a pronounced influence on the dynamics of the microbial community structure and function in soils (Lombard *et al*., 2011). This close interplay between abiotic conditions and the soil biosphere is one of the most fascinating issues as far as earth sciences are concerned, with huge implications on environmental as well as human health (Van Elsas *et al*., 2008). Due to the complex interactions, it is not surprising that the formation of soils with a high level of fertility is a result of more than hundreds of years of soil “evolution” (Harrison and Strahm, 2008). Another one factor is that plants play an important role in the stabilization of the slope (Korner, 2004) and the soil development, as their root exudates and decaying litter material are the main sources of organic matter at initial sites of the glacier fore field (Duc *et al*., 2009). The microbial biomass is much lower at initial sites, the decomposition of plant material is as fast as it is at developed sites over a time period of twelve weeks (Esperschutz *et al*., 2011).

Studies from Lawrence & Neu, 2003; Besemer *et al*., 2007, showed that a variety of physical and chemical determinates, including nutrient availability, light,

hydrology and temperature, as well as biological processes (i.e. grazing) are dynamically shaping the biofilm. Paul & Duthie, 1989; Pringle, 1990, proposed that the Physical and chemical characteristics may differentially affect biofilms of different ages. Previous studies have shown that thick, and mature biofilms differ from thin and young biofilms in their ability to escape from the conditions in the overlaying water.

Materials and Methods

Carbon, nitrogen and phosphorus content

Carbon, nitrogen and phosphorus content was analyzed in microbial communities associated to leaves, branches and fine and coarse inorganic substrata. Attached microbial communities in leaves and branches, each sample was thawed inside glass vials, and 5 ml of purified water were added. Biofilms on fine and coarse inorganic substrata were detached by sonication to remove the attached community in leaves and branches. The resultant suspension of the microbial community was filtered on pre-combusted and pre-weighed filters (0.7 µm, glass fiber filter Whatman). Then, filters were dried for 48h at 80 ºC, and later on, analyzed for CNP. C and N content of filters was determined by means of a CN Elemental Analyzer using vanadium pentoxide as the oxidation catalyzer. P content was determined after the basic digestion (NaOH) of filters in an autoclave (110ºC for 90 min; Grasshoff *et al.*, 1983). Digestion of filters transformed all organic phosphorus forms into inorganic forms, and then, total P content was determined according to APHA (1989). Based on total C, N and P content of biofilms, C: N, C: P and N: P biofilm molar ratios were calculated.

Extracellular polymeric substances (EPS) extraction and polysaccharide content analysis

For EPS extraction, complete biofilm from three tiles for each replicate was detached (sterile silicone scraper) and taken up in 1.5 ml of phosphate buffer (1.7 g/L KH_2PO_4; 4.5 g/L $Na_2HPO_4.12\ H_2O$; pH-value 7.0). Phosphate buffer was used to counteract the decrease in pH-value caused by treatment with a (Na^+) cation exchange resin. The resin was conditioned before application, Conditioned resin was added to each vial (0.5 g per vial) and EPS extraction was performed for one hour on a shaker at 300 rpm and 4°C. The extraction time and resin concentration used, minimize microbial cell disruption and maximize EPS extraction (Frølund *et al.*, 1996). Crude EPS extracts were centrifuged at 12000*g* for 15 minutes to remove solid parts of the biofilm. The resulting clear EPS extracts were analyzed for polysaccharide content.

Polysaccharide content of the whole biofilm and EPS fraction was determined following the protocol described by Dubois *et al*., (1956). For the whole biofilm, biofilm from 1 tile was detached in 0.5 ml phosphate buffer (pH 7), while for EPS, 0.5 ml of EPS extract was used. Phenol-solution (80%) was added immediately (12.5 µL), and then 1.25 ml of concentrated sulfuric acid was pipetted rapidly into the tubes to ensure sufficient mixing. The samples were allowed to stand for 10 minutes before mixing and were later incubated in a water bath at 30°C for 20 minutes. Absorbance was measured at 485 nm against a reagent blank using Spectrophotometer. The polysaccharide content was calculated using glucose for the standard curve (0-200 µg ml^{-1}).

Results

Physicochemical characteristics

During the study period (n=7) water temperature was moderate (11.3 ± 1.2 ºC) and decreased steadily. Underwater light was very low (19.9 ± 5.1 µmol photons $m^{-2}\ h^{-1}$). Water conductivity (278.2 ± 16.2 µS cm^{-2}), pH (7.2 ± 0.02) and dissolved oxygen (7.9 ± 0.4) did not highly differ from average values.

C, N and P composition of stream water

C: N molar ratios of the dissolved fractious in the stream water were similar during the study (29.2 ±1.2 and 28.1 ±2.3, respectively Figure:1-a). However, the N: P molar ratio of the dissolved fraction in the water was initially increased in sand (22.4 ±1.3) (Fig:1-b). Those large differences were caused by the high N concentration, especially in dissolved organic nitrogen forms in the stream water.

