

Development of periphytic bacteria associated with detritus of the Amazonian aquatic macrophyte *Oryza glumaepatula*

by

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Abstract

In analyses of the decomposition of *Oryza glumaepatula*, fresh culms were collected in Lake Batata, an Amazonian clear water lake, and allowed to decompose in litterbags for 120 days. Bags were withdrawn in triplicates after 3, 10, 20, 39, 59, 88, 102, and 120 days and weight loss, nitrogen and phosphorus content, and bacterial density, biovolume and biomass were determined. Loss of detritus of *O. glumaepatula* was rapid (>50 % of dry weight during the first 20 days of decomposition). Nitrogen content in *O. glumaepatula* detritus increased during the study, while phosphorus content initially decreased and later increased. Detritus C:N:P ratios suggest that periphytic bacteria may be limited by nitrogen and not phosphorus. Bacterial biomass and density remained constant from day 3 to day 20, and increased from day 20 to day 39. The percentage of carbon lost by the detritus and metabolized (assimilated + respired) by bacteria was estimated and suggests that bacteria play a minor role in the decomposition of *O. glumaepatula* as they metabolize <6 % of all carbon lost by detritus.

Keywords: **Decomposition, bacteria, periphyton, aquatic macrophyte, Amazon.**

Resumo

Na análise da decomposição de *Oryza glumaepatula*, colmos verdes foram coletados no Lago Batata, um lago Amazônico de águas claras, e acondicionados para decomporem em "litter bags" durante 120 dias. Os "litter bags" foram retirados em réplicas após 3, 10, 20, 39, 59, 88, 102 e 120 dias, sendo determinados o peso seco, o conteúdo de fósforo e nitrogênio e a densidade, biovolume e biomassa bacterianas em cada momento de coleta. A perda de massa dos detritos de *O. glumaepatula* foi rápida (>50 % do peso seco durante os 20 primeiros dias de decomposição). O conteúdo de nitrogênio nos detritos de *O. glumaepatula* sofreu incremento durante o estudo, enquanto os teores de fósforo inicialmente decaíram e posteriormente sofreram incremento. As razões C:N:P dos detritos sugerem que as bactérias perifíticas podem ter sido limitadas por nitrogênio e não por fósforo. A biomassa e densidade bacterianas permaneceram constantes do dia 3 ao dia 20 e sofreram incremento do dia 20 ao dia 39. A percentagem de carbono perdido pelos detritos e metabolizado (assimilado + respirado) pelas bactérias foi estimado e sugere que as bactérias desempenharam papel pouco significativo na decomposição de *O. glumaepatula*, tendo metabolizado <6 % de todo carbono perdido pelos detritos.

Introduction

The periphytic community has an important role in nutrient cycling and energy transfer in aquatic ecosystems, especially in shallow environments with high densities of aquatic macrophytes (WETZEL 1990). In these areas, periphyton production may exceed that of the aquatic macrophytes (WETZEL & SØNDEGAARD 1998). The community of microorganisms on aquatic macrophytes may be termed epiphyton, biofilm, or periphyton. In the present report, we use the term proposed by WETZEL (1983), where the periphyton is defined as a complex association of microorganisms and organic and inorganic detritus attached to living or dead substrate.

Bacterial density on 1 cm² of colonized surface is about two orders of magnitude greater than the density found in 1 cm³ of water (HAMILTON 1987). In the initial stages of periphyton development, growth is mainly sustained by external nutrients, whereas in more advanced stages is based on internal processes of organic matter regeneration (SAND-JENSEN 1983). At this stage the periphyton polysaccharide matrix is more developed, and the resistance to changes in the environment is stronger (FREEMAN & LOCK 1995).

It is generally accepted that the periphytic microbial community play a major role in the decomposition of aquatic macrophytes. However the quantitative importance of each of these groups in the decomposition has been a matter of discussion for some years. It has been found that fungi decomposition of aquatic macrophytes is negligible (e.g. MANN 1988; BLUM & MILLS 1991), while others have shown that fungi are the main decomposing organisms (NEWELL et al. 1995; GESSNER & NEWELL 1997). KUEHN et al. (2000) compared the role of both groups of microorganisms during decomposition of *Jucus effusus* and showed that fungal decomposers are the dominant microbial assemblage associated with decaying plant litter and that they accounted for 99 % and 91 % of total microbial biomass and production respectively. However, these authors suggest that bacterial productivity may increase with temperature and therefore be significantly higher in subtropical and tropical environments.

