



## **Organic matter availability structures of microbial biomass and their activity in Pachamalai Forested stream in Tamilnadu.**

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### **Abstract**

Microbial biomass (bacteria, fungi, algae) and the activity of extracellular enzymes used in the decomposition of organic matter among different benthic substrata over one hydrological year in a Pachamalai stream. Microbial heterotrophic biomass (bacteria plus fungi) except during short periods of high light availability in the spring and winter. Bacterial and fungal biomass increased with the decomposition of cellulose and hemicellulose compounds from leaf material. Later, lignin decomposition was stimulated in fine (sand, gravel) and coarse (rocks, boulders and cobbles) substrata by the accumulation of fine detritus. In the drought provoked an earlier leaf fall. The resumption of the water flow caused the weathering of riparian soils and subsequently a large increase in dissolved organic carbon and nitrate, which led to growth of bacteria and fungi.

**Keywords:** Microbial diversity; microbial population; bacterial community; organic matter.

### **Introduction**

Microbial heterotrophs (bacteria and fungi) can produce a broad range of substrate – specific enzymes that enable allochthonous and autochthonous organic matter (OM) mineralization (Arnosti, 2003). This substratum is decomposed by physical, chemical, and biological factors. Lignocelluloses the major components of biomass are degraded by that enzyme itself. Natural disturbance, from seasonal changes rainfall and tree fall, to hurricane damage, cause population shifts and changes to communities of bacteria (Lyautey, *et al.*, 2005). To understand the ecology of these microorganisms, their interactions, and the functions they perform, it is important to study them in their natural habitats (Sterflinger, *et al.*, 1998).

Leaf breakdown in streams is caused by the joint action of physical factors, the activity of microorganisms, such as aquatic hypomyces (Barlocher, 1992).

Microorganisms like bacteria and fungi are among the few organisms that secrete enzymes that can break down large molecules, such as cellulose, chitin, and lignin, into smaller compounds that can be taken up by the biota (Sinsabaugh, and Linkins. 1990.). The untapped diversity of microorganisms is a resource for new genes and organisms of value to biotechnology. The diversity patterns of microorganisms can be used for monitoring and predicting environmental change.

In present study water sediments and leaf litter samples were collected from streams of Mayiluthu and Koraiyar streams in Pachamalai hills situated in Trichy Dt. Tamil Nadu. To study the role of different microbial groups as decomposers in the littoral area and their metabolic and structural responses of sediment in microbial communities to the addition of different carbon substances have been assessed.

## Materials and Methods

### Study area

In the study area are Pachamalai hills, located in Trichy District. Tamilnadu, india. situated 3000 feet above sea level lying between 78° 31' East and 11° 28' North and 11° 10' South and 78° 20' West Latitude. In the Pachamalai forested streams several streams are present. In present work all samples were collected in Koraiyar and Mayiluthu streams.

### Sampling

Monthly samples were taken (from March 2015 until February 2016) at different scales of coarse particulate organic matter (CPOM), POM transported by the water, and water nutrient concentration. The visually estimated the relative cover (%) of coarse substrata (cobbles, boulders and rocks), fine substrata (gravel and sand), leaves (distinguishing the relative cover of *Pongamia pinnata*, *Morinda tinctoria* and *Acacia nilotica*), branches and fine detritus.

At the habitat scale, collected distinct benthic substrata (leaves, sand and tiles) Analysed them for microbial biomass (algae bacteria and fungi) and extracellular enzyme activity ( -glucosidase, - xylosidase, leucine-aminopeptidase phosphatase, cellobiohydrolase and phenoxidase activity). Benthic substrata were classified as coarse (rocks, boulders and cobbles), fine (sand) or leaves (the three dominant species in the reach; *Pongamia pinnata*, *Morinda tinctoria* and *Acacia nilotica* ).

**In this sample the following parameter was analyzed**

### I. Physical and chemical parameters

In this water sample dissolved oxygen, PH, conductivity, were measured temperature, dissolved organic carbon (DOC), dissolved inorganic carbon (DIC), dissolved N and P concentration and then total nitrate and phosphate concentrations were determined by the standard methods (APHA, 1998).

## II. Coarse particulate organic matter (CPOM)

During the period March 2015- February 2016, total CPOM input in the two streams was analysed by a monthly collection of leaf and plant materials accumulated in five traps distributed along the reach and suspended 1 cm above the water surface. The traps consisted of a square wooden frame (1X1 m) and a nylon net the CPOM materials collected were dried at 80° C for 48h and then weighed.

