Heterotrophic and autotrophic metabolism in Mediterranean streams

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HETEROTROPHIC AND AUTOTROPHIC METABOLISM IN MEDITERRANEAN STREAMS

Ph. D. Thesis

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3.2. Organic matter utilization in a forest stream sediment: surface versus subsurface zones

Abstract

The heterotrophic metabolism of a forest stream sediment was studied right after an important flood which completely washed and homogenized the sediment to the first 10-12 cm in depth. After this event, leaf fall began. Differences were found between the surface sediment (a depth of 0-3 cm) and the subsurface sediment (a depth of 7-10 cm). Higher extracellular enzymatic and respiratory activities were measured in the surface sediment as compared to the subsurface sediment. The higher heterotrophic activity has been related to the higher quantity and quality of the organic matter which accumulates in the surface sediment (with higher chlorophyll-a and bacterial densities) Furthermore, the heterotrophic metabolism in the surface sediment followed a marked seasonal pattern which correlated with the variations in the environmental parameters (discharge, nutrients). However, no time pattern was observed in the subsurface sediment where all activities were rather steady over the study period. The few cm in depth of the subsurface sediment studied showed a significant decrease in heterotrophic metabolism, which was slower and steadier than in the surface sediment.

Introduction

The main function of microbial populations living in stream sediments is the uptake and subsequent removal of organic substances from the catchment area and/or from the autochthonous material (Naiman et al. 1987), and their conversion into a particulate form (Kaplan and Bott 1983, Bott et al. 1984). In the hyporheic zone, defined as the sediments hydrologically linked to the open stream channel (Findlay 1995) and above groundwater (White 1993), microbial metabolism and organic carbon cycling are as tightly coupled as in surface sediments (Hedin 1990, Bärlocher and Murdoch 1989). Despite the fact that there are few microbial studies (Hendricks 1993), high metabolic and chemical activity have been measured for the hyporheic zones (Grimm and Fisher 1984, Pusch and Schwoerbel 1994). It has been concluded that freshwater sediments are an important site for bacterial production (Moran and Hodson 1992).

Sediment metabolism is affected by physical factors such as hydrology and permeability (Hakenkamp et al. 1993, Findlay 1995), leading to heterogeneity in biological and physicochemical patterns in the hyporheic zone (Palmer 1993). Stream bed permeability, mainly controlled by grain size (Findlay 1995), favours the water exchange between the surface and the hyporheic sediments (Grimm and Fischer 1984).

This study focus on the heterotrophic metabolism in the sediment of a Mediterranean forest stream (Riera Major), distinguishing a surface and a subsurface zone. Remarkably high heterotrophic activity has been measured on sandy biofilms in this stream (section 3.1). In sandy stretches (which may account for 40% of the stream bottom) the hyporheic zone ranges

from a few cm to 50 cm in depth. The important input of allochthonous organic matter from the riparian forest and the existence of a hyporheic zone made us think that this sediment was an important site for carbon cycling.

This study started right after the severe flood occurred at Riera Major in October 1994 that completely removed all stored materials from the stream bottom and homogenized the stream sediment to a depth of 10-12 cm. This event took place before the leaf fall and provide an opportunity to compare the evolution of the surface sediment (a depth of 0-3 cm) and the subsurface sediment (a depth of 7-10 cm) from the same starting point (few material accumulated in both zones). The main purpose was to find out whether differences in utilization of organic matter by the heterotrophic community of the surface and subsurface sediment were significant or not. Differences in time pattern after such a flood were also investigated. To this purpose, the ectoenzymatic (β-glucosidase, β-xylosidase and phosphatase) and respiration activities were measured, as well as bacterial density and chlorophyll-a and organic matter content in both sediments over an eleven month period.

Methods

Site and sampling

This study was carried out in Riera Major. During the study period (October 1994-August 1995) average flow was 60 L s^{-1}, but the mid-October flood increased discharge to 722 L s^{-1}. Previous experiments performed in this stream (chapter 3.1) provided background data about the heterotrophic metabolism of the sediment before the flood (Table 1), which allowed to control the removal effect of the flood on the benthic activity and biomass.

Sand samples were taken every fifteen days for the first two months after the flood (October-December 1994), and monthly till the end of the experiment (December 1994-August 1995). A metallic corer-sampler (7 cm diameter) was used for collecting sand samples (six replicates) in a stretch of the stream where fallen leaves accumulated. Sediment at a depth of 0-3 cm was considered surface sediment, while at a depth of 7-10 cm was subsurface sediment. The sediment at a depth of 4-6 cm was discarded to avoid edge effects. Sand samples (with a volume of about 2 ml) were placed in sterile glass vials and kept cold (on ice) in the dark until arrival at the laboratory. Sand samples for bacterial counting were fixed with 2% formalin.

At each sampling date, temperature, pH, dissolved oxygen and conductivity were measured in stream and subsurface water. A manual pump was used to sample subsurface water using two bores previously installed in the stream. Water samples were filtered through precombusted Whatman GF/F filters and analyzed for inorganic nutrients (nitrate, ammonium and soluble reactive phosphorus, SRP), dissolved organic carbon (DOC) and dissolved inorganic carbon (DIC). (Three samples per sample analysed). The DIN:SRP ratio was calculated as the molar ratio of dissolved inorganic nitrogen to soluble reactive phosphorus. Discharge was also measured in the field at each sampling date. Analyses were performed following the procedures described in chapter 2.

Particulate organic matter (POM) transported by the stream was collected with a net (230 μm pore, 20 cm x 20 cm) placed in the stream for 2 min. The material accumulated was dried for two days at 110°C and weighed, and expressed as mg L^{-1}.

TABLE 1. Biofilm metabolism and biomass in Riera Major sediments before the flood (01/94-09/94) and right after the flood (11/94-01-95). Values are means of monthly averages and standard deviations.

	Before flood		**After flood**	
	Mean (n=8)	SD	Mean (n=4)	SD
Bacterial density (10^{10} cm^{-2})	1.60	1.20	0.19	0.11
Chlorophyll-a (μg cm^{-2})	1.78	1.13	0.98	1.00
Organic matter (mg AFDW cm^{-2})	6.31	2.78	3.74	8.05
β-glucosidase (nmol cm^{-2} h^{-1})	20.52	8.67	11.15	1.77
β-xylosidase (nmol cm^{-2} h^{-1})	9.24	4.60	4.79	1.44
Phosphatase (nmol cm^{-2} h^{-1})	22.10	16.8	11.93	10.96
ETS (μg cm^{-2} h^{-1})	1.23	0.72	0.44	0.16

Bacterial density, algal biomass, and organic matter content

Bacterial density (DAPI stain, epifluorescence microscopy) and chlorophyll-a (acetone extraction) were measured using six replicates per sample type following the procedures described in chapter 2. Organic matter in surface and subsurface sand samples (six replicates per type) was measured as ash-free dry weight (AFDW), after combusting the sand samples at 450°C for 4 h, and expressed as mg cm^{-2} .

Metabolism measurements

Extracellular β-D-glucosidase, β-D-xylosidase, and phosphatase potential activities were determined spectrofluorometrically using MUF-substrate analogues. Surface and subsurface sand samples (six replicates per type), controls (killed samples), and MUF-substrate blanks were incubated. Respiratory activity (ETS) was assayed using six replicates per sample type and two controls. The incubation procedures for all the assays were those described in chapter 2.

All activity and biomass measurements were related to cm^2 of sand grain surface area, after dry weighting (2 days at 110°C) each sand sample, as described in chapter 3.1, using the conversion factor calculated for mid-channel sand (1.4798 cm^2 g^{-1}).

Data analyses

Correlation analysis of the metabolic parameters with environmental variables was performed using product-moment Pearson coefficient. Correlation was also used to elucidate differences in the time pattern of microbial metabolism between surface and subsurface sediment. Differences in metabolic activities and biomass between sampling dates were analyzed with a one-way analysis of variance (ANOVA) and compared using a Tukey's multiple comparison test. Differences between surface and subsurface sediment metabolism and biomass were analyzed using an analysis of variance (ANOVA, two factor with replicates).

Results

Physical and chemical parameters

Physical and chemical parameters in stream water and subsurface water (Table 2) were not significantly different (*t*-test, $p>0.05$). A significant correlation was found between DOC and discharge in surface water ($r=0.75$, $p<0.001$, $n=51$).

TABLE 2. Physical and chemical characteristics of Riera Major stream water and subsurface water during the study period.

	Surface water				Subsurface water			
	min.	max.	average	SD	min.	max.	average	SD
Temperature (°C)	3.1	16	8.53	4.22	3.25	13.2	8.47	3.0
Conductivity ($\mu S\ cm^{-1}$)	126	205	175.8	30.3	129.5	209	177.86	30.2
pH	7.4	8.29	8.01	0.31	7.59	8.08	7.93	0.2
Oxygen ($mg\ L^{-1}$)	9.2	12.35	10.51	1.05	7.8	12.4	10.63	1.71
NO_3-N ($\mu g\ L^{-1}$)	269	601.2	426.54	120.	254.7	629.5	423.84	140.
NH_4-N ($\mu g\ L^{-1}$)	2.16	46.78	17.93	14.9	0.37	51.14	18.67	17.0
SRP ($\mu g\ L^{-1}$)	2.17	18.75	9.94	5.98	2.96	20.75	12.23	6.96
DOC ($mg\ L^{-1}$)	1.49	5.95	3.02	1.38	2.46	6.09	3.95	1.22
DIC ($mg\ L^{-1}$)	11.16	23.99	18.46	5.23	11.33	23.27	18.59	4.53

The discharge dynamics and POM transported in Riera Major is shown in Fig. 1. After the main flood in October (722 $L\ s^{-1}$), two minor successive floods took place in early November (264 $L\ s^{-1}$) and in early December (175 $L\ s^{-1}$). Afterwards, discharge decreased gradually. POM transported in the stream water was very high during a short period of time (2-3 weeks), reaching values of 6-7 mg dry weight L^{-1}.

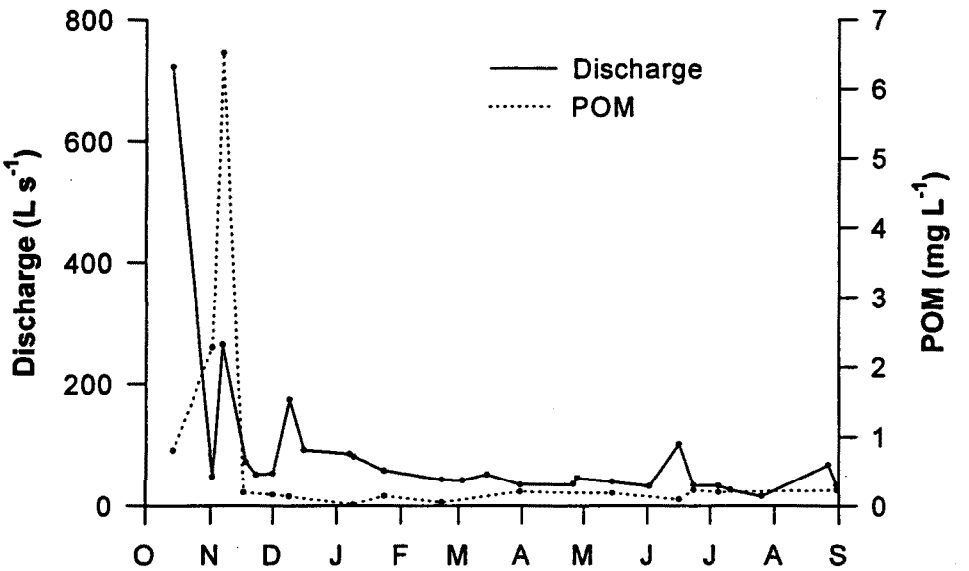


Fig. 1. Temporal variation of discharge and transported POM in the Riera Major stream, October 1994-August 1995.

Benthic organic matter and biomass

Benthic organic matter content (Fig. 2a) was slightly but significantly higher in the surface zone (ANOVA, Table 3). In the surface sediment, it reached a peak two weeks after the main flood coinciding with the fall of leaves and the highest POM value in stream water, but due to the large fluctuations, no significant differences were found between sampling dates (ANOVA, $p=0.37$). In the subsurface sediment, benthic organic matter was rather constant over the study period, with no significant differences observed between sampling dates (ANOVA, $p=0.41$).

Bacterial cell density and chlorophyll-a content were significantly higher in the surface sediment (Table 3), where both parameters followed a similar pattern (Fig. 2b and c, Table 4). Differences between sampling dates were significant for the surface sediment for both chlorophyll-a and bacterial density (ANOVA, $p<0.0001$); the values increased to a significant peak in June (Tukey's test, $p<0.05$), decreasing again in July. Both bacterial density and chlorophyll-a fluctuated less in the subsurface sediment (Fig. 2b and c), with no significant differences observed between sampling dates for chlorophyll-a (ANOVA, $p=0.06$) and only a significant peak of bacterial density in August (Tukey's test, $p<0.05$).