The Amazon region is characterized by relatively high annual temperatures (around 30 °C) throughout the year and a huge number of floodplain lakes. These lakes usually have large stands of aquatic macrophytes with a life cycle determined by the flood pulse. In addition the flood pulse influences the area colonized and the amount of biomass produced by the aquatic macrophytes (JUNK & PIEDADE 1993).

A literature survey on the ecology of aquatic macrophytes in the Amazon region reveals a paucity of data on the periphytic bacteria. The few studies on periphytic bacteria focus mainly on nitrogen fixation and denitrification (e.g. DOYLE & FISHER 1994; ENRICH-PRAST & ESTEVES 1998), whereas studies on bacterial biomass and productivity are restricted to pelagic bacteria (e.g. SCHMIDT 1970; BENNER et al. 1995; ANESIO et al. 1997). The present study followed changes in density, biovolume, and biomass of the periphytic bacterial community and in the nitrogen and phosphorus detritus content during decomposition of *Oryza glumaepatula*. The importance of the bacterial community in carbon mineralization associated with the detritus of *O. glumaepatula* was also estimated.

Study site

Lake Batata is a typical clear water lake located on the right bank of the Trombetas River (1°35'S, 56°25'W), a tributary of the left bank of the Amazon River. A detailed

map from Lake Batata can be found in ANESIO et al. (1997). In Lake Batata, as in most Amazonian lakes, the water level fluctuates markedly, in some years more than 8 m. The rise in water level results in flooding of the surrounding forest, called igapó forest. The water level variation can be categorized in 4 hydroperiods: low water, filling, high water, and drawdown. High water occurs June-July. Drawdown is characterized by a reduction in the water level and a simultaneous reduction of allochthonous organic matter input into the lake from the igapó forest and occurs usually from August to October. Low water occurs in November-December, and is characterized by very low water levels in rivers and lakes, and complete drying of the littoral zone (igapó forest). The filling period begins in December-January, with a rapid increase in the water level (up to 10 cm d⁻¹), and a subsequent re-flooding of the igapó forest. During the filling period, floodplain lakes receive large amounts of allochthonous carbon in the form of seeds, leaves, and other organic materials that originate in the floodplain forest.

Much of the flooded area in Lake Batata is colonized by the aquatic macrophyte *Oryza glumaepatula* Steud (Poacea), locally known as "wild rice". This species germinates when the sediment in the previously flooded areas is exposed. The terrestrial phase of its life cycle lasts for about three months. As the individual plants are flooded, a period of intense development of the culms follows, and these culms are colonized by periphyton. At this stage, the growth rates of *O. glumaepatula* may reach 4 cm d⁻¹. After the establishment of anoxic conditions near the sediment, the plants begin to decompose progressively from the bottom close to the sediment to the top of the plant. As the water level decreases, the detritus is carried away to other parts of the lake, or settles on to the sediment.

Material and methods

Fresh culms of *O. glumaepatula* were collected in Lake Batata in the filling period (March 1995), and then allowed to decompose in litterbags (plastic bags with 5 mm mesh) until the high water period (July 1995). Approximately 30 g wet weight (ww) of fresh plant material was placed in each litterbag. Bags were withdrawn in triplicates after 3, 10, 20, 39, 59, 88, 102, and 120 days for weight loss, nitrogen and phosphorus content and bacterial determinations. On each sampling day (except on day 10 and 120), at the sampling moment, small sub-samples (ca. 1 cm) of detritus were carefully cut and transferred to plastic vials that contained 10 ml of a solution of sodium pyrophosphate (final concentration 0.01 %) and paraformaldehyde (final concentration 3.7 %). In the laboratory, the content of each bag was dried for 72 h at 80 °C and weighed to determine dry weight (dw) loss. Then the detritus was ground and the contents of total nitrogen (ALLEN et al. 1974) and total phosphorus (FASSBENDER 1973) were determined. The phosphorus content was only determined until day 88, since there was not enough material available for chemical analyses on days 102 and 120. The organic carbon content in the detritus of *O. glumaepatula* was assumed to be 46.5 % of dw remaining throughout the period of decomposition (WESTLAKE 1965). The rate of detritus decay was measured as dw loss over time. The ww was determined in each litterbag before the beginning of incubation. Ratios of ww: dw were determined using the procedure proposed by BLUM & MILLS (1991). Wet fragments of culms were weighed (n=30), dried for 72 h at 80 °C, and reweighed to obtain a conversion factor to estimate the decay rate. The factor obtained was 1 g ww = 0.098 g dw. Determination of bacterial population density and biovolume (also known as mean cell volume) was performed according to THOMAZ & ESTEVES (1997a). Culms were carefully scraped with a spatula, and their area was determined. The detached periphyton was diluted in 10 ml of buffered formalin and sonicated in a TRANSONIC 890-T (ELMA) for 3 minutes. Since there was a high variation in the amount of suspended material in the sonicated samples, they were diluted in a range from 2 to 10 times using 0.22 µm filtered-sterilized water. Afterwards the diluted material was filtered with NUCLEOPORE 0.22 µm black filters together with a solution of acridine orange (0.01 %). Filters were counted in an inverted