C, N and P composition of microbial communities

The nutrient molar ratios (C: N and N: P) of microbial communities varied considerably between streambed substrata (Sand, gravel, Branches, *Pongamia* fresh leaves, and decaying leaves, *Morinda* fresh leaves and decaying leaves). C: N molar ratios in leaves and branches communities were significantly higher in *Pongamia* (29.2 ± 1.2; 28.1 ± 2.3) and *Morinda* (22.3 ± 0.9; 16.3 ±0.7) compared to branches (Figure:1-a), average value (n=7) than those developed on sand and

gravel. In contrast high N: P molar ratios characterized the microbial communities on inorganic substrata sand (22.4 ± 1.3) gravel (15.4 ± 0.6) as well as these colonizing branches (18.2 ± 0.3) and closer to the values measured for the stream water (Figure:1-b). In contrast, *Pongamia* and *Morinda* microbial colonizing communities showed in average N: P molar ratios of (1.1 ± 0.2) respectively (Figure:1-b).

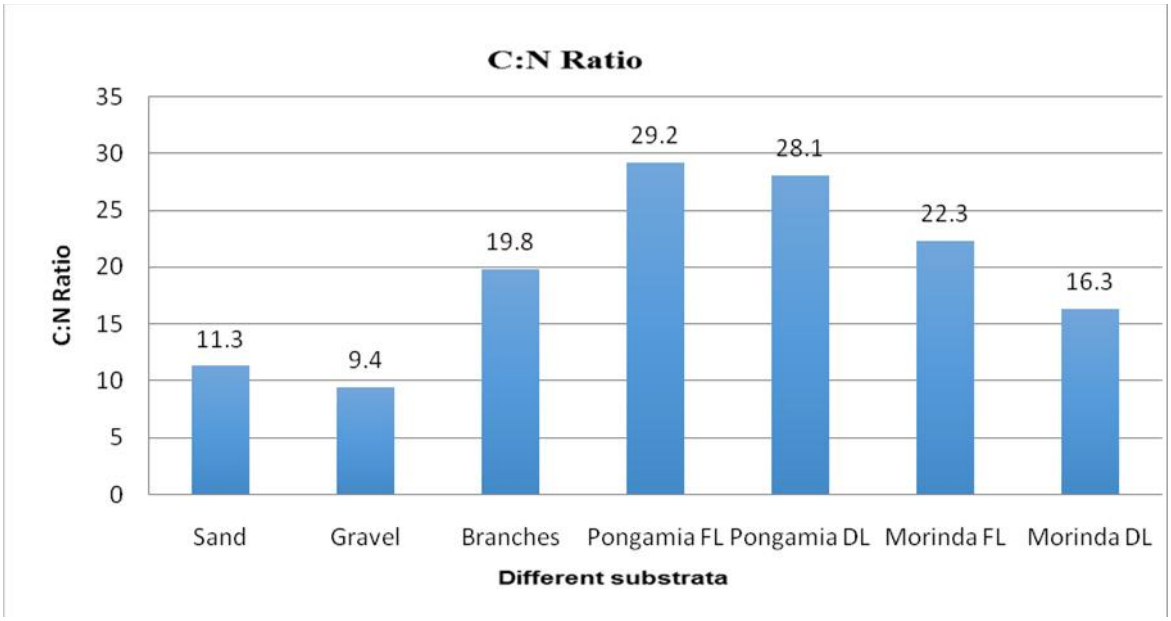

Figure: 1- a): C: N molar ratios of microbial communities colonizing different substrata and those from the particulate and dissolved fractions of streams water. Values are means and stranded errors of seven sampling dates. Significantly different group (a>b>c) obtained with the Tukey's test (α=0.05) are also shown