## III. Extra cellular enzymes activity

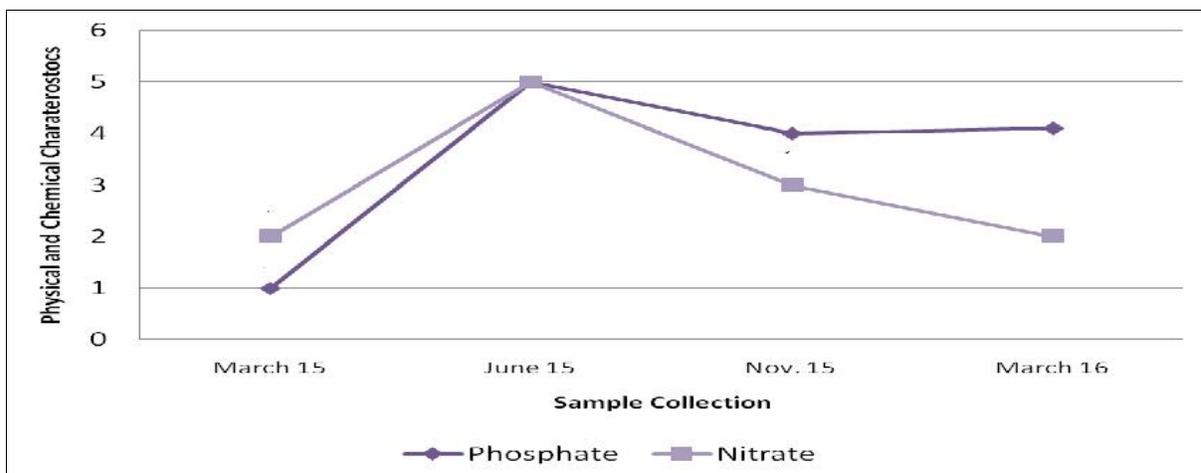
In the study, several hydrolytic and oxidative extracellular enzymes activities were analyzed the samples consisted of 1 leaf disk, 1 piece of branch 1ml of sand volume, 1 gravel grain and 1 liter placed in glass vials filled with stream water (5 ml) and kept cold (4° C) until arrival at the laboratory. A total of six different hydrolytic enzymes assays were performed by polysaccharide compounds degradation by means of the  $\beta$ - glycosidase,  $\beta$ - Xylosidase, Cellobiohydrolase, Phosphatase. Peptidase and Phenol oxidase using the methodology described by (Romani et al., 2001). In the all three parameters sample was collected by triplicate and the data was carried out in statistical analysis.

## Results

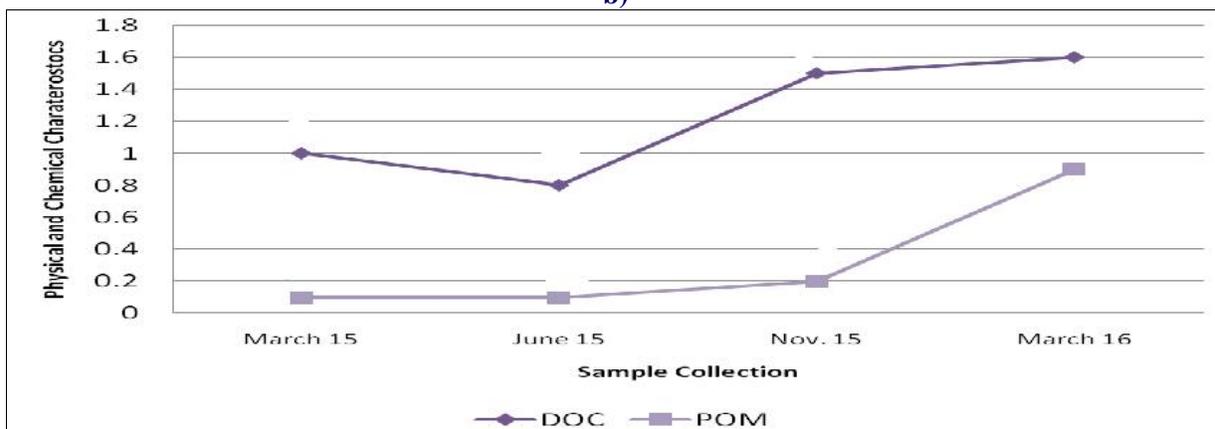
### Environmental variables and stream characteristics

Rainfall is generally highest in spring and autumn, however total rainfall can vary from year to year. During the study period temperature varied between 16° C and 26 °C. Stream discharge was highest in the autumn (in October) and dramatically decreased in early summer (in June) to zero flow in July and August. The first rainfall in September restored the stream's water flow and increased its dissolved nutrient content (nitrate, phosphate and DOC) (Figures .1 a,b,c). Stream water was low in P and high in N most of the year, the majority of CPOM input occurred during autumn (especially in October), but there was a secondary peak in spring (March). In both cases, the largest input of CPOM came from *Pongamia pinnata* (L). CPOM standing stock and POM transport were particularly high in September, shortly after water flow resumed. However spates in October rapidly washed the accumulated CPOM downstream (Fig- 1c).