epifluorescence microscope ZEISS Axiovert (1600 x magnification). In each slide 30 fields were counted and 50 bacteria cells were randomly selected for biovolume determination. These cells were divided in three categories and their average biovolume were estimated. Biovolume was converted to biomass with the conversion factor proposed by BJØRNSSEN (1986), assuming $1 \mu\text{m}^3 = 3.5 \times 10^{-13} \text{ g C}$. Limnological parameters have been sampled as described in ANESIO et al. (1997).

Results

The water column characteristics analyzed (temperature, dissolved oxygen, pH, conductivity, and alkalinity) did not change much during the rise in the water level between the filling and high water periods (Table 1). The most pronounced change was the decrease in chlorophyll-*a* (chl-*a*) and total suspended solids (TSS) in the water column. The decrease in the values of chl-*a* was indicative of a decrease in phytoplankton primary production. This decrease could result in a decrease in periphytic bacterial production, due to the smaller amount of dissolved organic carbon (DOC) leaching by phytoplankton. Nonetheless, as individuals of *O. glumaepatula* in Lake Batata are densely colonized by periphytic algae (ENRICH-PRAST et al., unpubl.), it can be assumed that the DOC used by periphytic bacteria is formed in the periphyton itself, and that changes in the phytoplankton would have only a small influence on the dynamics of the periphytic bacterial community.

The decomposition of *O. glumaepatula* could be divided into three phases based on the decrease in dw with time of incubation (Fig. 1a). During the first phase, from day 0 to day 3, 27 % of the initial dw was lost. During the second phase from day 3 to day 20, 28 % of the dw was lost (rate of $1.65 \text{ \% dw d}^{-1}$, $r^2 = 0.99$). A reduced loss during the third phase from day 20 to day 120, with a rate of dw loss of $0.37 \text{ \% dw d}^{-1}$ ($r^2 = 0.97$). The decay rate constant derived from an exponential function for dw loss versus time during all 120 days was 0.016d^{-1} ($r^2 = 0.96$). By the end of the experiment, 10 % of the original material remained in the litterbags. The nitrogen content remained constant ca. $350 \mu\text{mol N g dw}^{-1}$ until day 20 after which it increased significantly (ANOVA, $p < 0.05$) to ca. $1200 \mu\text{mol N g dw}^{-1}$ by the end of the experiment (Fig. 1b). There was a 45 % decrease in phosphorus content from day 0 to day 20, after which it increased significantly (ANOVA, $p < 0.05$) and reached the initial content of ca. $15 \mu\text{mol P g dw}^{-1}$ at day 88 (Fig. 1c). On day 88 the phosphorus content were significantly higher than those on day 20. The C:P ratio gradually increased from 2570 on day 0 to 4590 on day 20 and then gradually decreased until it reached its lowest value 2220 on day 88 (Table 2). The C:N ratios remained high during the first 20 days of incubation (89-104). After day 20 there was a decrease in the C:N ratio to 28 by the end of the incubation (Table 2).