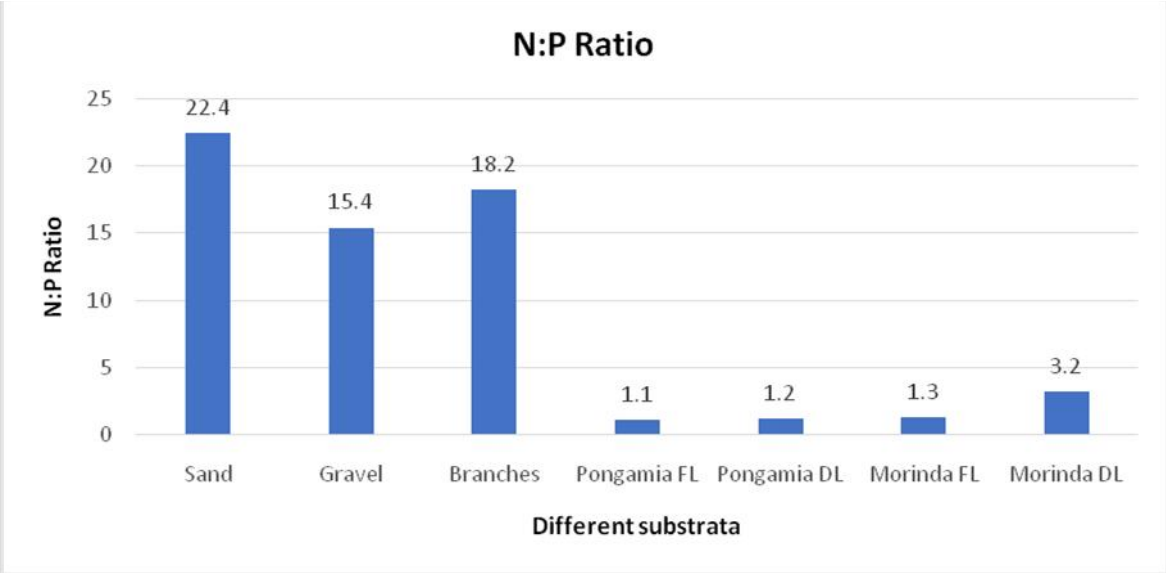

Figure: 1- b): N: P molar ratios of microbial communities colonizing different substrata and those from the particulate and dissolved fractions of streams water. Values are means and stranded errors of seven sampling dates. Significantly different group (a>b>c) obtained with the Tukey's test (α=0.05) are also shown

Microbial communities and polysaccharide concentration

The bacterial density, chlorophyll-*a*, and ergosterol concentrations varied between stream substrata. Bacterial density was higher on sand biofilms (1.9 ± 0.6) while densities on decaying leaves (DL) (0.7 ± 0.2; 1.0 ±0.3 respectively) and branches (0.8 ± 0.1) exceeded those measured on the recently deposited leaves (FL) (Figure:.2-a). Ergosterol was higher in branches (6.2 ± 0.1) (Figure:2-b), while chlorophyll-*a* was higher on sand and gravel (Figure:2- c). Leaf microbial communities, and especially those formed on recently deposited *Morinda* fresh *leaves*, showed the higher polysaccharide content (3.2 ± 1.0) than *Pongamia* (1.1 ±0.6) (Figure:2-d). In contrast it will show that degradation of polysaccharide content.

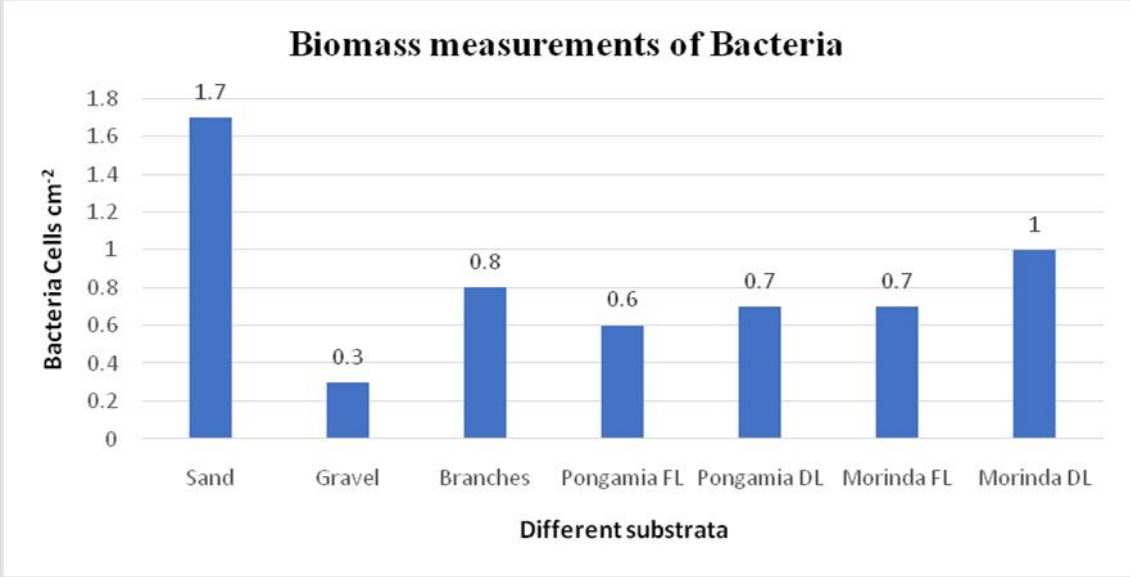

Figure: 2- a): Biomass measurements of bacteria in microbial communities colonizing the different substrata analyzed (means and standard errors). Significantly different groups obtained with Tukey's test (a>b>c, α= 0.05) also shown.