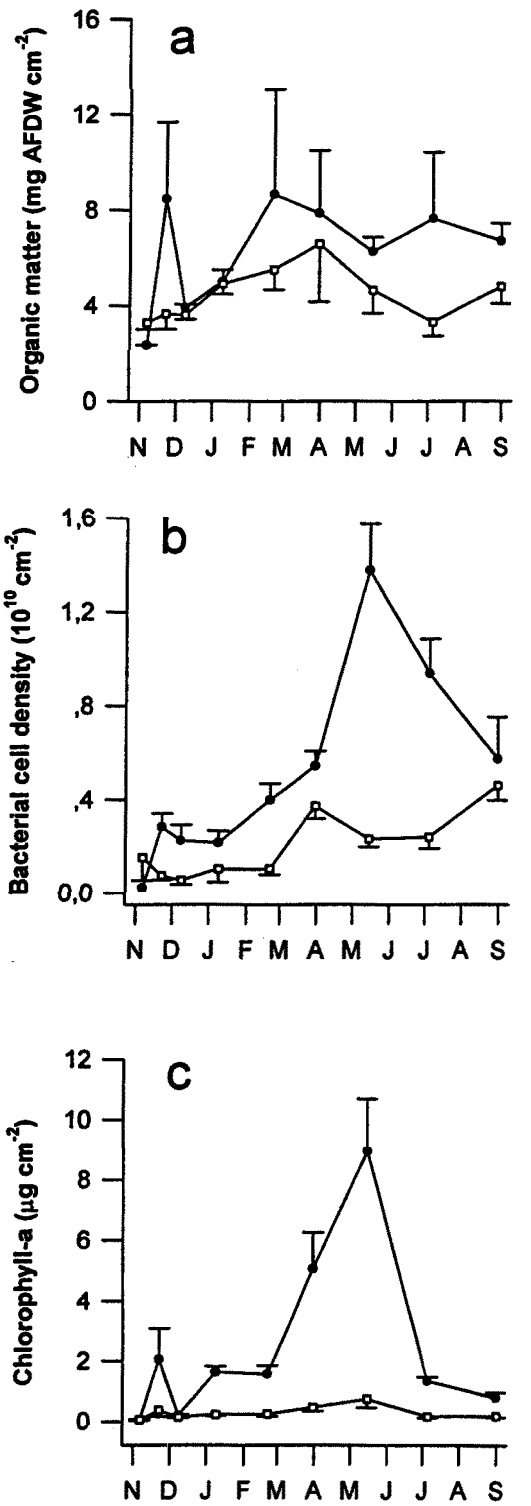


Fig. 2. Temporal variation of benthic biomass and organic matter in surface sediment (black circles) and subsurface sediment (empty squares) in Riera Major stream, October 1994-August 1995. Means ± standard errors (vertical bars) are shown, n=6.

In the surface sediment, both chlorophyll-a and bacterial density correlated negatively with discharge and DOC, whereas in the subsurface sediment only bacterial density correlated with discharge (Table 4). Bacterial density in both habitats correlated with temperature (Table 4).

The chlorophyll-a/organic matter ratio (Chl/OM) was calculated for both surface and subsurface zone as an indicative of the "quality" of the material accumulated in the sediment and related to the source of autochthonous/allochthonous organic matter. Significantly higher values of this ratio were observed for the surface sediment (Table 3).

TABLE 3. Extracellular enzymes and respiration activities and biomass on surface and subsurface sediments from Riera Major. Values are means and standard deviations for the study period. Differences between the two habitats are expressed by the probability of the F-Fisher after the ANOVA analysis. The star (*) indicates the significantly higher values for the surface sediment.

	Surface sediment		**Subsurface sediment**		ANOVA
	Mean (n=51)	SD	Mean (n=51)	SD	probability
Bacterial density (10^{10} cm^{-2})	0.56*	0.46	0.20	0.17	$9\ 10^{-13}$
Chlorophyll-a (μg cm^{-2})	2.48*	3.31	0.30	0.35	$1.6\ 10^{-11}$
Organic matter (mg AFDW cm^{-2})	8.05	11.12	4.54	2.53	0.031
Chl/OM ratio (10^{-4})	4.17*	5.46	1.10	3.39	$2.4\ 10^{-5}$
β-glucosidase (nmol cm^{-2} h^{-1})	12.49*	5.08	6.68	3.15	$2.2\ 10^{-10}$
β-xylosidase (nmol cm^{-2} h^{-1})	5.44*	2.07	2.56	1.50	$5.4\ 10^{-14}$
Phosphatase (nmol cm^{-2} h^{-1})	22.98*	14.35	15.94	8.62	$2.2\ 10^{-8}$
ETS (μg cm^{-2} h^{-1})	0.55*	0.49	0.24	0.12	$1.4\ 10^{-7}$

Microbial metabolism

Enzymatic and respiratory activites were significantly higher in the surface than in the subsurface sediment (Table 3).

β-glucosidase and β-xylosidase activities in the surface sediment followed a similar trend, with significant differences between sampling dates (ANOVA, $p=0.009$ for β-glucosidase, $p=0.0001$ for β-xylosidase) and a peak in June (Tukey's test, $p<0.05$) (Fig. 3a and b). In subsurface sediment, no significant differences were found between sampling dates (ANOVA, $p=0.31$ for β-glucosidase, $p=0.23$ for β-xylosidase). β-glucosidase and β-xylosidase activities correlated with chlorophyll-a density and bacterial density in surface sediment, but only with chlorophyll-a density in subsurface sediment (Table 4). In both habitats, β-glucosidase activity correlated with β-xylosidase activity (Table 4).

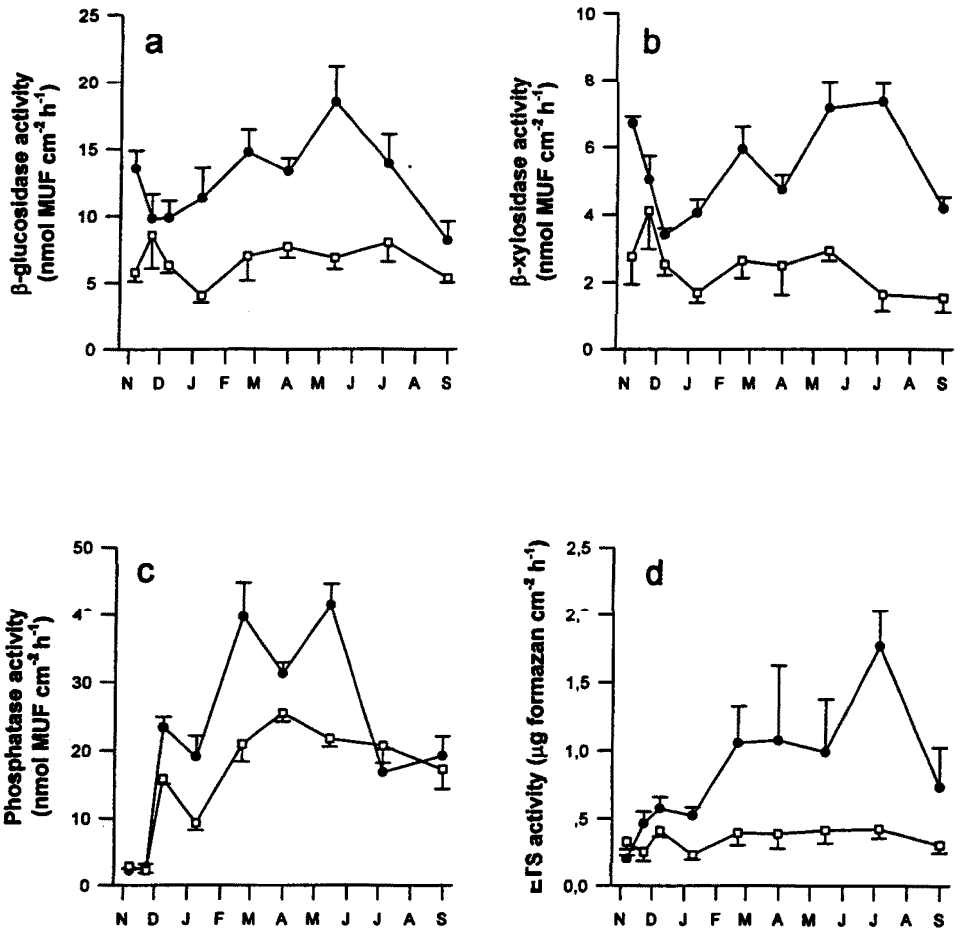


Fig. 3. Temporal variation of ectoenzymatic and respiratory activities in surface sediment (black circles) and subsurface sediment (empty squares) in Riera Major stream, October 1994-August 1995. Means ± standard errors (vertical bars) are shown, n=6.

However, a similar pattern was observed for phosphatase activity in the surface and subsurface sediments ($r=0.84$, $p=0.004$, $n=9$, Fig. 3c), with significant differences between sampling dates in both habitats (ANOVA, $p<0.0001$). The lowest values were measured in November, increasing then till spring. This activity correlated with the bacterial density, the DIN:SRP ratio and the discharge but also with chlorophyll-a density and DOC in surface sediments (Table 4). Phosphatase activity in the subsurface zone also correlated negatively with SRP (Table 4).

Respiratory (ETS) activity (Fig. 3d) was significantly higher in surface than in subsurface sediments (Table 3). In the surface, community respiration increased gradually to a significant peak in July (Tukey's test, $p<0.05$), whereas the subsurface values were not

significantly different over the study period (ANOVA, p=0.47). In the surface sediment, ETS activity correlated with bacterial density, DOC and discharge while no significant correlation was found in the subsurface sediment (Table 4).

TABLE 4. Significant Pearson correlation coefficients between the heterotrophic activities, biomass and physico-chemical parameters in surface and subsurface sediments from Riera Major. The level of signficance is also indicated: *** = P<0.001, ** = P<0.01, * = P<0.05, no star = P<0.01; n=51.

Surface sediment	Bact.	Chl-a	β-gluc	DOC	SRP	DIN: SRP	Temp.	Disch.
Bacteria				-0.53***			0.42**	-0.43**
Chlorophyll-a	0.62***			-0.36**				-0.35*
β-glucosidase	0.59***	0.46**						
β-xylosidase	0.40**	0.33*	0.59***					
Phosphatase	0.39**	0.51***		-0.53***		0.28*		-0.33*
ETS	0.42**			-0.33*				-0.31*
Subsurface sediment								
Bacteria							0.37*	-0.35*
β-glucosidase		0.49***						
β-xylosidase		0.44**	0.76***					
Phosphatase	0.44**				-0.32*	0.23	0.41**	-0.42**

Discussion

The cleaning effect of the flood provided excellent conditions for this investigation. The flood caused an important decrease (ca. 50%) in extracellular enzymatic activity, organic matter and chlorophyll-a, and an even more drastic reduction in respiration activity (ca. 70%) and bacterial cell density (ca. 80%) in the stream sediment (Table 1). Thus, the organic matter utilization would be caused mainly by the new material accumulated. Although leaf fall may be especially relevant in such a forest stream in autumn, a high amount of the accumulated leaves were transported downstream due to the short increase in discharge which occurred two weeks before the high flood event (Fig. 1) (Snaddon et al. 1992).

After the flood, clear differences were found between the organic matter utilization in each sediment habitat. The heterotrophic activity and biomass values were significantly higher in the surface sediment than in the subsurface sediment (Table 3). A decrease in microbial enzymatic activities with depth has been observed in marine and freshwater sediments (Meyer-Reil 1986, Poremba and Hoppe 1995, Sala 1995) and has been related to sedimentation and

accumulation of particulate organic matter produced in the photic zone (e.g. Cole et al. 1988). Few investigations have focused on depth variation of hydrolytic enzymes in stream sediments, where other factors should play a more relevant role than in planktonic environments.

The lack of significant differences between the chemical characterisitcs in surface and subsurface waters reflects the important water exchange that takes place, partially caused by the coarse sediment grain size (Table 2 in chapter 3.1). Thus, other variables apart from chemical are probably causing the metabolic differences observed between the surface and the subsurface sediment.

Two reasons are suggested for these metabolic differences that may be acting together. Firstly, the higher organic matter accumulated in the surface sediment is providing a major *quantity* of organic carbon to the heterotrophic organisms and secondly, the higher chlorophyll-a levels in the surface sediment indicates that there is also a provision of higher *quality* organic compounds.

It has been described that the organic matter of a sediment is important for bacterial colonization and/or a source of nutrients (Cammen 1982, Bott and Kaplan 1985, Findlay et al. 1986 and 1993). This would explain the higher bacterial density found in the surface sediment. It also seems obvious that, if there are not other limitations, a higher quantity of substrate for the heterotrophs enhances their metabolic processing (e.g. Marxsen 1996).

Concerning the suggested higher quality compounds reaching the upper sediment, it has been observed that the quality of the organic matter affects microbial activity and growth (Kaplan and Bott 1985, Bärlocher and Murdoch 1989, Hedin 1990), which is enhanced by the more labile compounds (Middelboe and Sondergaard 1993). The higher quality of the benthic organic matter accumulated on the surface sediment is revealed by the higher Chl/OM ratio (Table 3). Algal material has been defined as "high quality" organic matter (Kaplan and Bott 1985) and preferentially utilized by bacteria instead of allochthonous materials (Haak and McFeters 1982a). The importance of chlorophyll-a as a source of organic matter is shown by the significant correlation between the enzymatic activities and chlorophyll-a levels (Table 4). Furthermore, the greater amount of benthic chlorophyll-a in the surface sediment reveals that detritic algae/plant remains can also be a better physical substrate for bacterial attachment than a more decayed (less fresh) material.