a)



b)



c)

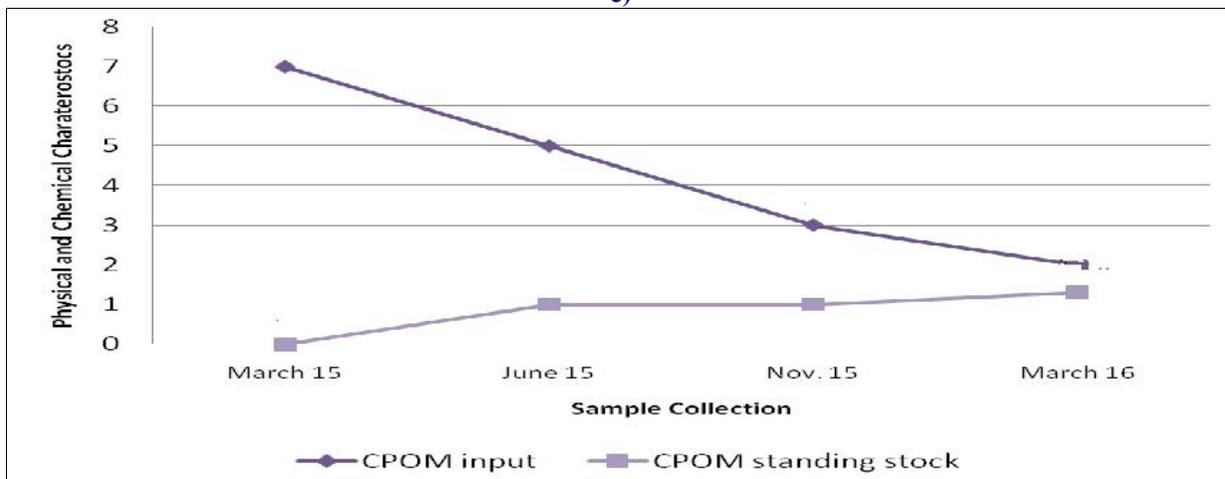
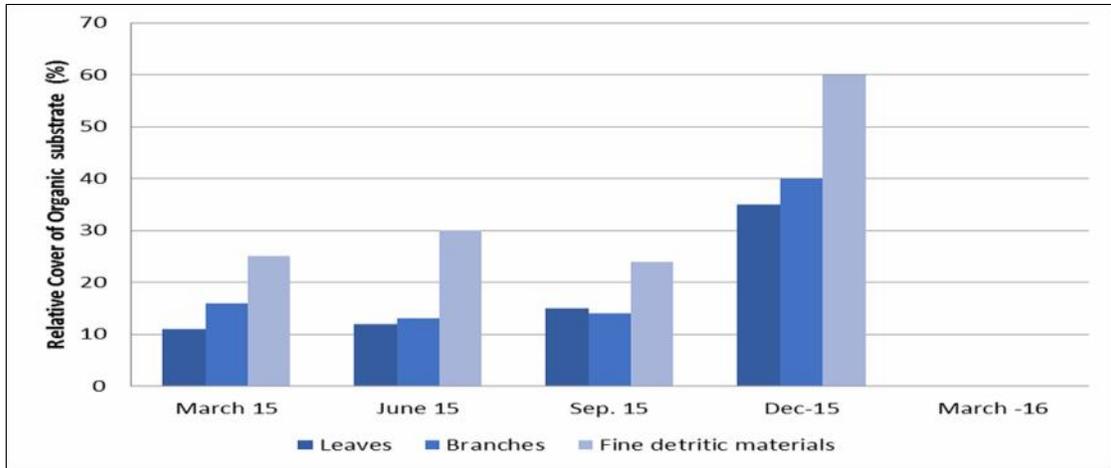


Figure – 1 Physical and chemical characteristics of the Pachamalai stream water during the study period. Values of phosphate (g) and nitrate (mg) (a), dissolved organic carbon (mg) and particulate organic matter (POM) in transport (b), and CPOM input and standing stock (c) are shown. Low or Zero flow occurred from the end of June to early September- 2015.