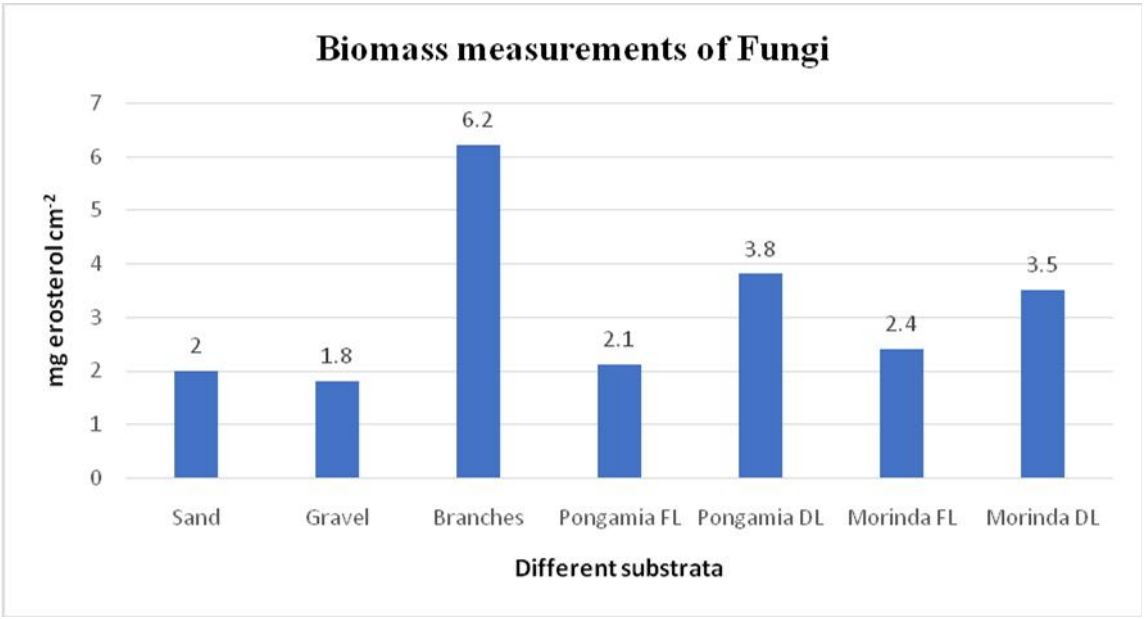

Figure:2-b): Biomass measurements of fungi in microbial communities colonizing the different substrata analyzed (means and standard errors). Significantly different groups obtained with Tukey's test (a>b>c, α= 0.05) also shown.

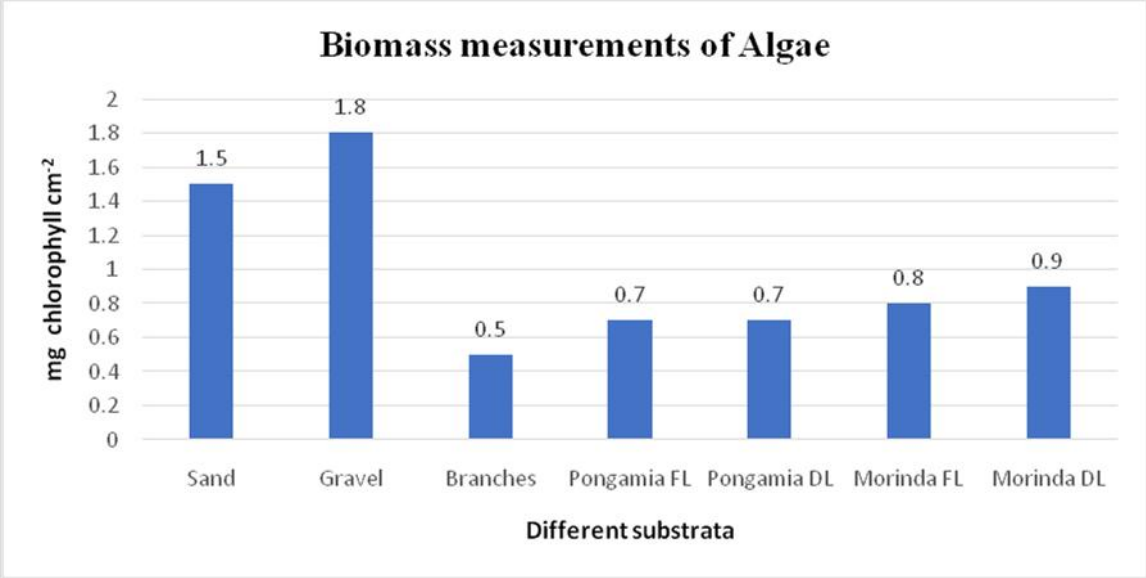

Figure:2-c): Biomass measurements of algae in microbial communities colonizing the different substrata analyzed (means and standard errors). Significantly different groups obtained with Tukey's test (a>b>c, α= 0.05) also shown.