In contrast, in the subsurface sediment, Chl/OM ratio is about one third of that detected in the surface sediment. The chlorophyll-a is possibly more degraded, indicating that less favourable (more recalcitrant) material accumulates in this habitat (McKinley and Vestal 1992). This would also be affected by the reduced mechanical abrasion in the subsurface zone (Metzler and Smock 1990). The lower respiration and enzymatic activities in the subsurface sediment could be also a result of their inhibition by the more recalcitrant DOC in this habitat (Hedin 1990, Freeman et al. 1990).

Apart from empirical differences in the heterotrophic metabolism and biomass, the time pattern in both habitats was drastically different. The surface sediment showed seasonal fluctuations while a rather constant pattern was observed for the subsurface, as determined by

the ANOVA analysis results. The physical parameters, especially discharge and DOC, affect the benthic metabolism and biomass of the surface sediment (Table 4). However, β-glucosidase and β-xylosidase activities in the surface sediment seem to be independent of discharge and DOC. It is possible that these enzymatic activities are proportionally more affected by POM input caused by accumulation of leaves. Both enzymes contribute to the degradation of decaying plant material (Sinsabaugh et al. 1994a and 1994b) and a peak of activity was observed at the beginning of the experiment, coinciding with maximum POM.

The effects of the physical parameters (e.g. discharge and organic matter input) on microbial activity are much weaker in the subsurface zone than in the surface sediment. Discharge only correlated with bacterial density and phosphatase activity and DOC did not correlate with any variable (Table 4). However we observed that in both sediments bacterial density depended on water temperature (Table 4).

Surprisingly, phosphatase activity was the only parameter which followed a similar pattern in both sediments (Fig. 3c). Phosphatase activity correlated positively with the DIN:SRP ratio in both sediments and negatively with SRP in the subsurface sediment (Table 4), thus suggesting that this activity is mainly controlled by nutrients and it is enhanced when inorganic phosphorus is scarce (Whitton 1991, Klotz 1992). The non-significant differences between surface and subsurface nutrient concentrations indicate that this regulation may act in the same manner in both sediments. Decomposing leaf detritus and the organic material accumulated may be used as substrate by this enzyme in both surface and subsurface sediments (Elwood et al. 1988, Hansson 1989).

It can be concluded that metabolic processes are slower and steadier in the subsurface sediment, less affected by physical changes (e.g. discharge) that affect surface sediments. The study of the few cm of sediment depth considered as subsurface sediment revealed a significant decrease in heterotrophic metabolism. Although this forest stream showed a great allochthonous input from the riparian vegetation and a lower algal activity than open streams (Guasch and Sabater 1994), the algal accumulation is a source of high-quality organic matter for the heterotrophs living in the surficial stream sediment.

3.3. Effects of removal of riparian vegetation in algae and bacteria of a Mediterranean stream

Abstract

The effect of riparian removal of a riparian strip on algae and bacteria was monitored in a Mediterranean stream during the canopy growing period. Community composition, biomass and metabolic activities were compared with those recorded during a pre-riparian removal period and in a forested stretch downstream. Higher irradiance was related with *Cladophora* increase in the logged section. Algal biomass increased up to ten times, and productivity was up to four times higher than in the pre-removal period and the forested section. Bacterial communities showed higher ectoenzymatic activities (β-glucosidase, β-xylosidase) in the logged section than in forested conditions. Moreover the coincidence between the maxima of β-glucosidase and chlorophyll-a suggests that bacterial activity was enhanced by the higher availability of high-quality algal material. Responses of environmental variables and biotic communities indicate that the changes occurring in the stream because of the riparian removal could be considered bottom-up controlled, being increased illumination the main mechanism responsible.

Introduction

The importance of riparian vegetation to the functioning of the river has been longly recognized (Ross 1963, Vannote et al. 1980, Newbold et al. 1981). It contributes to the input of organic matter (Naiman et al. 1988) but has the capacity to retain nutrients which may enter from diffuse sources (Pinay et al. 1992). The control of water temperature is favoured by riparian vegetation (Ward 1984), a fact especially important for fishes (Tait et al. 1994).

Removal of riparian vegetation is a human disturbance that affects river systems widely (Gregory et al. 1991). In the last 200 years more than 80% of the original riparian corridor of North America and Europe has disappeared (Décamps and Naiman 1989). Because riparian vegetation determines the amount of light energy, primary producers are the group most immediately affected (Bott et al. 1985, Naiman 1983); changes include both the composition of algal communities (Hansmann and Phinney 1973) and biomass (Triska et al. 1983). When temperature and nutrients are also affected, synergetic effects on algal biomass are observed (Peterson et al. 1985, Lowe et al. 1986, Hill and Knight 1988). In a multivariate analysis, Leland (1995) determined that algal biomass was related to composition and density of the riparian vegetation, but not to any of the chemical variables measured. Changes in the autochthonous production influence consumer trophic levels within the stream (Bilby and Bisson 1992). Logging may also affect the metabolism of bacterial communities. It is likely that alterations of primary production and organic matter transport (Webster and Waide 1982) might affect bacterial activity. Finally, logging might also affect macroinvertebrate communities, because of the quality and quantity of the organic matter available (Cummins et al. 1989, Hawkins et al. 1982) or

through their changing interactions with the periphyton (Hawkins and Sedell 1981, Feminella et al. 1989, Rosemond 1993).

Removal of riparian vegetation may be especially significant in semiarid regions (Fisher 1995). In Mediterranean streams the canopy development of the riparian corridor coincides with the increase in ambient irradiance and air temperature. Cloud cover in spring and summer is reduced, and photoperiod increases. Hydrological conditions are then greatly affected by riparian evapotranspiration, as well as by the reduced rainfall. This interaction of environmental factors creates potential for great change in Mediterranean stream systems (Sabater and Sabater 1992).

This paper compares the biological changes between pre- and post- riparian removal periods in an oligotrophic Mediterranean stream. In the post-removal period a cleared stretch is also compared with another that remained unharvested. The effect of the riparian vegetation removal can be more important during the period of steep light and temperature increase (spring and summer). Therefore, the possible implications the riparian removal could have on autotrophic and heterotrophic activities during that period were explored. Few modifications caused by riparian removal have been analyzed in the relationships between algae and bacteria, especially to follow the changes occurring from the dormant to the growing season.

Materials and Methods

Site and sampling

The study site was an unconstrained reach, ca. 500 m length and 2 % slope, of Riera Major stream (coinciding in part with the stream stretch for chapter 3.1). Original riparian vegetation in that stretch during 1994 was made up by mature (about 50 years old) alders (*Alnus glutinosa* (L.) Gaertn), which formed a dense gallery over the stream bottom. Several stretches of the stream were affected by logging of riparian vegetation in February 1995 (Fig. 1). Trees, shrubs and all riparian vegetation was removed from the saturated zone of the stream (6-8 m wide). The adjacent terrestrial forest was left intact. The area studied during 1994 (chapter 3.1) was partially affected by riparian removal. Observations in 1994 (pre-removal period) were compared with those on the same section of Riera Major after riparian vegetation removal.

A strip 300 m long was cleared, while another 200 m long located immediately downstream was left undisturbed. Therefore this downstream stretch was also monitored for changes in the biological communities. Observations in these two stretches (logged and forested) in the post-removal period were performed during early spring (days 1, 15 and 59 after riparian removal) and increased in frequency during late spring and summer (days 94, 113, 127, 149 and 174). The observations finished in late August 1995 (day 174), when light and temperature started to decline.

Fig. 1. Picture of the study site at Riera Major stream after the removal of the riparian vegetation (February 1995).

Artificial substrates (ceramic tiles, 0.64 cm^2) were used to measure biomass and activities of the algal and bacterial biofilm. Six-to-eight-week-old tiles were employed for each sampling occasion (chapter 3.1). The collected tiles for bacterial and algal metabolism (ectoenzymes, primary production) and chlrophyll-a measurements were placed in sterile glass vials with stream water and kept cold (on ice) and in the dark for its transport to the laboratory. Activity measurements were performed in the laboratory two to three hours after sampling. Tiles for bacterial counting and for description of the algal community were fixed with 2% formalin.

Underwater light (PAR) immediately above the stream substrates, water temperature, pH, conductivity and dissolved oxygen were measured at each sampling date. Filtered (precombusted Whatman GF/F filters) water samples were taken to analyze inorganic nutrients (nitrate, ammonia and soluble reactive phosphorus (SRP)), as well as dissolved organic carbon (DOC) and dissolved inorganic carbon (DIC) following the procedures described in chapter 2.

Algal measurements

Chlorophyll-a was measured (acetone extraction) in triplicate. $H^{14}CO_3$ incorporation was measured on five replicates which were incubated under a battery of fluorescent tubes (irradiance of 210 µmol photons m^{-2} s^{-1} for the communities growing under light conditions, 50 µmol photons m^{-2} s^{-1} for the communities growing under shade conditions), two control (substrate killed) tiles, and dark tubes (two replicates). The procedures described in chapter 2 were followed.

Algal community composition and relative abundance was analyzed from 2-3 replicates (tiles) from both the logged and the forested sections in June-August 1995. Observations were performed with light microscopy at 400 magnification. Results were expressed as relative percentages of the total algal cells. Up to 400 cells were counted for each sample. Algal samples were seldomly collected in 1994, and observations therefore refer to those by Guasch and Sabater (1994) during 1991-1992 at the same stream site.

Bacterial densities and ectoenzymatic activities

Bacterial densities were estimated on four different dates (days 113, 127, 149 and 174) in both the logged and the forested reaches. Counts of cell numbers were performed in triplicate following the procedures described in chapter 2.

Extracellular enzyme potential activities (β-D-glucosidase and β-D-xylosidase) were determined with the periodicity described above using triplicate samples and one control (substrate killed) sample. Two blanks of filter-sterilized stream water for each enzyme were also included in the incubation. The incubation procedure was that described in chapter 2.

Results

Physical and chemical characteristics in the pre- and post-removal periods

Environmental characteristics in 1994 (February-April, June-August) and in the logged and forested sections in 1995 (March-August) are shown in Table 1. Spring-summer 1994 were drier and warmer than those of 1995. Within the post- riparian removal period, the cleared and the forested reaches exhibited similar water temperatures, with a maximum difference of 1°C between them.

The removal of the riparian forest produced an average increase of 94%-98% of ambient irradiance in the logged section with respect to the forested conditions of 1995 and 1994 respectively. Nitrate and phosphorus (as SRP) concentrations experienced moderate increases in 1995. Ammonia did not show apparent variations between the two periods. DOC did not change substantially after the riparian removal.

Algal occurrence

Cladophora glomerata (L.) Kütz. was relatively abundant in spring during the pre-riparian removal period (Table 2), but markedly decreased in summer. The algal succession was then characterized by the dominance of the encrusting red alga *Hildenbrandia rivularis* Lemm. and several cyanobacteria, along with several diatom taxa.

TABLE 1. Physical and chemical measurements in Riera Major in the pre-riparian removal period (spring and summer 1994) and in the post-riparian removal period (1995, logged section and forested section). Data are averages; standard deviation of the means are indicated in brackets.

	Spring 1994	Summer 1994	Logged section 1995	Forest. section 1995
Temperature (°C)	9.4 (1.1)	16.2 (1.1)	12.1 (1.1)	11.9 (1.5)
Discharge (L s^{-1})	35 (6.8)	15 (4.2)	37.8 (5.8)	37.5 (4.2)
Conductivity (µS cm^{-1})	196 (14.5)	238 (8.3)	205 (13.3)	204 (10.2)
Underwater irradiance (µmol m^{-2} s^{-1})	274 (251)	9.15 (4.8)	698 (244)	40 (7.8)
pH	8.3 (0.15)	7.6 (0.5)	8.3 (0.25)	n.d.
DOC (mg L^{-1})	1.8 (1.5)	1.6 (0.8)	1.4 (0.2)	1.5 (0.2)
SRP (µg L^{-1})	4.2 (3.3)	7.2 (4.1)	10.8 (3.2)	11.8 (4.1)
NH_4-N (µg L^{-1})	21.9 (7.2)	6.7 (6.7)	9.2 (3.6)	9.2 (4.9)
NO_3-N (µg L^{-1})	261 (77)	343 (30)	407 (89)	461 (322)

The algal community in the post-riparian removal period was remarkably different between the logged and the forested sections (Table 2). In the forested reach was dominated by the diatom *Cocconeis* spp. (spring 1995) and by the encrusting green alga *Gongrosira* (summer 1995). In the logged reach *Cladophora glomerata* and several Zygnematales (*Mougoetia* sp. and *Spirogyra* sp.) accounted for 60 % on some occasions. Diatoms (mainly *Achnanthes minutissima* Kütz., *Cocconeis placentula* Ehr., *Synedra ulna* (Nitzsch.) Ehr. and *Cymbella* spp.) were also abundant. *Cladophora* mats were completely covered by epiphytic diatoms (mainly *Cocconeis pediculus* Ehr.) at the end of July. Even though not recorded on the ceramic tiles, small colonies of *Nostoc verrucosum* Vaucher were scattered on the stream bottom of both the logged and the forested reach.