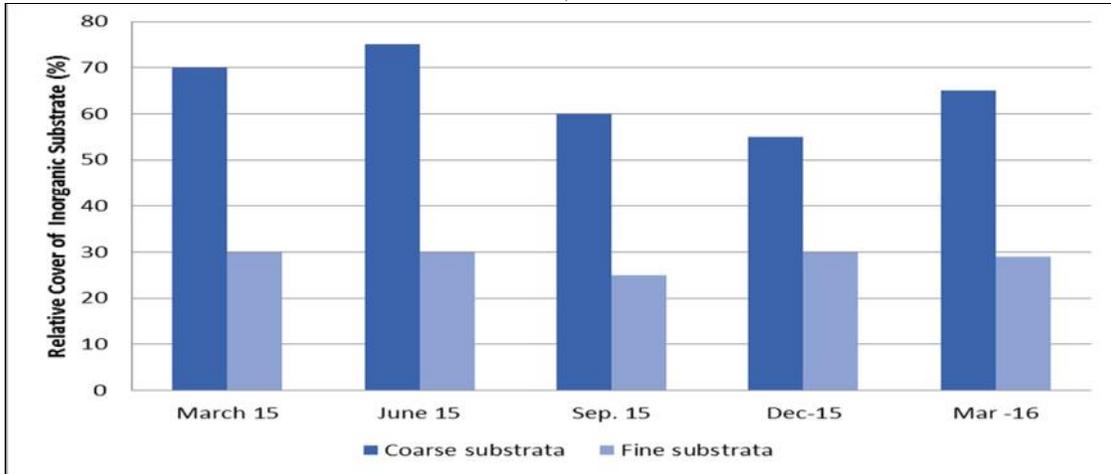
Mean wetted surface area was  $71.6 \pm 4.2$ . Coarse substrata covered 70% of this area, while 30% was covered by fine substrata (Figure .2 a, b). Coarse substrata dominated in the centre of the channel, while gravel and sand accumulated in pools and in the depositional zones near the banks. October rainfall increased the fine substrata cover to 52% of the total surface area of the reach. The decrease in water level from June onwards narrowed the wetted channel and

resulted in the loss of depositional zones and in the subsequent predominance of coarse substrata (up to 75% of reach surface). Leaf accumulation in the autumn covered as much as 80% of the total wetted surface area, while fine detritus were more abundant in spring and winter (up to 28% of reach area). The highest accumulation of branches occurred in autumn.

a)



b)



**Figure : 2 Relative coverage (%) at each sampling time of the inorganic (coarse and fine substrata) and organic (leaves, branches, fine detritic materials) benthic substrata in the studied**

***Comparison of microbial biomass among substrata***

Reach-scale values of bacterial, fungal and algal biomass in the different benthic substrata varied seasonally (ANOVA, T effect,  $P < 0.005$ ; Figure. 3). Bacterial and fungal biomass peaked in September, although their temporal variations also depended on the substratum type. Bacterial standing stock on fine substrata and tiles was highest during spring, which

coincides with the higher algal biomass episode (Figures. 3 a&c). Each microbial group showed certain preferences in substratum colonization (Figure .3). In general, bacteria mainly accumulated on tiles, except in September when submerged leaves were preferred, fungi colonised leaves while algae accumulated on tiles. Fungi dominated in the biomass of the microbial

community (up to  $9 \text{ gC m}^{-2}$ , in autumn), followed by algae (up to  $6.9 \text{ gC m}^{-2}$  in spring) and bacteria (up to  $0.21 \text{ gC m}^{-2}$ , in autumn; Figure.3).

The microbial autotrophic heterotrophic C ratio shifted from being highly autotrophic in spring and winter (ratio of 3 and 2.1, respectively) to highly heterotrophic in autumn (ratio of 0.1).