The bacterial biomass showed a similar pattern to that of bacterial density (Fig. 2), with a strong correlation between these two variables ($r^2 = 0.83$, $p < 0.05$). On day 3, bacterial density and biomass were respectively $0.32 \times 10^7 \text{ cm}^{-2}$ and $0.05 \mu\text{g C cm}^{-2}$, being the significantly (ANOVA, $p < 0.05$) lowest values found in the course of this study. The significantly highest values (ANOVA, $p < 0.05$) for bacterial density and biomass were found on day 39, being respectively $3.38 \times 10^7 \text{ cm}^{-2}$ and $0.29 \mu\text{g C cm}^{-2}$. From day 3 to 20 and from day 20 to 39, bacterial density increased by 140 and 300 % respectively. In the same interval, it was an increase in 65 and 125 % in bacterial biomass respectively. After day 39, bacterial density and biomass decreased significantly (ANOVA, $p < 0.05$) until day 88 and between days 88 and 102 no significant differences were observed (ANOVA, $p > 0.05$). The bacterial biovolume decreased significantly

(ANOVA, $p < 0.05$) from $0.42 \mu\text{m}^3$ at day 3 to $0.16 \mu\text{m}^3$ at day 59. The lower bacterial density on days 3 and 20 coincided with the higher bacterial biovolume values.

Discussion

The detritus of *O. glumaepatula* lost about 30 % of its total weight within 3 days, and may be classified, as proposed by BELOVA (1993), as a fast decomposing species. It can be assumed that on the first three days of decomposition, the loss of dw was due to physical processes, specifically leaching (VALIELA et al. 1985). The action of bacteria on the detritus may be considered negligible because of their low density. According to TANAKA (1991) and BLUM & MILLS (1991) the action of fungi on detritus is also negligible during the first days of decomposition. Although the effect of macroinvertebrates on the detritus of aquatic macrophytes in the Amazonian region was shown to be important by WALKER (1986), she concluded that this effect is not significant during the first 10 days of decomposition. We can therefore assume that most biological activity occurred only after day 3, when 70 % of the *O. glumaepatula* detritus remained. The values for dw loss found in this investigation were similar to those found by HOWARD-WILLIAMS & JUNK (1976), who studied the decomposition of six species of aquatic macrophytes in the Amazon region. The results of these authors, as well as those obtained in our study, show that the decomposition rates for aquatic macrophytes in amazon areas are much higher than in temperate areas (MORRIS & LAJTHA 1986; EMERY & PERRY 1996), probably due to higher temperatures.

The increase in the nitrogen content per gram dw remained in the detritus of *O. glumaepatula* agrees with a phenomenon observed in most studies on the decomposition of aquatic macrophytes and litter (e.g., VALIELA et al. 1985; TANAKA 1991). The decrease and later increase in phosphorus content in the detritus of *O. glumaepatula* support the hypothesis that microorganisms recycle P in the periphyton. The initial decrease in phosphorus content can be explained by the fact that most of this element present in the plant occurs in labile compounds (BURKHOLDER & WETZEL 1990). The later increase in phosphorus content is an indication that the phosphorus present in more refractory molecules is released when the periphytic community is more developed, being then absorbed and recycled by the periphyton microbial community. HIETZ (1992) obtained a similar result, but attributed the later increase in nitrogen and phosphorus contents to uptake of nutrients from the water column.

GOLDMAN et al. (1987) showed that the ideal C:N and N:P ratios for bacterial metabolism are 5 and 9, respectively. Throughout the experiment the N:P detritus ratio was 3-8 times higher than the ideal ratio for bacterial metabolism, while the C:N detritus ratio was 6-21 times higher than the ideal C:N ratio. The C:N and N:P ratios in the detritus of *O. glumaepatula* suggest, therefore, that nitrogen and not phosphorus is limiting the periphytic bacterial community associated to *O. glumaepatula* detritus.

The bacterial biomass and density associated with the detritus did not increase noticeably from day 3 to day 20, in spite of the fact that in this period the available carbon is more labile than in any other period of the decomposition process (GODSCHALK & WETZEL 1978). However, from day 20 to day 39 there was a noticeable increase in bacterial density, which could be explained by the decrease in the C:N ratio of the detritus. This results from the fact that as the C:N ratio decreases and approaches bacterial ratio, carbon conversion efficiency increases (LINDLEY & NEWELL 1984). This decrease in C:N ratio can not be attributed to an bacterial biomass increase as the

role of bacteria in the detritus nutrient pool can be considered negligible. Estimates of the bacterial carbon content and the total mass of detritus found in our study (based on values per culm surface) indicate an average bacterial carbon biomass <0.01 % of total carbon. This result is similar to those of THOMAZ & ESTEVES (1997a) and BLUM et al. (1988), who studied the decomposition of other species of aquatic macrophytes.