Algal biomass and productivity

Chlorophyll-a ranged from 1.6-6.6 µg cm^{-2} in the pre-removal period (1994, Table 3) and from 1.6-7.7 µg cm^{-2} in the forested section (1995) (Fig. 2). The logged section reached up to ten-times the concentrations in the forested reach (Fig. 2). The maximum was at day 113 (83.4 µg cm^{-2}), and corresponded with the dominance of *Cladophora*. High values of chlorophyll-a extended until *Cladophora* senesced.

TABLE 2. Community composition and abundance of the algal and cyanobacterial community observed in Riera Major during the pre-removal period (spring and summer 1991, data from Guasch and Sabater 1994), and the post-riparian removal period (1995), both in the logged and in the forested sites. Data are given as percentages.

	Spring 1991	Summer 1991	Forested spring 95	Forested summer 95	Logged spring 95	Logged summer 95
Achnanthes minutissima	4.5	2	0	8.5	0	15
Cymbella spp.	5	2	2	1	1	13.5
Cocconeis spp.	4	11	90	30	1.5	10
C. pediculus	14	2	1.5	5	14	7.5
Diatoma vulgare	12	0	0	0	3.5	0
Melosira varians	13	0	0	0	2.5	1
Gomphonema spp.	4	2	0	1	0	5.5
Navicula spp.	0	2	4.5	6	2	11
Nitzschia spp.	0	2	2	0	0	0
Synedra ulna	0	2	0	1	15	2.5
Meridion circulare	0	9	0	0	0	0.5
Cladophora glomerata	14	7.5	0	1	45	11.5
Gongrosira incrustans	0	7	0	39	0	4.5
Zygnematales	0	0	0	0	8	7.5
Scenedesmus spp.	0	0	0	5	7.5	2.5
Hildenbrandia rivularis	4	26	0	0	0	0
Phormidium spp.	23	11	0	0	0	2.5
Chamaesiphon sp.	2.5	15	0	2.5	0	5

The photosynthetic activity (measured as ^{14}C incorporation per surface area) ranged in 1994 between 2.4-3.7 μg C cm^{-2} h^{-1}. This was slightly lower than that recorded in the forested site (Fig. 3a), which ranged 2.1- 11.5 μg C cm^{-2} h^{-1} , with a maximum of 37.6 μg C cm^{-2} h^{-1} at day 147. Values in the logged reach were much higher (1.8-31.5 μg C cm^{-2} h^{-1}) with a maximum at the end of July (day 149; 112 μg C cm^{-2} h^{-1}). Photosynthesis per unit of chlorophyll-a had a maximum at day 149 that was higher in the forested reach (Fig 3b). The maximum in the forested reach was due to the dominance of the encrusting green alga *Gongrosira* on the tiles, and probably associated with low light adaptation of this shade-adapted community. A smaller maximum in photosynthesis per unit of chlorophyll-a occurred in the logged reach in March (day 15).

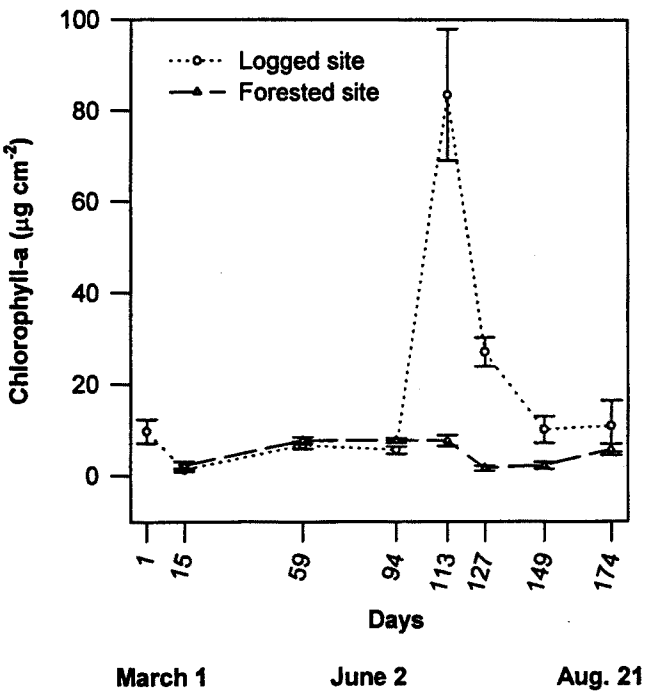


Fig. 2. Chlorophyll-a density in the logged site and in the forested site of Riera Major during spring-summer 1995. Bars indicate standard deviation of the mean (n=3).

TABLE 3. Average values of algal biomass (as chlorophyll-a), algal photosynthesis (as ^{14}C incorporation), and ectoenzymatic activities (β-glucosidase, β-xylosidase) during the pre-riparian removal period (March- August 1994). Standard deviation is indicated in brackets.

	Chlorophyll-a ($\mu g\ cm^{-2}$)	**^{14}C-incorporation** ($\mu g\ cm^{-2}\ h^{-1}$)	**β-glucosidase** ($nmol\ cm^{-2}\ h^{-1}$)	**β-xylosidase** ($nmol\ cm^{-2}\ h^{-1}$)
March 1994	6.64 (1.31)	2.8 (0.75)	5.3 (1.4)	1.02 (0.99)
April 1994	4.78 (2.81)	2.4 (3.6)	3.9 (1.3)	111.9 (14.1)
June 1994	2.73 (0.22)	3.4 (0.81)	5.2 (2.7)	2.9 (0.88)
July 1994	3.44 (0.82)	2.7 (0.88)	15.1 (3.2)	1.7 (1.9)
August 1994	1.67 (1.37)	3.7 (0.26)	4.6 (0.9)	3.6 (1.2)

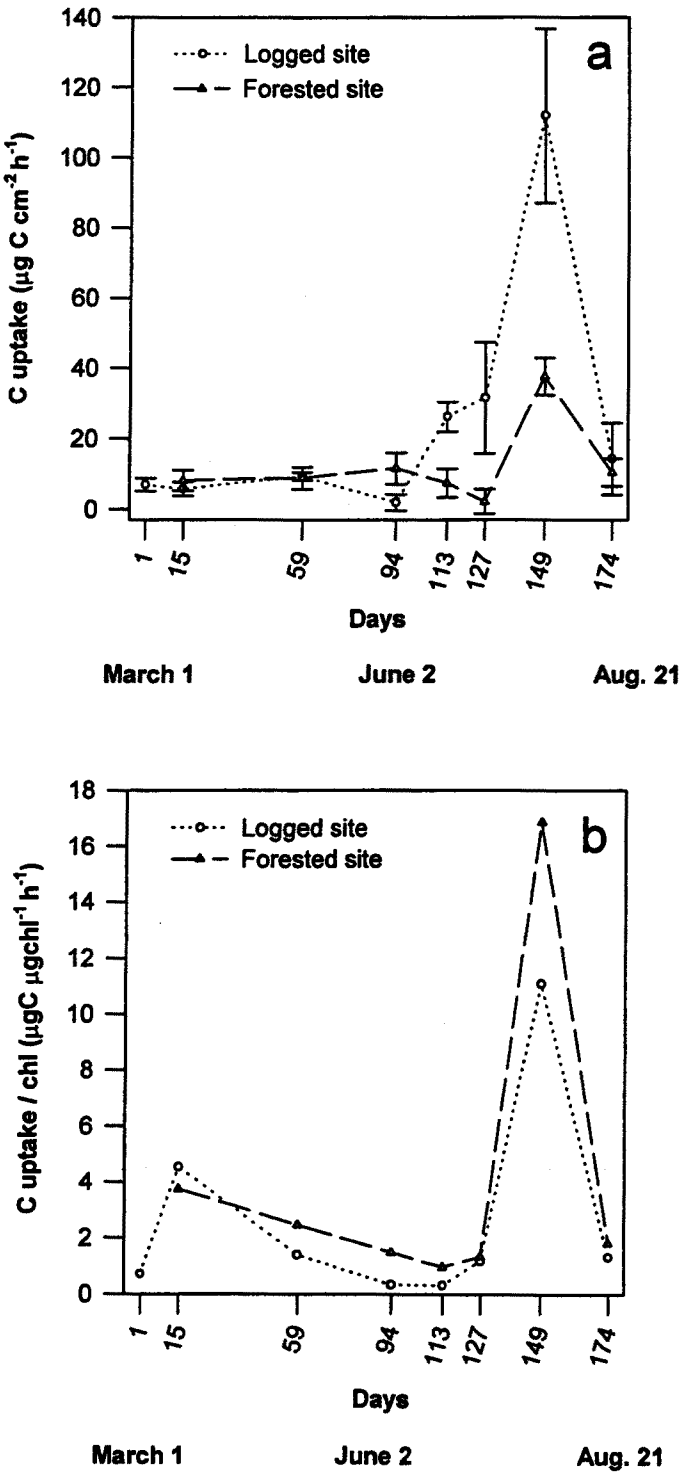


Fig. 3. a) Photosynthetic activities (as $H^{14}CO3$ incorporation) per unit area, and b) photosynthetic activities per unit chlorophyll-a during spring - summer 1995 in the logged and in the forested site of Riera Major. Bars indicate standard deviation of the mean (n=5).

Bacterial density and ectoenzymatic activities

Bacterial densities were slightly higher in the logged reach (average 3.56 10^{10} cells cm^{-2}, n=6) than in forested conditions (2.65 10^{10} cells cm^{-2}, n=18 in the pre-riparian removal period, 2.82 10^{10} cells cm^{-2}, n=9 in the post-riparian removal period).

β-glucosidase activity in the pre-removal period (1994) ranged between 3.9-15.1 nmol cm^{-2} h^{-1} (Table 3). In the forested section (post-removal period) it reached 5.4- 15.1 nmol cm^{-2} h^{-1}. β-glucosidase was also low (3.5-10.5 nmol cm^{-2} h^{-1}) in the logged reach from March to early June 1995 (day 94, Fig. 4a) but increased from day 113 to the end of July (day 149; maximum value of 254 nmol cm^{-2} h^{-1}). β-glucosidase was significantly correlated with chlorophyll-a only in the logged reach ($r= 0.86$, $p< 0.05$).

β-xylosidase activity was 1-3.6 nmol cm^{-2} h^{-1} during 1994, but a maximum of 112 nmol cm^{-2} h^{-1} occurred in April 1994. β-xylosidase in the post-riparian removal period exhibited maxima at the end of spring both in the logged (22 nmol cm^{-2} h^{-1}) and in the forest reaches (25 nmol cm^{-2} h^{-1}) (Fig. 4b). Maxima were slightly delayed in the forest reach.

Discussion

Removal of riparian vegetation favoured the dominance of *Cladophora glomerata* mats, therefore replacing the diatom-dominated community of pre-riparian removal conditions (Guasch and Sabater 1994, chapter 3.1). This pattern was also observed with respect to the forested reach. *Cladophora* dominance lasted until the growth of the epiphytes was too high and impaired the development of the macrophyte. *Cladophora* (and Zygnematales) development caused remarkable increases of algal biomass (as chlorophyll-a) and photosynthetic activities.

Cladophora success in the logged reach of the stream is strongly related to light availability. Before the riparian removal *Cladophora* was abundant only in spring (Guasch and Sabater 1995), when the vegetation significantly reduced the light reaching the stream bed. Light was one of environmental variables which increased most because of the riparian removal (Table 1), while nutrients as a whole were not remarkably modified. Prolific growth of *Cladophora glomerata* has been associated with nutrient enrichment (Whitton 1970, Lohman and Priscu 1992), but low nutrient levels can also maintain high standing crops. Lorenz and Henderdorf (1982) observed a remarkable development of *Cladophora* in the littoral zone of the North American Great Lakes with phosphorus concentrations between 0.02-0.4 μM, a similar value to that observed in Riera Major. On the other hand, *Cladophora* is favored by high light intensities (Whitton 1970). Graham et al. (1982) determined the minimum light intensity of 35 μmol m^{-2} s^{-1} to support net photosynthesis, light saturation being reached around 300-600 μmol m^{-2} s^{-1}. The mean irradiance value measured in the forested reach was ca. 40 μmol m^{-2} s^{-1}, near levels documented as thresholds for light compensation. Water temperature, which has