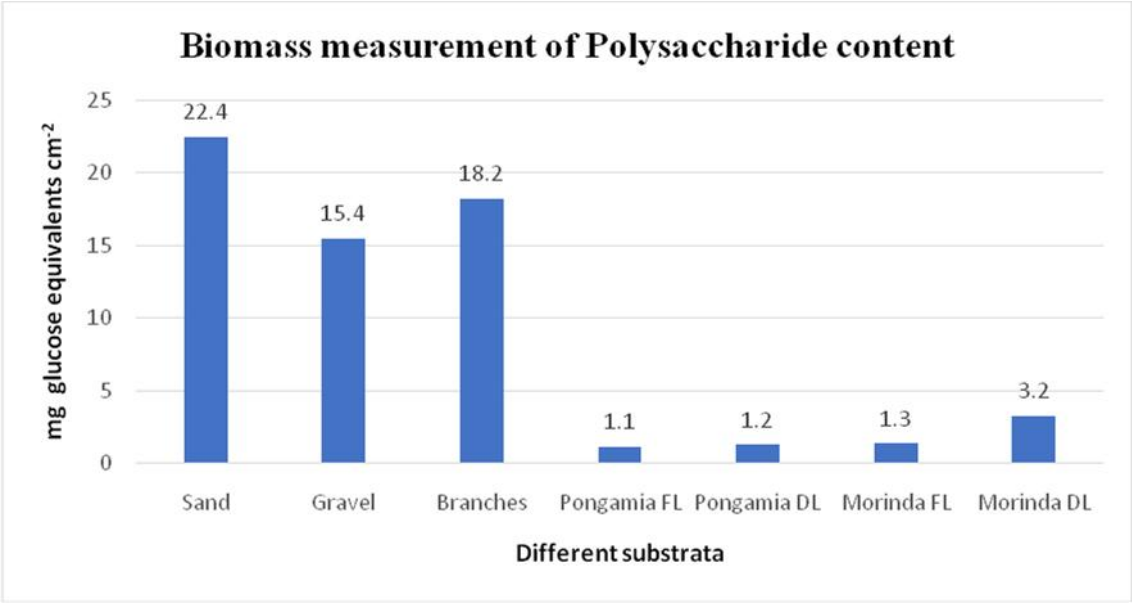

Figure:2-d): Biomass measurements of Polysaccharide content in microbial communities colonizing the different substrata analyzed (means and standard errors). Significantly different groups obtained with Tukey's test (a>b>c, α= 0.05) also shown

Extracellular enzyme activities

Activities involved in the degradation of polysaccharides (β-glucosidase, β - xylosidase and cellobiohydrolase) were higher in microbial communities developing on decaying leaves (DL), especially in those of *Morinda* (Figure :3 -a). In contrast, those on sand and gravel showed higher capacity to degrade lignin (Figure:3- b). Peptidase activity was significantly higher in sand and gravel biofilms, but also in *Morinda* decaying leaves (Figure :3- c), while phosphatase activity was higher in decaying leaves, branches and sand (Figure :3- d).

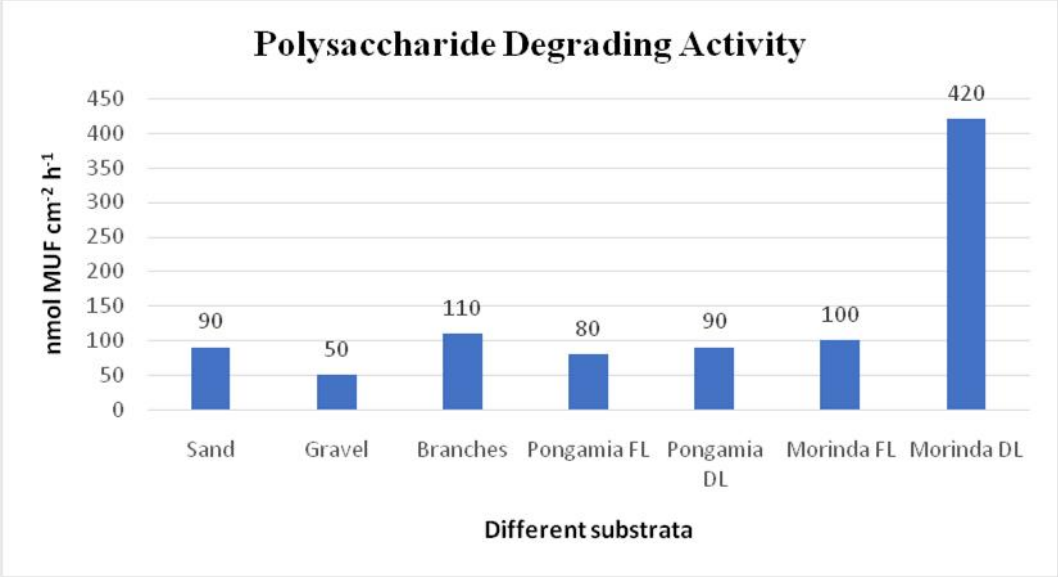

Figure :3-a): Extracellular enzyme activities measured in each substratum during the study. The polysaccharide degrading activity is the sum of β -glucosidase, β-xylosidase, cellobiohydrolase activities. Values are means and standard errors of the seven sampling dates. Significantly different groups (a>b>c) obtained by means of Tukey's test (α=0.05) are shown.