A hypothesis to explain the decrease in bacterial density after day 39 is a possible increase in predation by flagellates. According to GASOL et al. (1995) and SIMEK et al. (1994), planktonic flagellates and ciliates usually prefer to prey on larger bacteria. ABREU & ODEBRECHT (2000) obtained a similar result in a study of the decomposition of *Scirpus maritimus* in Lagoa dos Patos, Brazil. These authors attributed the reduction in bacterial density and biovolume to "top-down" processes, which is basically predation pressure.

An analysis of the values reported in the literature shows that the density of periphyton bacteria can vary by up to two orders of magnitude (WETZEL & SØNDEGAARD 1998). Our results (compared with other studies where the bacterial density was determined by epifluorescence counting with acridine orange or DAPI) show that the bacterial density in detritus of *O. glumaepatula* is within the range of most reported values. Most investigations of the decomposition of aquatic macrophytes have been done in temperate environments, but THOMAZ & ESTEVES (1997a, 1997b) and BRUM & ESTEVES (2001) studied the density and biomass of periphyton bacteria in tropical coastal lagoons of Rio de Janeiro. These investigators found densities close to those observed in our study.

From some data obtained in this study and some values obtained in the literature, we estimated the percentage of carbon lost by the detritus of *O. glumaepatula* that was metabolized (assimilated + respired) by the bacterial community. This estimation was based on the following formulas:

$$\text{C release rate} = (0.465 * (dw_{da} - dw_{db}) * 0.01 / (\Delta t)) \quad (1)$$

C release rate (g C cm⁻² d⁻¹): rate of carbon that is release by the detritus in a sampling interval

0.465: assumed value that 46.5 % of detritus biomass content is carbon

dw_{da} (g dw plant): dry weight value obtained in sampling day a from Figure 1a

dw_{db} (g dw plant): dry weight value obtained in sampling day b from Figure 1a

0.01: conversion factor of g dw⁻¹ in cm²; 1 g dw⁻¹ = 99 cm² (n=20)

Δt (d): difference in days between the sampling interval

$$\text{C uptake rate} = BB * CIE * Dt \quad (2)$$

C uptake rate (g C cm⁻² d⁻¹): rate of carbon that is metabolized (respired + assimilated) by bacteria in a certain sampling day

BB (μg C cm⁻²): bacterial biomass obtained from Figure 2

CIE (μg C): carbon incorporation efficiency. We used a value of 0.1 as explained further in the text

Dt (μg C d⁻¹): bacterial doubling time. We used a value of 10, as explained further in the text

$$\% \text{ C metabolised} = \text{C release rate} / \text{average of C uptake rate} \quad (3)$$

% C metabolised: % of carbon metabolised by bacteria in a certain sampling interval

C released rate: rate of carbon that is release by the detritus in a certain sampling interval

Average of C uptake rate: average between the initial and final C uptake rate in a

certain sampling interval.

Some values used in these calculations were obtained in the literature. WESTLAKE (1965) suggests that the carbon content in aquatic plants is around 46.5 % of its dry weight. Ten days was considered a reasonable doubling time estimation for periphytic bacteria as KUEHN et al. (2000) found a value of 13 days and this value may be up to 30 days (BLUM & MILLS 1991). We also assumed a carbon incorporation efficiency (CIE) of 10 %, indicating that 90 % of the total carbon uptake was respired. MANN & WETZEL (1996) reported CIE values of 17 % and BLUM & MILLS (1991) reported a range from 2 to 20 % for periphytic bacteria.