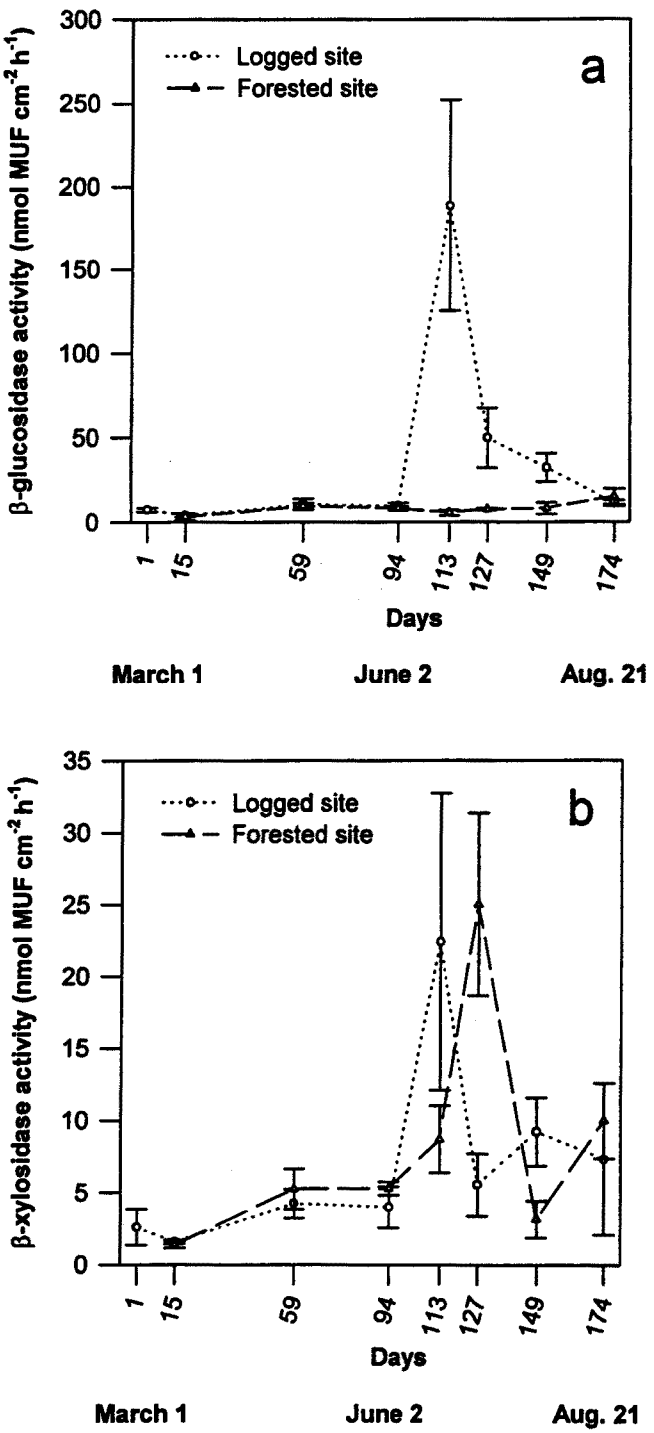


Fig. 4. Ectoenzymatic activities measured in the logged site and in the forested site of Riera Major during spring-summer 1995. a) β-glucosidase activity, b) β-xylosidase activity. Bars indicate standard deviation of the mean (n=3).

been also described as a limiting factor for *Cladophora* growth (Whitton 1970), differed by 1 °C at midday between the two reaches. These observations support the assumption that light is the key factor explaining the distribution and success of *Cladophora* in Riera Major.

The increase in herbivors at the end of the post-removal period (Sabater et al., in press) is very likely an important cause for algal biomass decline (together with the impairment effect of epiphytes) (Triska et al. 1982). In addition, *Cladophora* abundance at the logged site offered a potentially important shelter for invertebrates.

Temporal patterns of ectoenzymatic activities broadly follow those described for the algal biomass. Values recorded in the logged reach were usually higher than those observed in forested conditions. This difference accounted for a maximum of 14-fold for β-glucosidase (Fig. 4a, Table 3). Differences were substantiated by slightly higher bacterial densities in the logged reach. Haack et al. (1988) and Hudson and Roff (1992) also observed higher bacteria cell numbers and biomass in open than in forested streams. The coincidence between the maxima ectoenzymatic activities and that of chlorophyll-a (Figs. 2 and 4a) suggests that bacterial activity was enhanced because of the higher availability of substrate (Jones and Lock 1993).

Increased light affected the autotrophic production and biomass of Riera Major. The minor changes in the inorganic nutrients and in the DOC in this nutrient-limited stream (Martí and Sabater 1996) further indicate that light may have been a more important factor. The favourable conditions for the primary producers enhanced the bacterial ectoenzymatic activity and affected the structure of the macroinvertebrate community, favouring the scrapers and filterers (Sabater et al., in press). Therefore, most of the changes occurring in the unforested reach of the stream because of the riparian removal could be defined as bottom-up controlled during the study period. Peterson et al. (1993) showed a strong bottom-up effect of nutrient addition to a tundra stream. This study indicates a bottom-up effect, however, it is believed that increased illumination in the logged reach is the main mechanism responsible. The question of whether this control could be replaced or limited by predators or herbivores (top-down control) in a longer time period remains open to additional research.

4. LA SOLANA: A MEDITERRANEAN OPEN STREAM

Study site

La Solana, located in the north of Catalonia (NE Spain) (42°70'N 2°13'E), is an undisturbed second-order stream, tributary of the Ter river (see Fig. 6 in chapter 1), draining a calcareous basin (Martí et al. 1994). Its altitude ranges 980-500 m above sea level. The watershed area is of 16 Km^2, and the stream length is of 8.2 Km. The Mediterranean climate regime imposes variable discharge conditions with high flow in autumn and spring (12-32 L s^{-1}) due to strong rains, and reduced flow in summer (0-10 L s^{-1}). The riparian vegetation is not well developed, with only a sparse canopy made of willow (*Salix elaeagnus*, *Salix purpurea*) and hazel (*Corylus avellana*). In summer there is the maximum incident light (1600 μmol photons m^{-2} s^{-1}) and water temperature (20-25 °C). The nutrient content is very low, especially for phosphate (5-10 μg L^{-1}). The epilithic algal community is characterized by carbonate-encrusting algae (mainly Cyanobacteria), forming crusts 2-6 mm thick. Several algae developed over the crust such as *Rivularia biasolettiana* Menegh. and filamentous Chlorophyceae (*Mougeotia* sp., *Spirogyra* sp., *Zygnema* sp.) (Guasch and Sabater 1994).

Study site in La Solana.

4.1. Stromatolitic algal patches developing in a Mediterranean stream optimize organic matter use

Abstract

The bedrock of La Solana stream is covered by a thick calcareous cyanobacterial crust with a layered structure similar to a stromatolite. Different algal patches, which appear along with seasonal changes (especially discharge, temperature and light), characterize the stromatolite. This structure has a great capacity for organic matter utilization, as indicated by the high extracellular enzymatic activities (β-glucosidase, β-xylosidase and phosphatase) measured in the stromatolitic algal patches over an annual cycle. However, each patch showed a particular ability in the use of organic matter since a different hydrolytic potencial capacity was measured. In the *Zygnema-Spirogyra* community, autotrophic activity might enhance the β-glucosidase activity in spring, whilst in the diatom bloom, algal released polysaccharides and mucilagenous material from the diatom stalks might regulate the ectoenzymatic activities. The adaptation of the *Rivularia* community to oligotrophic conditions is shown by the extremely high phosphatase activity, related to the low phosphorus concentration in stream water. The highest β-glucosidase and β-xylosidase activities were measured in the mixed community (cyanobacterial crust with a sparsely developed overstorey), indicating that the understorey of this stromatolitic crust is highly active. The appearance of the different patches and the thick stromatolite can be seen as the adaptative response for surviving the drastic environmental changes characteristic of Mediterranean streams.

Introduction

Microbial mats are dense benthic communities of microorganisms which can give rise to laminated rocks known as stromatolites (Stal 1995). Stromatolites are formed through lithification processes such as binding and trapping of sediment particles, precipitated minerals (e.g. calcite, Winsborough et al. 1994) and accumulation of incompletely mineralized organic matter (Chafetz and Buczynski 1992). Bacteria, and especially cyanobacteria, play a significant role in producing stromatolites (Abdelahad and Bazzichelli 1989, Chafetz and Buczynski 1992). Extracellular polysaccharides released by diatoms are also involved (Winsborough and Golubic 1987). Algae and cyanobacteria living in microbial mats are distributed heterogeneously and change their pigment composition in response to the incident light, temperature, oxygen gradient, water current (Jorgensen et al. 1983, Winsborough and Seeler 1986, Stal et al. 1985, Hawes 1993, Pinckney et al. 1995b), and salinity levels (Mir et al. 1991, Esteve et al. 1992). Age is also a determining factor of mat structure (Oppenheim and Paterson 1990). Cyanobacteria-dominated microbial mats communities are restricted to oligotrophic environments, at high-nutrient levels diatoms may be competitive dominants (Pinckney et al. 1995a).

Microorganisms living in stromatolitic microbial mats have high metabolic activities (Cohen and Rosenberg 1989). The photoautotrophic and the heterotrophic communities of microbial mats are closely coupled, showing high rates of photosynthesis and organic matter

utilization (Canfield and Des Marais 1993, Paerl et al. 1993a), although a higher production : respiration ratio was observed in a stromatolitic mat (Pinckney et al. 1995b).

The calcareous bedrock of La Solana is covered by a lithified layer of cyanobacteria and diatoms, creating a crusted structure similar to a stromatolitic microbial mat. The distribution of different algal patches on the cyanobacterial crust changes during the year, due to variations in discharge and light (Guasch and Sabater 1994). This Mediterranean stream is subject to drastic seasonality, and large fluctuations in discharge and temperature have been observed (Martí et al. 1994). High photosynthetic and respiration rates were observed in La Solana (Guasch and Sabater 1994, 1995), which may require high rates of heterotrophic carbon utilization.

The main objective was to investigate the organic matter utilization in each algal patch in this stromatolitic microbial mat. Each algal patch has a characteristic photosynthetic behaviour (Guasch and Sabater 1995), and it was also possible that organic matter degradation processes may also be characteristic of each algal patch, the current hypothesis to be tested. The capacity to breakdown organic matter was studied by monthly analyzing ectoenzymatic activities (β-glucosidase, β-xylosidase, and phosphatase).

Materials and methods

Sampling

Stromatolitic patch samples were collected monthly from January 1994 to February 1995 from La Solana streambed. This study year was especially dry; the stream dried out completely for ca. 30 days (July 1994), and rainfall was also very low in August (30 mm).

The calcareous cyanobacterial crust is permanent over the year and the different algae developing over the crust characterize the different algal patches: the *Rivularia* community, the *Zygnema-Spirogyra* community, the Diatom bloom and the mixed community (Fig. 1).

Each patch, consisting of an understorey (the permanent crust) and an overstorey, was defined when appearing in the stream over more than 10% of the streambed surface. On each sampling date, pieces of each patch (0.5-1 cm thick) were placed in sterile glass vials with stream water. Samples were kept cold (on ice) and in the dark for transport. Samples from July and August 1994 (dry period) were transported wrapped with aluminium foil. The analyzed samples were approximately 1.1 cm^2 in surface area. Samples for chlorophyll-a analysis were frozen and kept in the dark until pigment extraction. For bacterial cell counts, samples were placed in sterile glass vials with 2% formalin. Activity measurements (extracellular enzyme analyses, photosynthetic activity and respiratory activity) were measured on the same day, two-three hours after sampling.

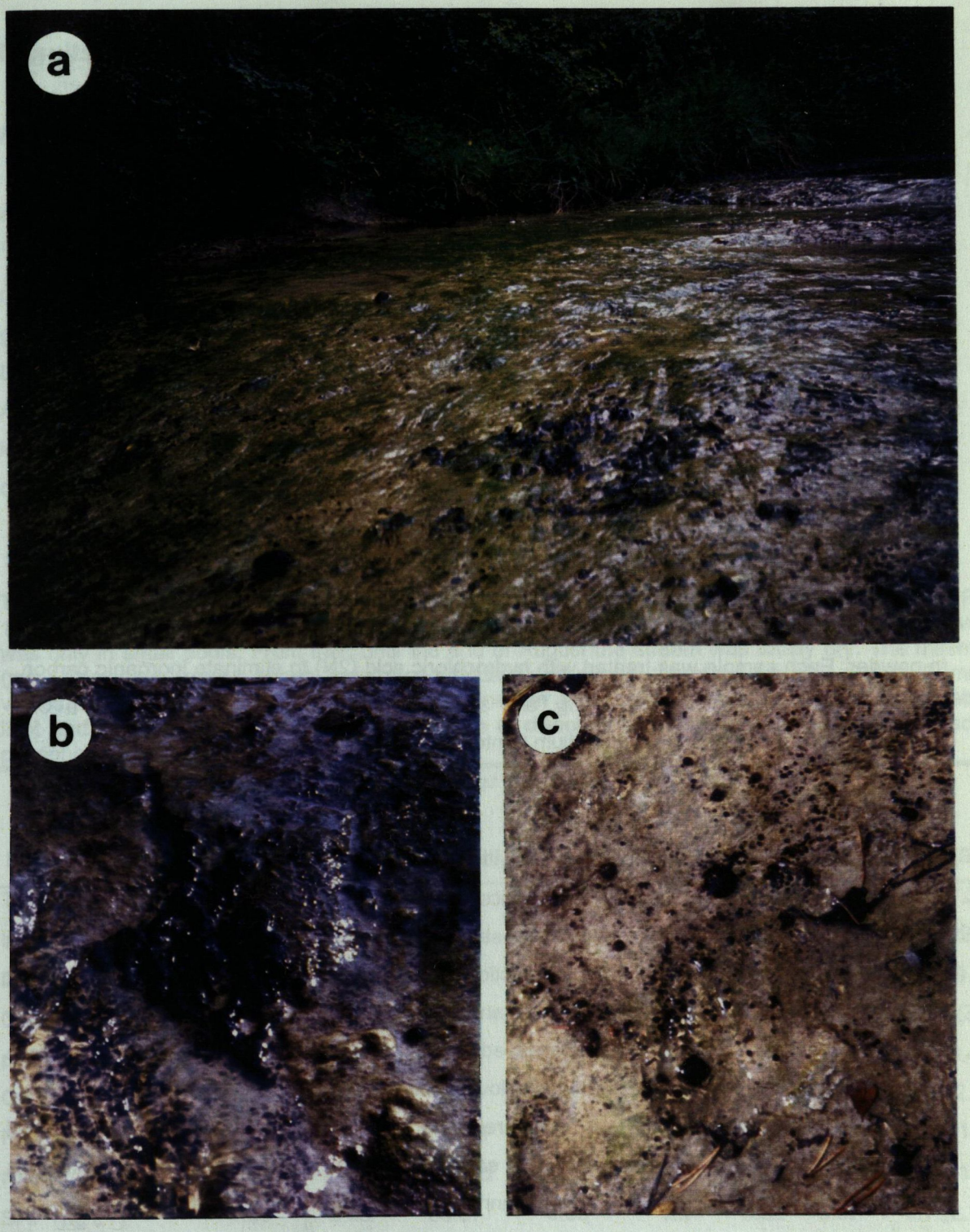


Fig. 1. Pictures of the stromatolitic algal patches observed in La Solana stream: a) View of the stream in June 1993 when the *Zygnema-Spirogyra* community was well developed, the green filaments from the Zygnematales cover the streambed, b) Aspect of the *Rivularia* community, a dark-geen colony of the cyanobacteria *Rivularia* can be seen (from July 1995), c) View of the mixed community, with a scarce development of its overstorey (few cyanobacteria spots and few green filaments) (from July 1995).