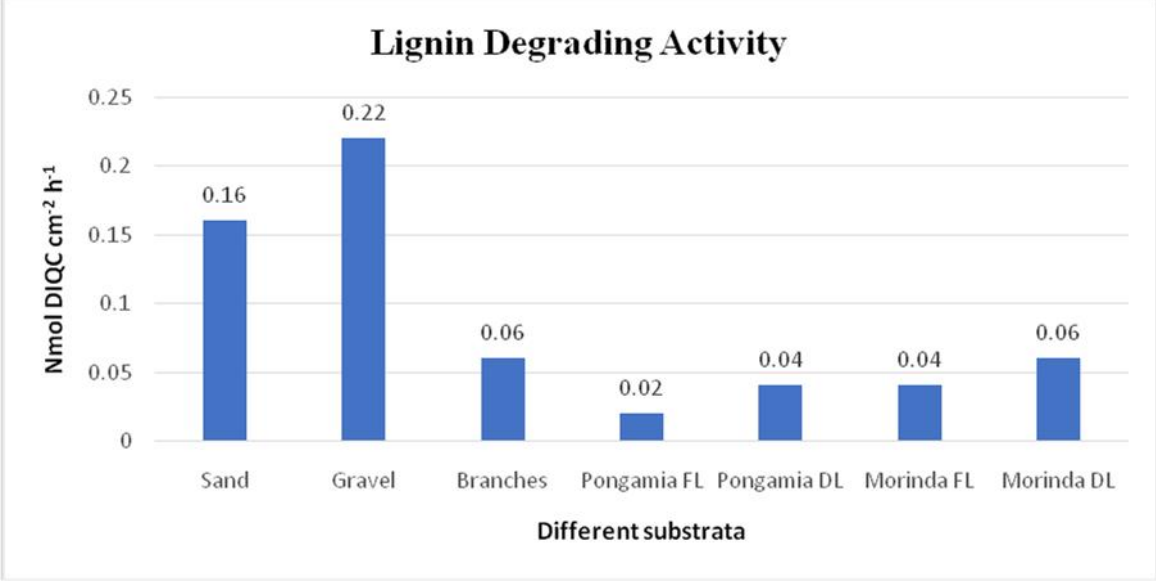

Figure :3-b): Extracellular enzyme activities measured in each substratum during the study. The lignin degrading activity is the sum of phenol oxidase and peroxidase activities. Values are means and standard errors of the seven sampling dates. Significantly different groups (a>b>c) obtained by means of Tukey's test (α=0.05) are shown.

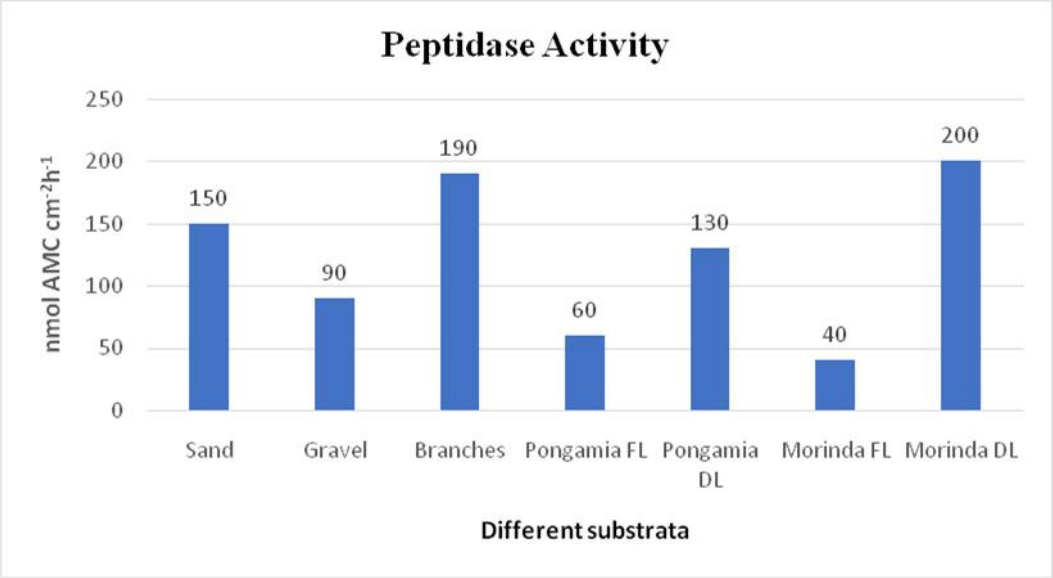

Figure :3-c): Extracellular Peptidase enzyme activities measured in each substratum during the study. Values are means and standard errors of the seven sampling dates. Significantly different groups (a>b>c) obtained by means of Tukey's test (α=0.05) are shown.