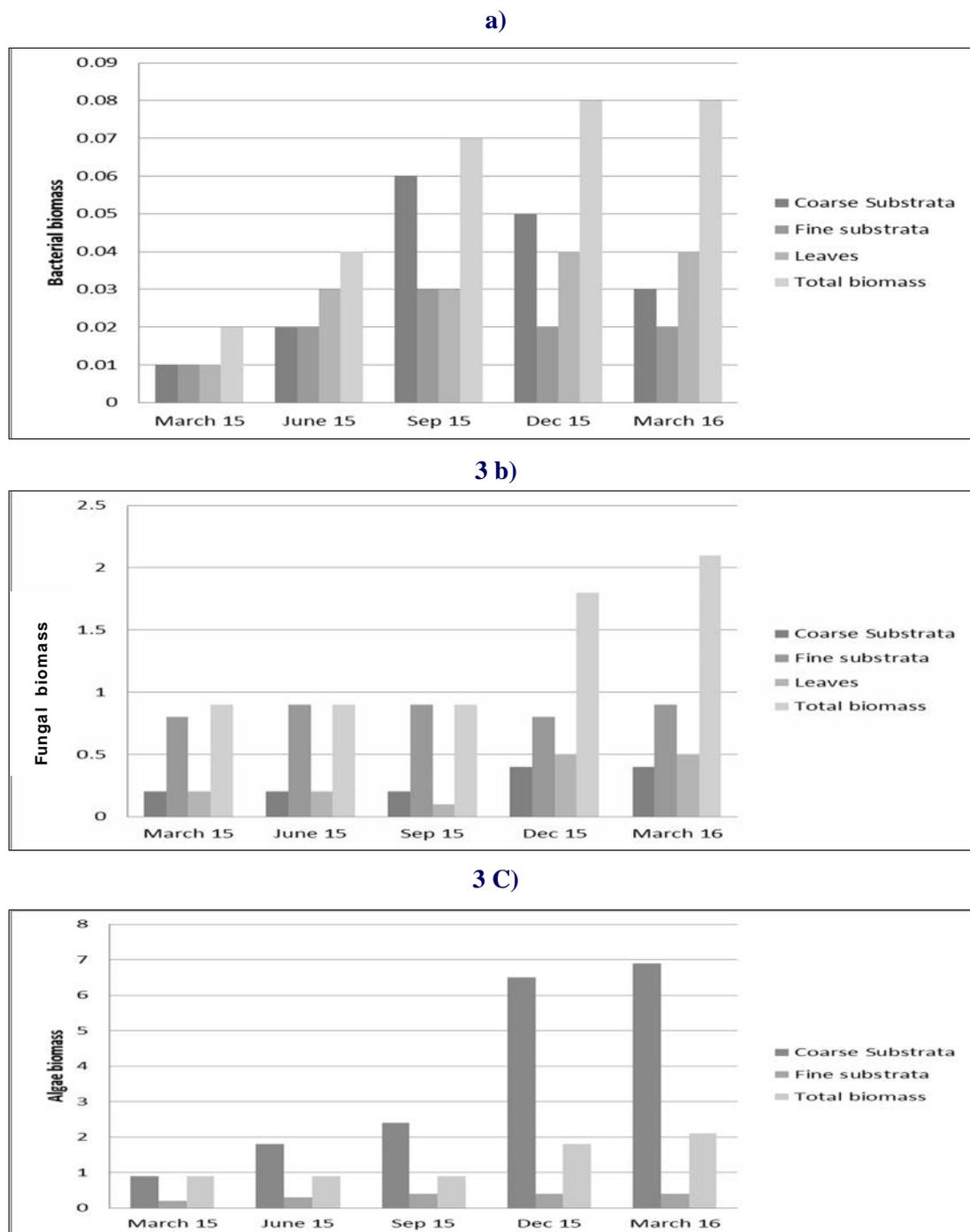


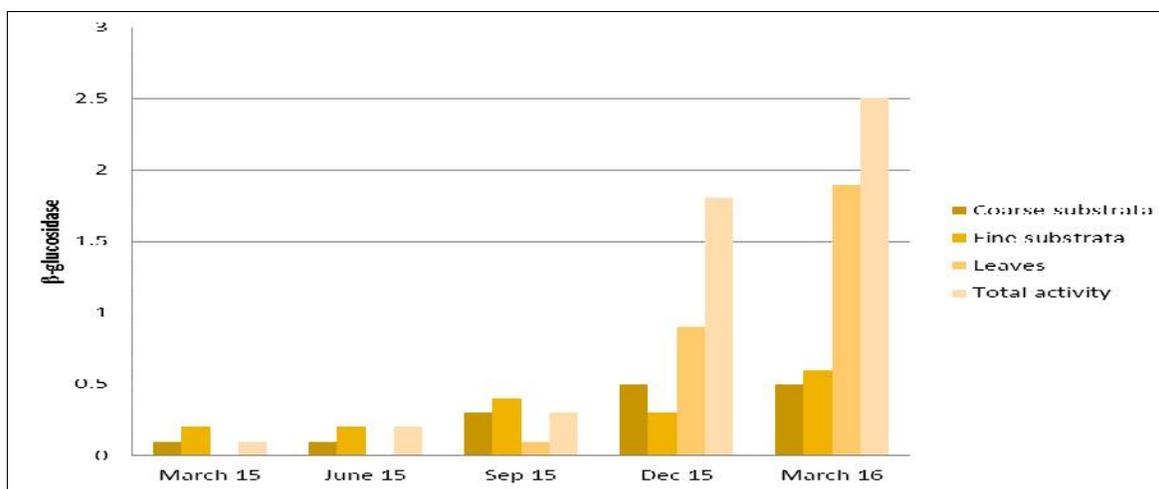
Figure – 3 Biomass of bacteria (a), Fungi (b) and algae (c) expressed in terms of carbon per unit of reach surface area on different benthic substrata (leaves, coarse and fine substrata) in the study reach. Total values of benthic microbial biomass accumulated in the reach ( biomass in coarse and fine substrata and leaves) are also shown.