On each sampling date, incident light, temperature, pH, dissolved oxygen and conductivity were measured in the field. Filtered (precombusted Whatman GF/F filters) water samples (three replicates for each analysis) were taken in order to analyse inorganic nutrients

(nitrate, ammonium and soluble reactive phosphorus), as well as dissolved organic carbon (DOC) and dissolved inorganic carbon (DIC). In summer 1994, when the stream dried out, water samples were collected from some pools in July and no water was collected in August.

Algal biomass and bacterial density

Chlorophyll-a was measured in triplicate for each algal patch, the ratio of chlorophyll to carotenoids and/or chlorophyll degradation products (OD430/OD665 ratio) being also measured (Margalef 1983). Algal composition and community structure was determined for all patches (light microscopy observations), distinguishing the different layers by slicing the structure horizontally (every 1.5-2 mm, when a change of colour was observed) with a razor blade. Bacterial density (DAPI stain, epifluorescence microscopy) was estimated in triplicate for each algal patch. The different bacterial morphotypes were also observed. All measurements were determined following the procedures described in chapter 2.

Organic Carbon and Nitrogen biofilm content

The stromatolite was completely dried (two days in the oven at 110°C) and then ground to powder. Each sample was treated with hydrochloric acid (2N) to eliminate inorganic carbon, placed under vacuum for total drying, and analyzed for carbon and nitrogen with a C/N Analyzer 1500 Carlo Erba using vanadium pentoxide as the oxidation catalyzer.

Metabolism measurements

The ectoenzymatic potential activities of β-D-glucosidase, β-D-xylosidase, and phosphatase were determined in five replicate samples and two replicate controls (killed samples) for each patch. Two blanks of filter-sterilized stream water for each enzyme were also included in the incubation. Primary production was measured in the mixed community in triplicate samples, substrate killed samples and including dark replicates. Respiratory activity (Electron Transport System, ETS) was assayed in five replicates and two killed controls for each patch. All measurements were determined following the procedures described in chapter 2.

All activities and biomass measurements were expressed per surface area of stromatolitic crust. The especific surface of each piece used for each different analysis was calculated by image analysis (IMAT) after scanning a picture of the stromatolitic pieces.

Data analyses

The *Rivularia* community, *Zygnema-Spirogyra* community and the Diatom bloom were compared with the mixed community on each sampling date using a two-tailed *t*-test. One-way analysis of variance was performed for each variable in the different patches. Differences between patches were analyzed by Tukey's multiple comparison test. Correlation analyses (Pearson coefficient) were performed with biological and environmental variables.

Results

Physical and chemical parameters

Physico-chemical features are summarized in Table 1. Minimum temperature (0.7 °C) and light (112 μmol photons $m^{-2}s^{-1}$) levels occurred in winter. Maximum oxygen (10.8-11 mg L^{-1}), nitrate (181-202 μg L^{-1}) and ammonium (26-35 μg L^{-1}) levels in the stream water were measured in winter and autumn. Soluble reactive phosphorus (SRP) concentration was in general very low, with the minimum values measured in winter (1.3-1.5 μg L^{-1}). The maximum DOC was found in spring and autumn (5.6-6.9 mg L^{-1}). During the drought period (July and August 1994), some water remaining in small pools contained a high nutrient concentration and low pH and oxygen. Although some rain occurred during August (30 mm), water in the stream did not flow again until September.

TABLE 1. Physical and chemical characteristics of La Solana stream during the study period (1994-1995). Values are seasonal means, except summer values which correspond to early-July, when water was only in small pools. From then until the end of August the stream was totally dry. The minimum (min.), maximum (max.), annual mean and standard deviation of the mean are also shown. Summer values are not included in the average, nor in minimum and maximum values except for temperature, light and discharge. DIN:SRP is the molar ratio of dissolved inorganic nitrogen to soluble reactive phosphorus.

	Winter 1994 (n=2)	Spring 1994 (n=2)	Summer 1994 (n=2)	Autumn 1994 (n=3)	Winter 1995 (n=2)	min.	max.	mean (SD)
Temperature (°C)	0.7	11.6	24.8	8.6	2.9	0.7	24.8	9.2 (7.3)
Light (μmol $m^{-2}s^{-1}$)	140	495.7	710	187.6	112.0	93	843	348 (292)
Discharge (L s^{-1})	10	12	0	26	18	0	42	15.7 (9.6)
Cond. (μS cm^{-1})	470	400	606	405	408	352	470	412.6 (36)
pH	8.3	8.2	7.65	8	8.5	7.63	8.9	8.2 (0.4)
Oxygen (mg L^{-1})	10.87	8.0	1.5	11	11.8	4.3	14.8	10.2 (2.9)
NO_3-N (μg L^{-1})	181.1	71.38	269.14	186.79	202.17	5.05	307	151.1 (102)
NH_4-N (μg L^{-1})	35.4	24.36	293945	26.56	29.06	11.7	48.5	27.4 (13)
SRP (μg L^{-1})	1.55	2.06	64.67	4.68	1.3	0.22	5.37	2.7 (2.1)
DIN:SRP	464.88	763.5	10059	101.5	1715.5	94.9	3315	721 (1166)
DOC (mg L^{-1})	1.80	6.91	128.48	5.68	4.69	1.79	15.3	5.4 (4.2)
DIC (mg L^{-1})	62.77	59.58	40.09	47.42	47.99	40.4	65.6	53.3 (8.1)

Description of patch communities

The patches of the stromatolitic cyanobacterial crust, embedded in calcareous material, consist of an understorey (the permanent crust) and an overstorey (algae and cyanobacteria covering the permanent crust). The understorey showed distinct layers. The upper layer (1-3

mm depth), green in colour, was composed mainly of *Rivularia biasolettiana* Menegh. and some filamentous cyanobacteria (*Schizothrix penicillata* (Kütz) Gom., *S. affinis* Lemm. and *Homeothrix* sp.). The green algae *Mougeotia* sp., *Spirogyra* sp. and *Zygnema* sp. and the diatom *Cymbella* sp. were also present in this layer. The second layer (2-6 mm depth), green-yellowish in colour, was exclusively made up of *Rivularia* sp. (with more empty sheaths and less heterocysts) and filaments of *Schizothrix penicillata*. The bacterial community within this structure was mainly composed (80%) of coccus and coccobacillus (0.2-0.4 μm diameter) but chains of rod-shaped bacteria embedded in a mucilagenous material and filaments were also observed. Any particular distribution of the bacteria morphotypes between the two layers was not observed. Some fungal hyphae were interspersed in the structure. The organic carbon content of this structure ranged between 3.2-17.2 %, while the organic nitrogen was 0.24-1.08 %.

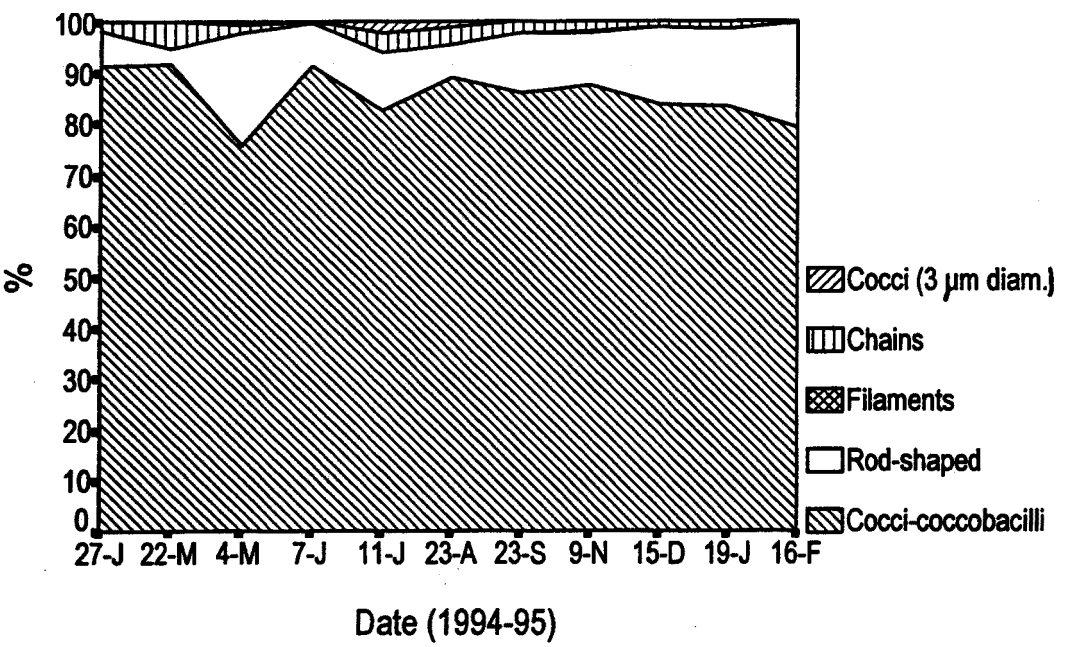


Fig. 2. Relative abundance (%) of the different bacterial morphotypes in La Solana mixed community observed during the study period (January 1994-February 1995).

The differences in the four patches were attributable to differences in the overstorey. In the mixed community, small spots of *Rivularia* sp. and *Zygnema* sp. or *Mougeotia* sp. were found over the permanent crust (Fig. 1c). The bacterial community of this algal patch was mainly composed by cocci and coccobacilli (ca. 80%), and rod-shaped bacteria (7-12%) which increased in May to 23%. Chains and greater cocci (0.3 μm diameter) were scarce (Fig. 2). Thin filaments and spirochaeta shapes were also observed under the fluorescent microscope although being scarce (Fig. 3a and b).