The percentage of carbon lost by the detritus of *O. glumaepatula* that was metabolized by the bacterial community increased gradually until the end of the experiment. From day 59 to 88 and from day 88 to 102, respectively 9 % and 8 % of all carbon lost was metabolized (Table 3). From day 0 to 3, 27 % of detritus dw was lost and bacteria metabolized only 0.9 % of it. From day 3 to 20, bacteria metabolized only 1.7 % of the carbon lost. Integrating all the data from Table 3, it was estimated that from day 0 to day 102, the periphytic bacteria community metabolized <6 % from all carbon lost by the detritus of *O. glumaepatula*. These calculations suggest that periphytic bacteria have a small role in the decomposition process of *O. glumaepatula*. If we have assumed a higher carbon incorporation efficiency value, the percentage of carbon lost that is metabolized by bacteria would be even lower. On the other hand, if we have assumed a lower doubling time, this percentage would increase. LINLEY & NEWELL (1984) showed that during the decomposition process, the amount of refractory compounds in the detritus increases and the carbon incorporation efficiency drops. KUEHN et al. (2000) also concluded that the role of bacteria is negligible in the decomposition of *J. effusus*, while fungi play a major role. In this estimate we did not consider the bacterial carbon consumed by flagellates. If we assume that half of the bacterial assimilated carbon is consumed by ciliates and flagellates, the amount of carbon lost that is metabolized by bacteria would reach 10 %, which is still a relative low value.

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Table 1: Some environmental parameters at the beginning and at the end of the experiment at the sampling site. *Data obtained in the same studied area and hydrological periods but at different year from ANESIO et al. (1997).

	March/95	June/95
Temperature (°C)	30.0	29.4
Depth (m)	1.4	3.5
pH	5.5	5.0
Conductivity ($\mu\text{S cm}^{-1}$)	8.0	9.0
Oxygen (mg L^{-1})	5.4	5.7
Total Phosphorus ($\mu\text{g L}^{-1}$)	24.9	22.6
Nitrogen Kjeldahl (mg L^{-1})	0.5	0.4
Chlorophyll- <i>a</i> ($\mu\text{g L}^{-1}$)	4.2	2.1
Total Suspended Solids (mg L^{-1})	8.4	5.2
Alkalinity (mEq L^{-1})	0.23	0.19
Bacterial density (10^5 cells m L^{-1})*	4.0	3.5
Heterotrophic flagellates density (10^3 cells m L^{-1})*	2.1	1.7

Table 2: C:N:P and C:N ratios (in a molar basis) of detritus of *Oryza glumaepatula*. * Phosphorus concentrations have not been analysed at days 102 and 120.

Day of incubation	C:N:P	C:N
0	2570:25:1	104
3	2620:26:1	99
10	3260:31:1	104
20	4590:51:1	89
39	3940:73:1	54
59	3060:48:1	64
88	2220:39:1	57
102	*	48
120	*	28

Table 3: Percentage of carbon lost by the plant that is metabolised (assimilated + respired) by periphytic bacteria during the different phases of decomposition of *Oryza glumaepatula*.

Phase of decomposition (days)	% of C metabolised
0-3	0.9
3-20	1.7
20-39	6.1
39-59	9.0
59-88	6.5
88-102	7.9
Total	5.6