**Extracellular enzyme activity**

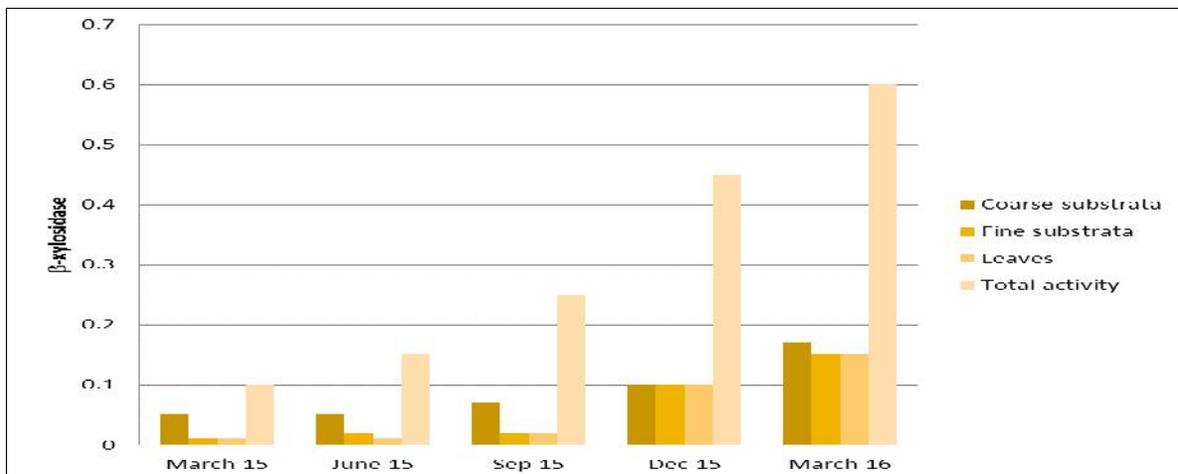
Enzyme activity in different substrata demonstrated a marked seasonally (Figure. 4). The activity of three enzymes involved in polysaccharide degradation (  $\beta$ -glucosidase,  $\beta$ -xylosidase and cellobiohydrolase) peaked in early autumn (September), coinciding with the rise in benthic heterotrophic biomass (Figures 4 a, b & c). However,  $\beta$ -glucosidase activity was highest between June and October, while  $\beta$ -xylosidase activity peaked between May and November. There was a sharp increase in cellobiohydrolase activity between June and September and again in February the following year. Phosphatase and phenol oxidase activity steadily increased in spring and peaked in September (Figures. 4 d & e), activity then decreased during the autumn and recovered in winter. Trends in peptidase activity contrasted with that of other enzymes (Figure.4 f).

The temporal dynamics of each enzyme activity varied with benthic substratum type, except in the case of the phenol oxidase activity ( $P = 0.33$ ). In general, polysaccharide-degrading enzymes were most active (  $\beta$ -glucosidase and  $\beta$ -xylosidase) in leaf material, while phosphatase and peptidase prevailed on tiles. However, cellobiohydrolase activity did not differ between substrata. The decomposition of lignocellulosic compounds was most common on fine substrata. In general, the capacity of benthic communities in the decomposition of simple C molecules (  $\beta$ -glucosidase, Figure. 4 a) was 100 times greater than their capacity to decompose hemicelluloses and celluloses (  $\beta$ -xylosidase and cellobiohydrolase, Figure .4 b & c). The greatest capacity was that measured for organic phosphorus compounds and peptide decomposition (Figures.4 d & f).

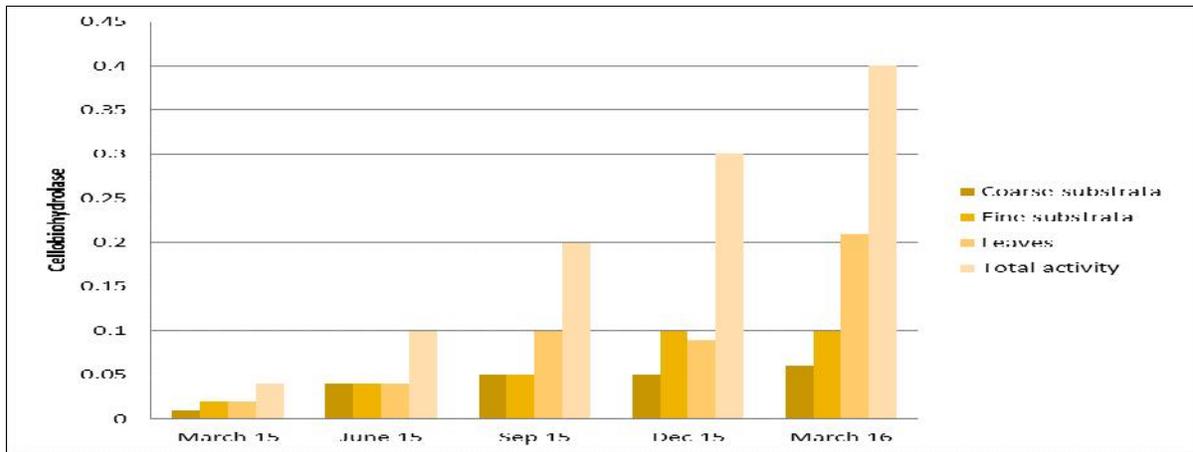
a)