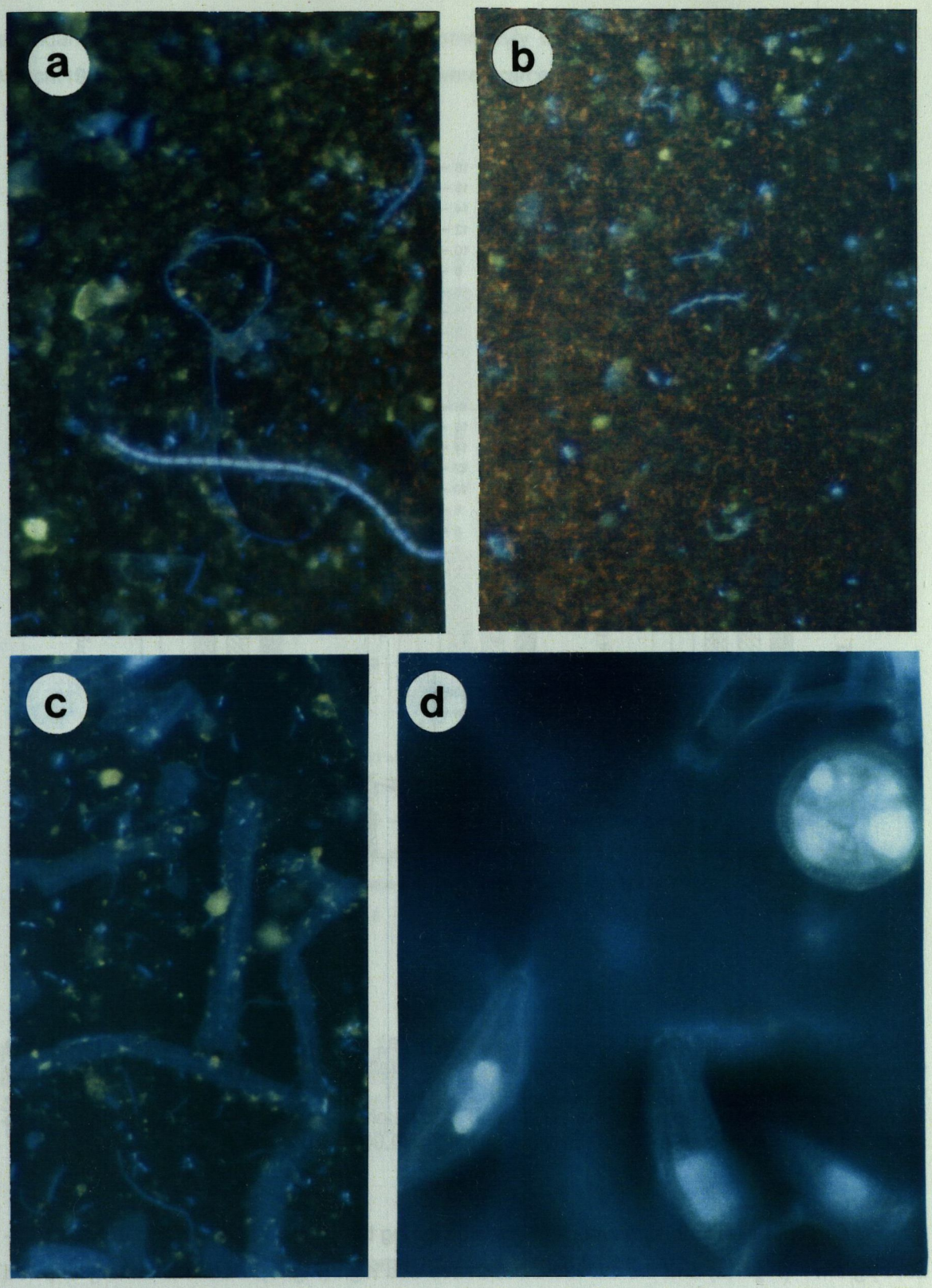


Fig. 3. Photographs under the fluorescent microscope of DAPI stained bacterial preparations from La Solana stromatolitic patches (a and b from a mixed-community sample collected in November 1994, c and d from a diatom bloom sample collected in May 1994). (a) A filament and a cyanobacteria chain can be observed. Small cocci and rod-shaped bacteria are also observed. (b) A spirochaeta is observed. (c) Mucilagenous material is observed in between the bacterial community of the diatom bloom. (d) *Cymbella* and *Cyclotella* were responsible for the diatom bloom.

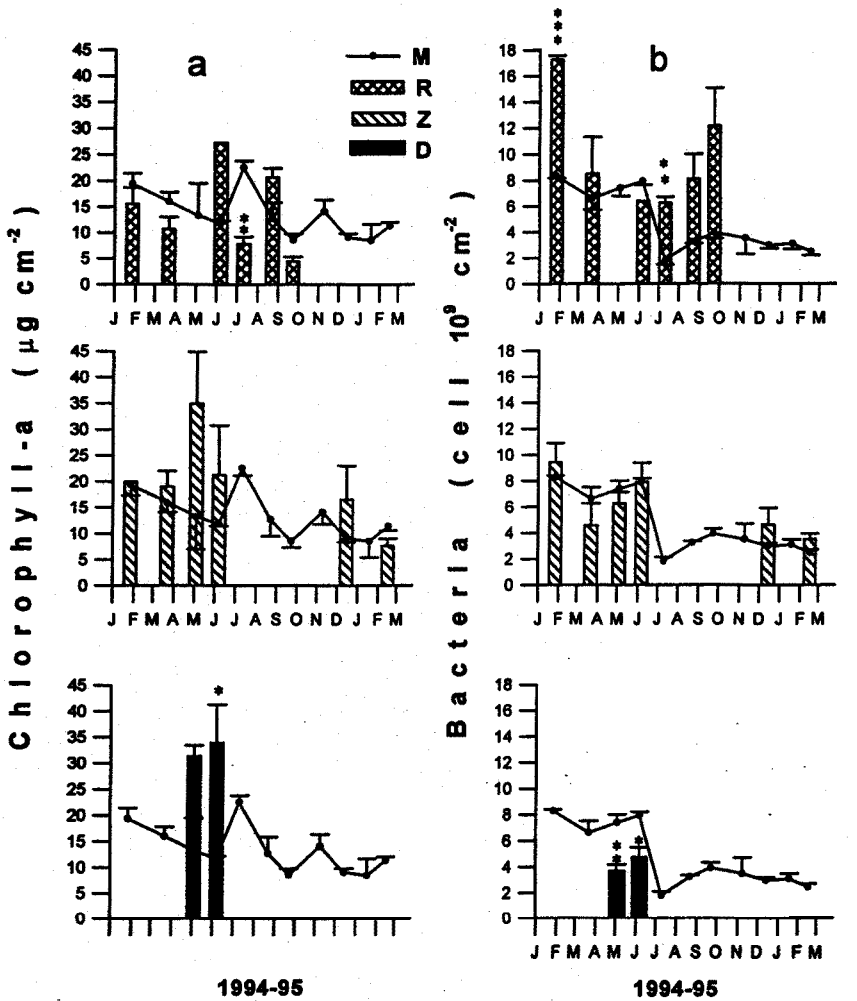


Fig. 4. Temporal variation of chlorophyll-a density (a) and bacterial cell density (b) in the different algal patches. M= mixed community, R= *Rivularia* community, Z= *Zygnema-Spirogyra* community, D= Diatom bloom. Error bars represent standard errors of the mean, n=3. Significant differences between the R, Z or D community and the M community on a given sampling date are indicated: * $p<0.05$, ** $p<0.01$, *** $p<0.001$ (two-tailed *t*-test).

Chlorophyll-a in this community fluctuated during the study period, ranging between 8.4-22.4 µg cm^{-2} (Fig. 4a) and the OD430/OD665 ratio was high (Fig. 5). Bacterial density showed two phases, being between 6.6-8.3 10^9 cell cm^{-2} in winter and spring 1994, and decreasing after the drought to 1.8-3.9 10^9 bacteria cm^{-2} (Fig. 4b).

The *Rivularia* community was made up of *Rivularia biasolettiana* colonies covering the permanent crust. This community had a different appearance in winter 1994 (clear-green colonies) compared to during summer and autumn (dark-green colonies). The bacterial community was mainly composed by small cocci and coccobacilli (ca. 75%). A higher

abundance of filaments than in the mixed community was found, especially in January and March 1994 (Fig. 6a). Chains and great cocci were scarce.

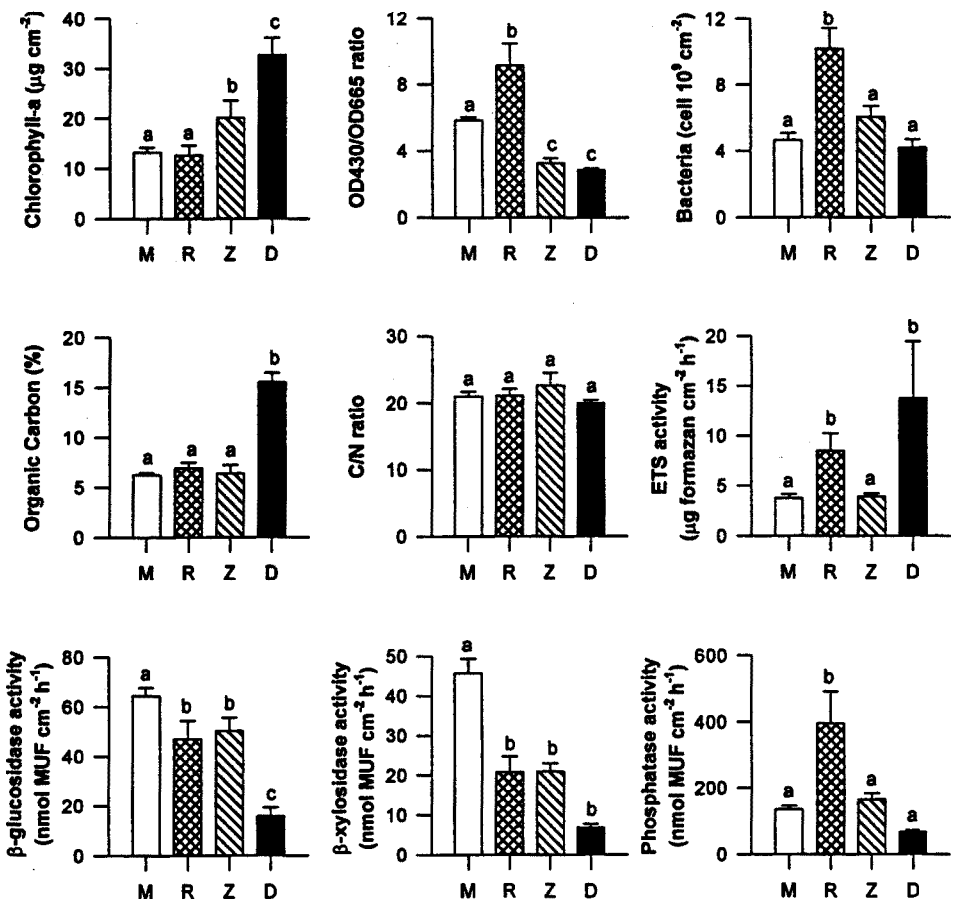


Fig. 5. Annual averages and standard errors (vertical lines) of the biological variables measured in the different algal patches. M= mixed community, R= *Rivularia* community, Z= *Zygnema-Spirogyra* community, D= Diatom bloom. Letters a, b, c mean significant different group found by Tukey's multiple comparison test ($p<0.05$).

Chlorophyll-a density in this community was similar to the mixed community, except for July 1994 when it was significantly lower (Fig. 4a). Bacterial density ranged between 6.2-17.3 10^9 cell cm^{-2}, being significantly higher than the mixed community in January and July 1994 (Fig. 4b). The *Rivularia* community had the highest bacterial density and the highest OD430/OD665 ratio of all the patches (Fig. 5).

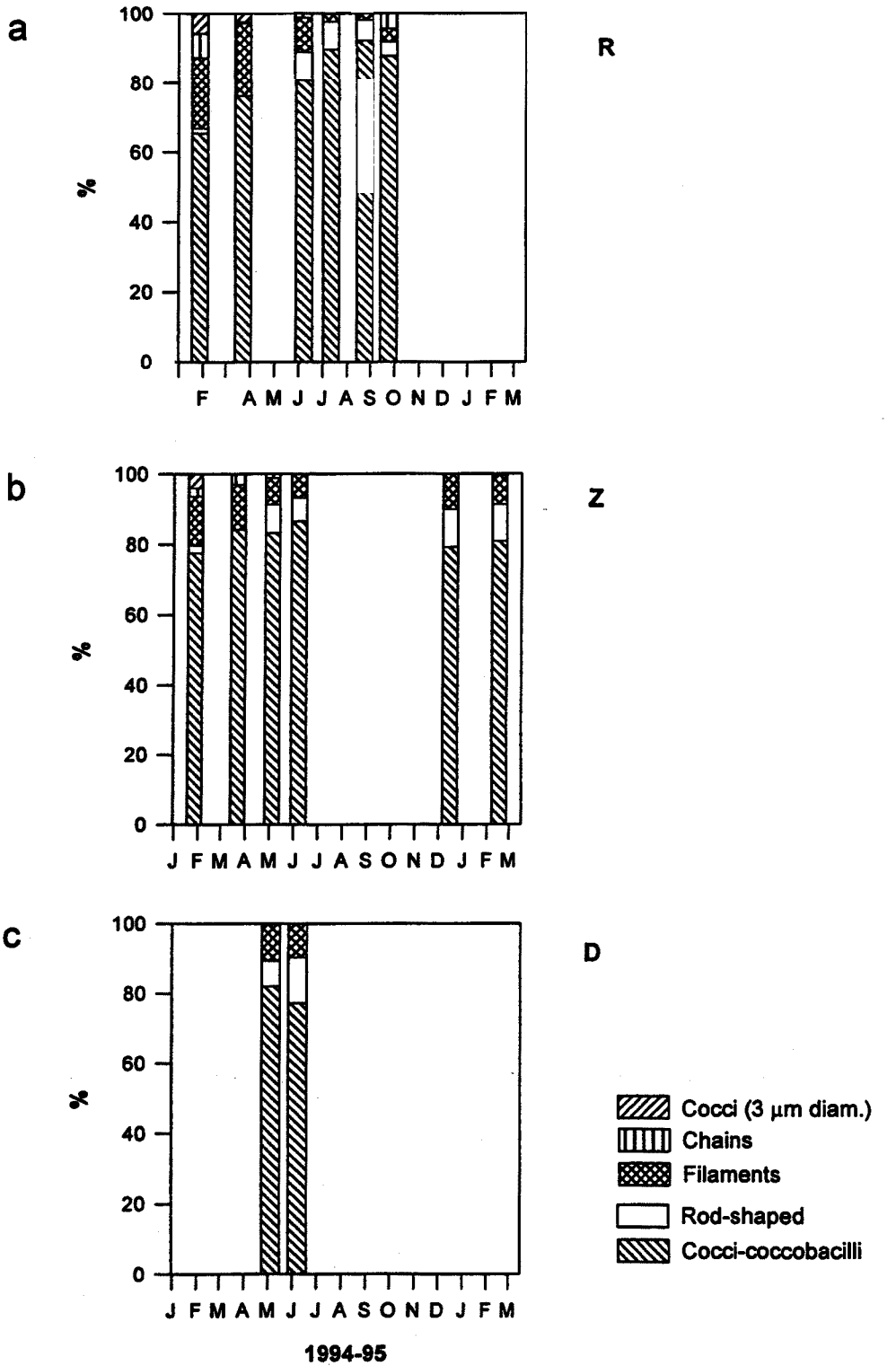


Fig. 6. Relative abundance (%) of the different bacterial morphotypes in La Solana *Rivularia* community, R (a), *Zygnema-Spirogyra* community, Z (b), and diatom bloom, D (c) observed during the study period (January 1994-February 1995).