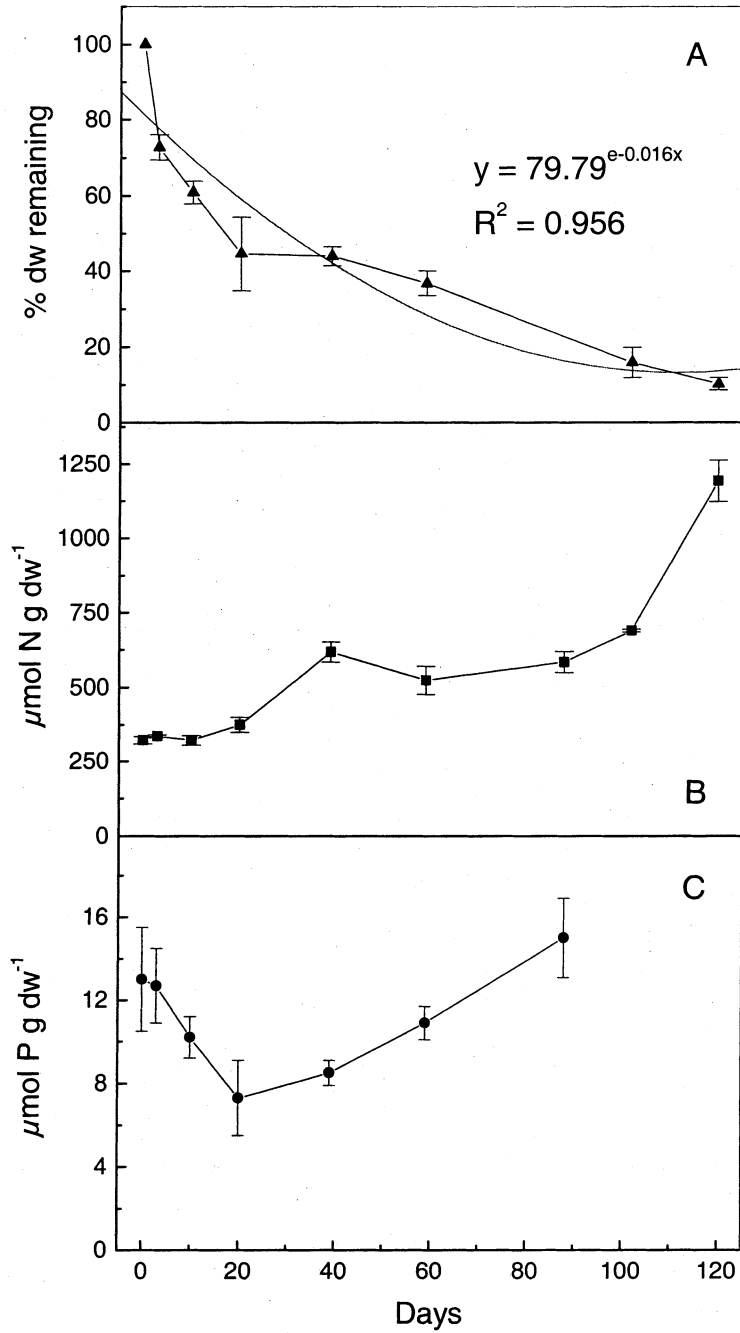


Fig. 1:
Weight loss (A) (expressed as percentage of dry weight remaining) nitrogen (B) and phosphorus (C)
content per g DW of detritus of *Oryza glumaepatula*. For all panels error bars are one SE (n=3).

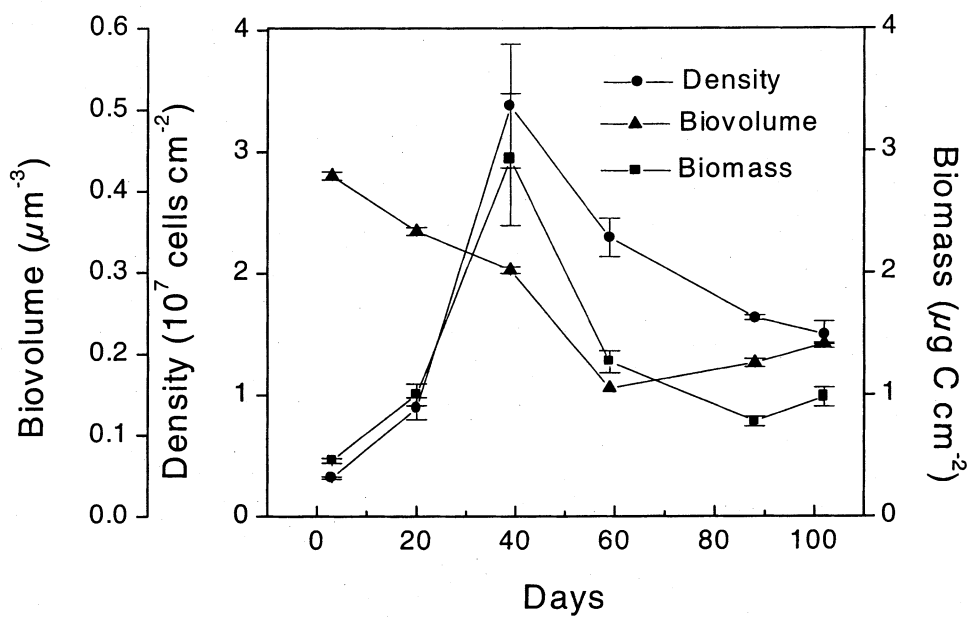


Fig. 2:
Density, biovolume and biomass of periphytic bacteria associated to detritus of *Oryza glumaepatula*.
For all panels error bars are one SE (n=3).