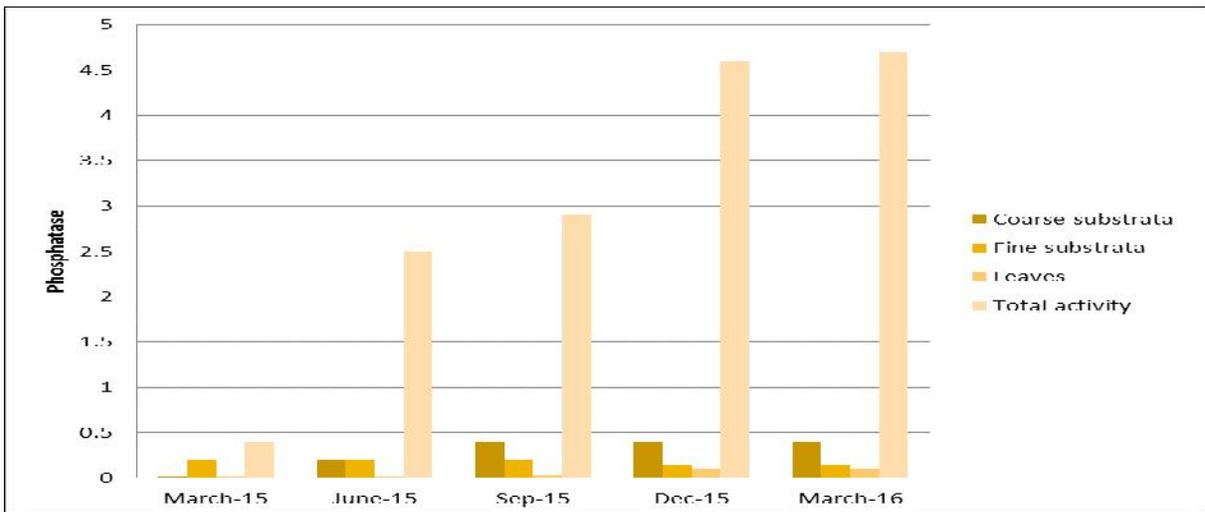
b)



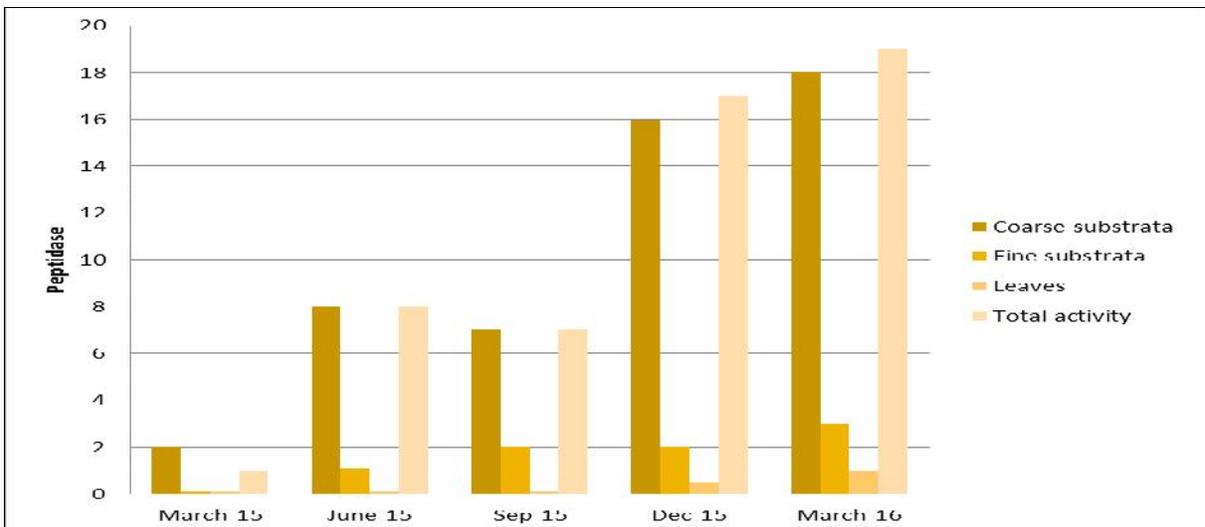
c)