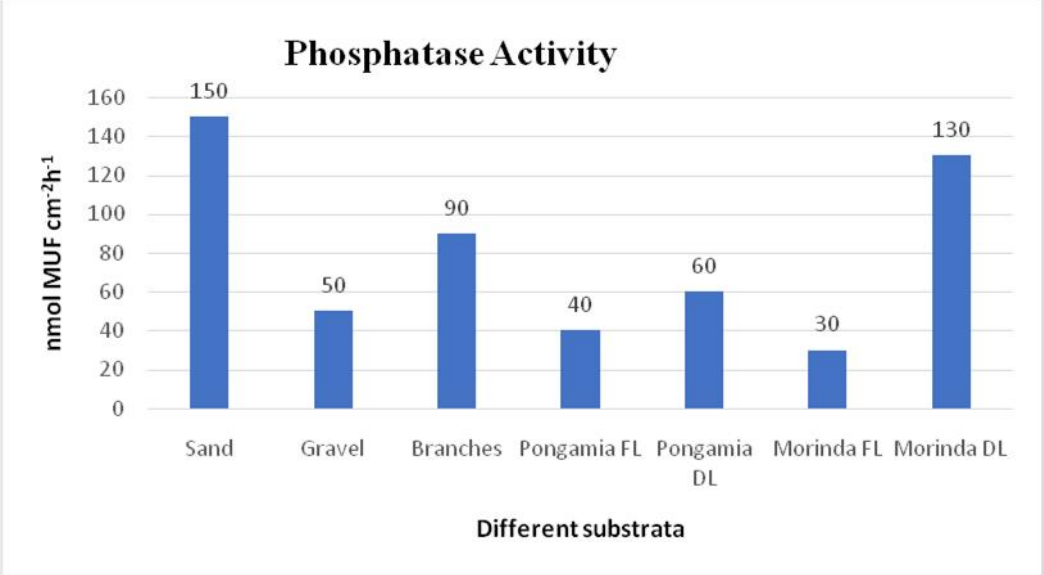

Figure :43-d): Extracellular Phosphatase enzyme activities measured in each substratum during the study. Values are means and standard errors of the seven sampling dates. Significantly different groups (a>b>c) obtained by means of Tukey's test (α=0.05) are shown.

The polysaccharide: lignin degradation ratio indicated that prevailing activities on microbial communities differed according to the substrata where they developed (Table -1). The ratio reached higher values in leaves and branches, especially in the microbial communities on *Morinda* leaves, and was lower in those on sand and gravel. The peptidase:phosphatase ratio indicated that in most substrata there was a preferential transformation of organic phosphorus compounds higher phosphatase activity).

Table- 1: Polysaccharide: lignin degradation ratio and peptidase: phosphatase ratio measured along with the values of enzymatic activities in the different stream substrata. Values are means and standard errors of the seven sampling dates

	Polysaccharide: lignin degradation		Peptidase: Phosphatase	
	Mean	SE	Mean	SE
Sand	0.4	0.05	0.6	0.03
Gravel	0.2	0.03	1.3	0.07
Branches	1.4	0.1	0.5	0.03
Pongamia pinnata FL	4.8	0.14	0.7	0.3
Pongamia pinnata DL	2.1	0.2	0.5	0.03
Morinda tinctoria FL	7.0	0.3	0.6	0.03
Morinda tinctoria DL	8.2	0.3	1.0	0.1

Discussion

The combined analysis of extracellular enzyme activities and nutrient molar ratios of benthic microbial communities developed on different stream substrata suggested a link between the differential capacity for organic matter decomposition and the C: N and N: P molar ratios of each microbial community. Biofilms on inorganic substrata (sand and gravel) showed a major lignin and peptide decomposition capacity and a higher proportion of N (lower C: N and higher N: P molar ratios). In contrast, higher C: N and lower N: P molar ratios were measured in biofilms developed on organic substrata (leaves and branches), which also showed major capacities for polysaccharide decomposition (cellulose and hemicellulose). This linkage could be related to the differential organic matter available at different streambed substrata but also to the quality and quantity of materials in the flowing water. However, the accrual of microbial biomass (algae, bacteria, fungi) as well as other biological groups i. e. micro- and meiobenthic fauna) colonizing the benthic substrata, may also be relevant in determining biofilm nutrient molar ratios.

The stream habitat (sand, gravel, accumulated leaves and branches) may affect the functioning and nutrient molar ratios of the attached microbial communities due to several reasons: 1) the quantity and quality of available organic matter sources (Findlay *et al.*, 2002), 2) the physical characteristics of the different substrata (Pusch *et al.*, 1998), 3) the changes of nutrient availability in the water (Masseret *et al.*, 1998; Stelzer *et al*., 2003), and 4) the interactions between different biological groups colonizing the substratum [i. e. algae-bacteria in the epilithon (Francoeur & Wetzel, 2003) and bacteria-meiofauna in leaves (Perlmutter & Meyer, 1991)]. In sand and gravel habitats, finer particulate organic matter with high lignin content (Yeager & Sinsabaugh, 1998) and low C: N molar ratio may accumulate, enhancing ligninolytic enzyme activities and a higher N content of the microbial communities. Cycling of nitrogenous compounds in microbial communities on sand and gravel is further showed by the high peptidase activity. High peptidase activity in aquatic biofilms has been usually related to the use of peptides released by algae (Romaní & Sabater, 2000; Francoeur & Wetzel, 2003; Rier *et al*., 2007), which might occur in benthic microbial communities developing on sand and gravel. The higher N concentration of sand and gravel microbial communities (lower C: N and higher N: P) might be in concordance to the major use of nitrogenous compounds (lignin and peptides).

In contrast to sand and gravel habitats, debris dams and leaf accumulation habitats are stream sites of high heterotrophic activity (Pusch *et al.*, 1998) due to the decomposition of plant material. Degradation of celluloses and hemicelluloses is an early process in the decomposition of leaf litter (Berg & McClaugherty, 2003), and this was shown by the high polysaccharide degrading activities (β-glucosidase, β- xylosidase and cellobiohydrolase) that microbial communities in leaves and branches exhibit (higher polysaccharide: lignin ratio). The major use of C compounds by bacteria and fungi on these substrata may determine the higher C: N, in contrast to that on sand and gravel. However, major differences were detected for the lower N: P molar ratios measured in *Pongamia* and *Morinda leaves* in contrast to sand and gravel.

The higher P content of these communities was not related to a higher phosphatase activity rate, but probably to the use of liable organic P compounds released during leaching. Finally, consistent differences between C: N and N: P molar ratios in leaves and branches versus those measured for sand and gravel could be further modulated by the specific composition of the microbial community developed in each substratum (algae, bacteria, fungi, micro and meiofauna) which might show differences in C, N and P content (Sterner & Elser, 2002).

Even the effects and differences between benthic habitats, microbial functioning might be also influenced by stream water and its nutrients molar ratios in dissolved and particulate fractions. While C: N molar ratios of attached communities were similar to those measured in the stream water (both dissolved and particulate), the N: P ratios were much lower than those measured in the dissolved water fraction. Several other studies have shown that dissolved N availability (mainly nitrate) in the stream is very high during punctual rainfall episodes, because of N mobilization from the watershed soils (Bernal *et al.*, 2002). The larger N bulk in stream water (mainly due to the dissolved organic nitrogen, DON) may exceed the demands by the microbial community and/or an important proportion of this DON might be less biodegradable. The availability of dissolved nitrogen in the water seem to influence some enzymatic activities, as suggested by the positive correlations between the C: N molar ratio of the water and the peptidase activity of the biofilm.

During the large autumnal input of particulate organic matter in forested streams, the microbial benthic community is distributed in two major habitats, which become dynamic and interconnected. The high availability of allochthonous OM (materials with a high C: N molar ratio) during the study period (autumn-winter) affects both the nutrient ratios and the enzyme activities of microbial communities. The POM, mainly leaves, may support a microbial community with a higher polysaccharide matrix and high C and P content, which feeds on the substrata itself. As decomposition process takes place, plant material is transformed into smaller organic particles, which get included to the stream bed sediments. Inorganic substrata in streambed support communities with high N content that rely upon fine detritic materials accumulated and on peptide molecules produced by algae within the biofilm. Even though microorganisms (bacteria, fungi and algae) are essential in shaping the nutrients imbalance and metabolism of stream biofilms, recent studies have demonstrated that micro fauna participate to a variety of tropic processes within the benthic community that can contribute to these differences (Robertson *et al.*, 2000; Schmid-Araya & Schmid, 2000). Probably the lower allochthonous OM input and higher autotrophic production (high authochthonous OM input) characteristic of open streams would determine different patterns between the community nutrient molar ratios and enzymatic activities. Further studies addressed at different stream ecosystems considering both microbial and other biological groups (i. e. micro- and meiobenthic fauna as well as macrofauna) will be essential to validate the relationships between function and nutrient molar ratios of attached communities.

Conclusion

In Pachamalai forested streams, relationships between microbial decomposition activities of β-glucosidase, β-xylosidase, Cellobiohydrolase, Phenoloxidase, Peroxidase, Peptidase and Phosphatase at different stream substrata (leaves, branches, sand and gravel) and total carbon (C), nitrogen (N) and phosphorus (P) content of attached microbial communities and stream water matter fractions (particulate and dissolved nutrient concentrations) were examined. Microbial communities associated to leaves and branches showed a higher C: N and lower N: P molar ratios (averaging 29.2 ± 1.2 and 28.1± 2.3, respectively) and higher polysaccharide degrading activity (sum of β-glucosidase, β- xylosidase and cellobiohydrolase activities). Instead, biofilms on sand and gravel, where algae accumulate and finer particulate materials were more available, showed lower C: N and higher N: P molar ratios (averaging 11.3 ± 0.6 and 22.4 ± 1.3, respectively) and greater ligninolytic (sum of phenol oxidase and peroxidase activities) and peptidase activities. However, similarities (C: N) and divergences (N: P) between stream water and nutrient molar ratios in microbial communities may also affect nutrient demands and in consequence, the expression of extracellular enzymes. The obtained results show a relationship between function (extracellular enzyme activities) and nutrient molar ratios of attached microbial communities.

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