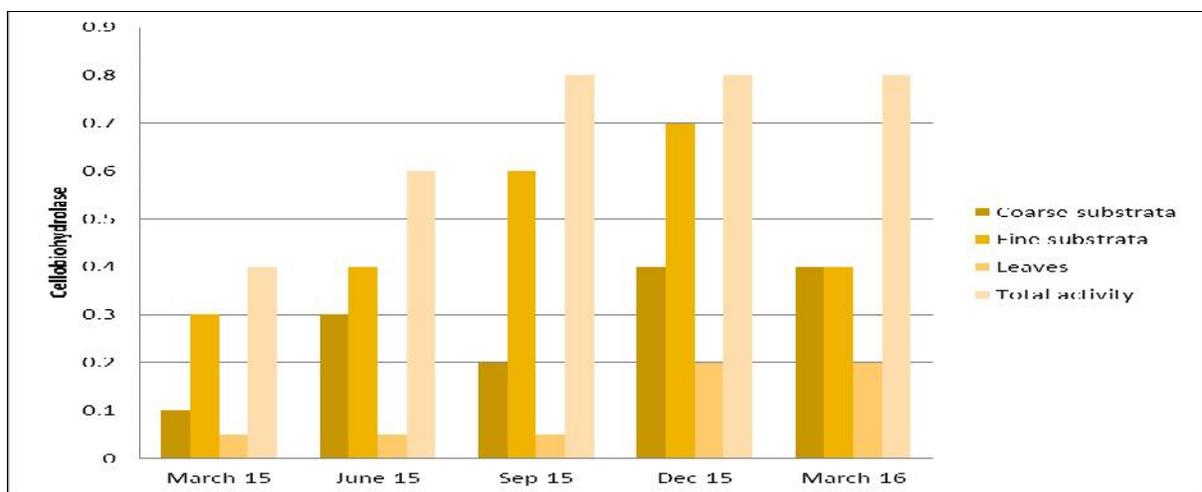
d)



e)



f)



**Figure – 4 : Extra cellular enzymes activity [(a)  $\beta$ - glucosidase, (b)  $\beta$ -xylosidase, (c) cellobiohydrolase, (d) phosphatase, (e) peptidase, and (f) phenol oxidase. Total values of different enzyme activity ( - activity in coarse and fine substrata and leaves) in the reach are also shown.**

## Discussion

Our observations on enzyme activity and microbial biomass reveal two distinct periods of high microbial enzyme activity: the first is in late winter-early spring, and the second in late summer-autumn. In late winter-spring, light reaching the stream bed was not restricted by canopy cover, which caused an increase in water temperature. For a short period, there was a sharp increase in autotrophic biomass, particularly on coarse substrata, resulting in a dense autotrophic population (high autotrophic: heterotrophic C ratio). The highest algal peak occurred in periods of relatively low dissolved inorganic nutrient concentration (June 2015), which is consistent with theories of light-dependant algal development in forested streams (Mallory & Richardson, 2005; Taulbee et al., 2005; Ylla et al., 2007). During this period, it is likely that algae outcompeted bacteria for dissolved inorganic nutrients (Rier & Stevenson, 2002). Peptidase activity was highest during periods of greatest algal biomass development in coarse substrata. This relationship may indicate the use of algal exudates (mainly peptidic molecules) by bacteria (Romaní et al., 2004), since bacterial enzyme production (especially peptidases) can be stimulated by active algal photosynthesis (Espeland et al., 2001). During this period, heterotrophic microbial activity may depend largely on the use of autochthonous sources.

The second episode of high microbial enzyme activity is based on allochthonous OM sources, especially leaf litter from the riparian forest. The activity of polysaccharide-degrading enzymes (involved in the degradation of cellulose and hemicellulose compounds) was associated mainly with the CPOM

standing stock in the reach. The magnitude and duration of high enzyme activity can be explained by temporal shifts in organic matter supply, as well as by changes in the storage capacity of sediments (Wilczek et al., 2005). Although this heterotrophic episode is correlated with leaf litter input and accumulation in the stream, microbial benthic communities also showed active decomposition activity during the winter. At this time, the decomposition of lignocellulose (phenol oxidase activity) and organic phosphorus compounds (phosphatase) was greatest in coarse substrata where fine detritus accumulated.

## Conclusion

Microbial heterotrophic biomass (bacteria plus fungi) was generally higher than autotrophic biomass (algae), except during short periods of high light availability in the spring and winter. During these periods, sources of organic matter (OM) shifted towards autochthonous sources derived mainly algae, which was demonstrated by high algal biomass and peptidase activity in benthic communities. Heterotrophic activity peaked in the autumn. Bacterial and fungal biomass increased with the decomposition of cellulose and hemicellulose compound from the leaf material. Later, lignin decomposition was stimulated in fine (sand, gravel) and Coarse (Rocks, boulders and cobbles) substrata by the accumulation of fine detritus. The Koraiyaru falls and Mayiluthu summer drought provoked an earlier leaf fall. The resumption of the water flow caused the weathering of riparian soils and subsequently a large increase in dissolved organic carbon and nitrate, which led to growth of bacteria and fungi.

## Acknowledgments

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