The *Zygnema-Spirogyra* community developed in spring 1994 and winter 1995 and its overstorey was dominated by *Zygnema*, *Spirogyra* and *Mougeotia* taxa, some diatoms being part of its periphyton (*Cyclotella* cf. *kutzingiana*, *Cymbella* cf. *helvetica*, *Achnanthes minutissima* Kütz, *Epithemia* sp.). Chlorophyll-a and bacterial density were not significantly different to the mixed community at any sampling date (Fig. 4a and b). In annual averages, chlorophyll-a was higher than in the *Rivularia* and mixed community (Fig. 5). The bacterial community was ca. 80% composed by small cocci and coccobacilli being similar to the bacterial community in M (Fig. 2 and Fig. 3a and b), but filaments were more abundant (Fig. 6b).

The diatom bloom appeared in spring 1994. It was a white-grey mucilagenous material, which covered nearly 80% of the cyanobacterial crust. It was mainly composed of the stalked diatoms *Cymbella affinis* Kütz and *Cymbella helvetica* Kütz, but also of *Cyclotella* cf. *kutzingiana*, *Fragilaria capucina* Desmazières, *Synedra ulna* (Nietzsche) Ehr and *Achnanthes minutissima* (Fig. 3c and d). Chlorophyll-a content was ca. 32 μg cm^{-2} (Fig. 4a), significantly higher than in the other patches (Fig. 5). However, bacterial density (ca. 4.2 10^9 bacteria cm^{-2}) was significantly lower than in the mixed community (Fig. 4b). The bacterial community was the less diverse of the algal patches, being mainly composed by small cocci and coccobacilli, rod-shaped bacteria and filaments (Fig. 3c, Fig. 6c). Organic carbon and nitrogen in the diatom bloom were significantly higher than in the other patches, but the molar ratio between organic carbon and organic nitrogen was not (Fig. 5).

Primary production and respiration

Photosynthetic activity, only measured in the mixed community, had an annual average of 8.18 μg C cm^{-2} h^{-1}. It was maximum in spring 1994 and increased again in winter 1995 (Table 2).

TABLE 2. Light and dark $H^{14}CO_3$ incorporation (μg C cm^{-2} h^{-1}) in the mixed community of La Solana stream during the study period. Values are seasonal means and standard deviations (n=6, but n=9 in autumn for light incorporation and n=4, but n=6 in autumn for dark incorporation).

		Winter 1994	Spring 1994	Summer 1994	Autumn 1994	Winter 1995
Light	Mean	0.034	12.72	9.76	1.90	11.21
	SD	0.013	15.30	11.20	1.78	5.44
Dark	Mean	0.012	6.04	7.73	1.63	11.82
	SD	0.024	2.68	4.90	1.86	6.07

Incorporation of ^{14}C in the dark was rather high (Table 2) suggesting that heterotrophic processes such a nitrification are occuring in this thick structure. For this reason a nitrification experiment was carried out after and addition of ammonia and phosphate and the measurement of nitrate during a four hour incubation period (Martí 1995). However, no clear conclusions were obtained from those experiments since nitrate disappeared very fast and was only detected in few replicates. If nitrification is occuring in La Solana, the process must be very quick (between the first 10-15 miutes of incubation). Experiments must be planned to further investigate the responsible for such a high ^{14}C incorporation in the dark.

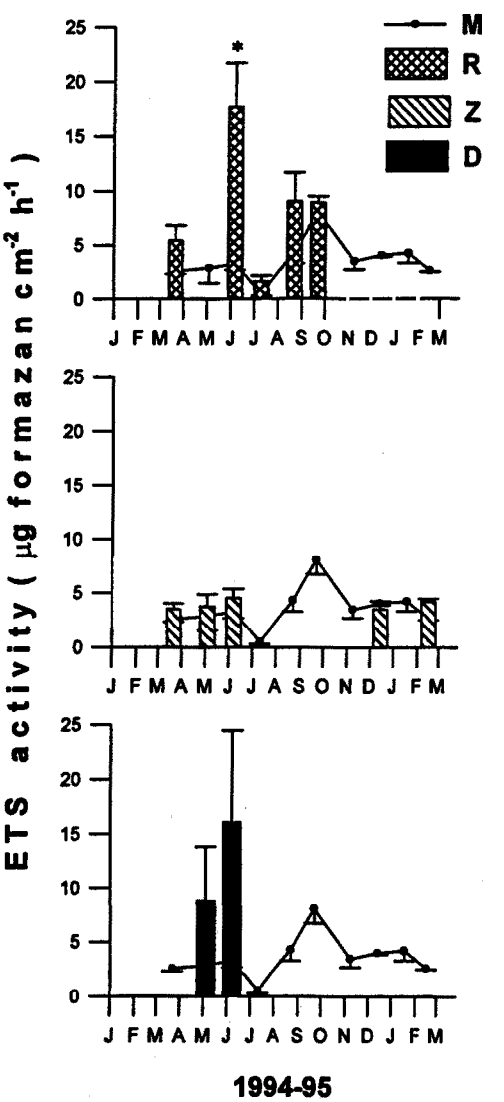


Fig. 7. Temporal variation of respiratory activity (ETS) in the different algal patches. M= mixed community, R= *Rivularia* community, Z= *Zygnema-Spirogyra* community, D= Diatom bloom. Error bars represent standard errors of the mean, n=5. Significant differences between the R, Z or D community and the M community on a given sampling date are indicated: * $p<0.05$, ** $p<0.01$, *** $p<0.001$ (two-tailed *t*-test).

ETS activity in the mixed community was lower in July 1994 and increased in September 1994 (Fig. 7). The *Rivularia* community showed a large peak in ETS activity in June, while values in the *Zygnema-Spirogyra* community were not significantly different from the mixed community (Fig. 7). When comparing annual averages, ETS activity was significantly higher in the *Rivularia* community and in the diatom bloom than in the mixed and *Zygnema-Spirogyra* communities (Fig. 5).

Extracellular enzymatic activities

β-glucosidase activity in the mixed community reached maximum values during the drought, and minimum values were measured in winter (1994 and 1995) and spring 1994 (Fig. 8a). In the *Rivularia* community, β-glucosidase was significantly lower than in the mixed community in January, March and June 1994, but no significant differences were found in the summer months (Fig. 8a). Values in the *Zygnema-Spirogyra* community were also lower in January and March 1994 but significantly higher in May (Fig. 8a). β-glucosidase activity in the diatom bloom increased from May to June, but values were significantly much lower than in the mixed community (Fig. 8a). Concerning annual averages, β-glucosidase activity was the highest in the mixed community and the lowest in the diatom bloom (Fig. 5).

β-xylosidase activity in the mixed community was lower in spring 1994 and increased during the drought, maintaining the high values until winter 1995 (Fig. 8b). The *Rivularia* community and the *Zygnema-Spirogyra* community showed a significantly lower β-xylosidase activity than the mixed community during winter and spring (Fig. 8b). In the *Rivularia* community, β-xylosidase was positively correlated to β-glucosidase ($r=0.96$, $p=0.002$, $n=6$). β-xylosidase activities in the Diatom bloom were also lower (Fig. 8b). Concerning annual averages, β-xylosidase activity was significantly higher in the mixed community than in the other patches (Fig. 5).

The β-xylosidase:β-glucosidase ratio was almost 0.5 except for the mixed community in winter 1994 (Table 3). Few variations in this ratio were detected in the *Rivularia* community over the study period. The other communities had higher seasonal fluctuations (Table 3).

TABLE 3. β-xylosidase:β-glucosidase ratio in the different algal patches. Values are seasonal means and standard error of the annual mean (except for the diatom blomm where the range is shown). M= mixed community, R= *Rivularia* community, Z= *Zygnema-Spirogyra* community, D= Diatom bloom.

Algal patch	**Winter 1994**	**Spring 1994**	**Summer 1994**	**Autumn 1994**	**Winter 1995**	mean ± SE	n
M	1.53	0.53	0.43	0.81	0.68	0.71 ± 0.10	11
R	0.40	0.39	0.40	0.55	--	0.42 ± 0.03	6
Z	0.90	0.34	--	0.53	0.50	0.49 ± 0.10	6
D	--	0.63	--	--	--	0.63 (0.3-0.9)	2

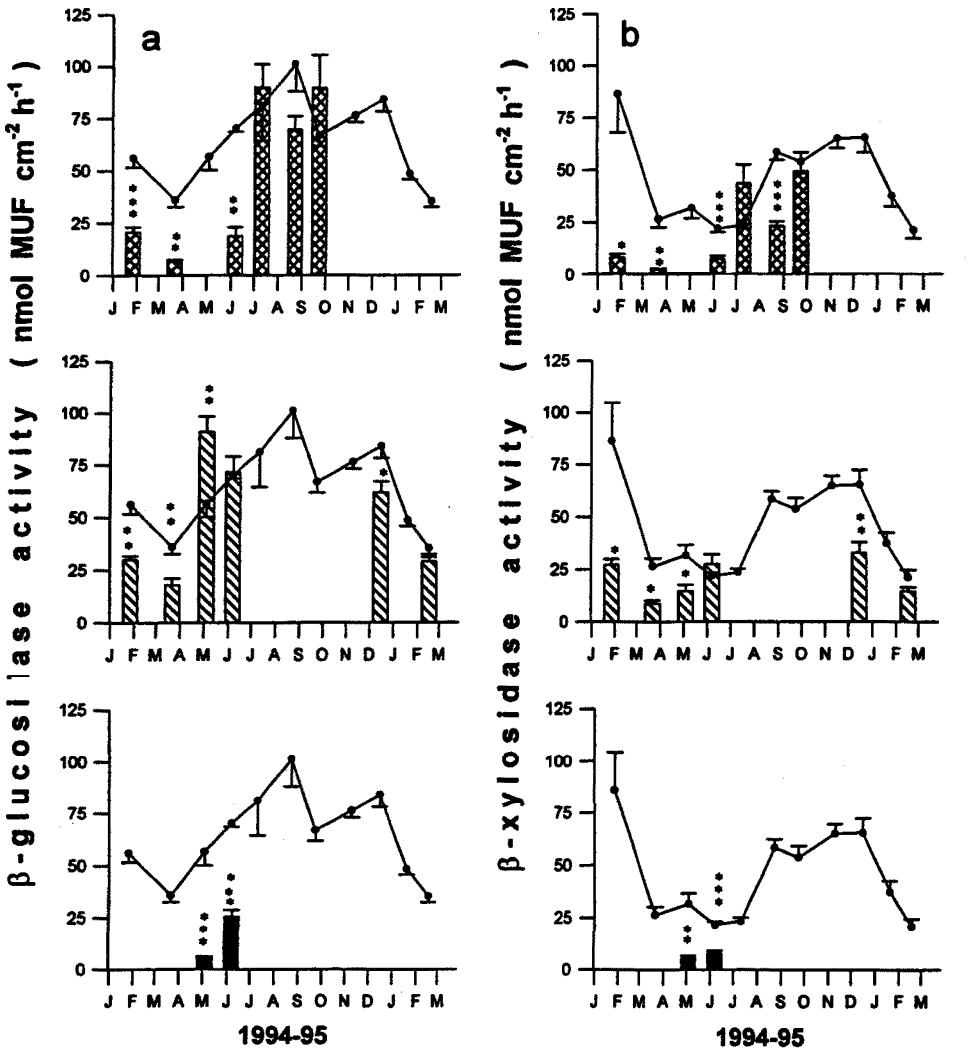


Fig. 8. Temporal variation of the β-glucosidase activity (a), β-xylosidase activity (b) in the different algal patches. M= mixed community, R= *Rivularia* community, Z= *Zygnema-Spirogyra* community, D= Diatom bloom. Error bars represent standard errors of the mean, n=5. Significant differences between the R, Z or D community and the M community on a given sampling date are indicated: * $p<0.05$, ** $p<0.01$, *** $p<0.001$ (two-tailed *t*-test).

Phosphatase activity in the mixed community had spring and summer values of between 100-150 nmol cm^{-2} h^{-1} (Fig. 9). The highest activities were measured in winter (1994 and 1995), and the lowest in autumn 1994. Phosphatase activity in the *Rivularia* community was extremely high in January and June 1994, whilst in the *Zygnema-Spirogyra* community, values for this ectoenzyme were not significantly different than for the mixed community (Fig. 9). Lowest phosphatase activity was found in the diatom bloom. Comparing the annual averages between the patches, phosphatase activity was significantly higher in the *Rivularia* community (Fig. 5).

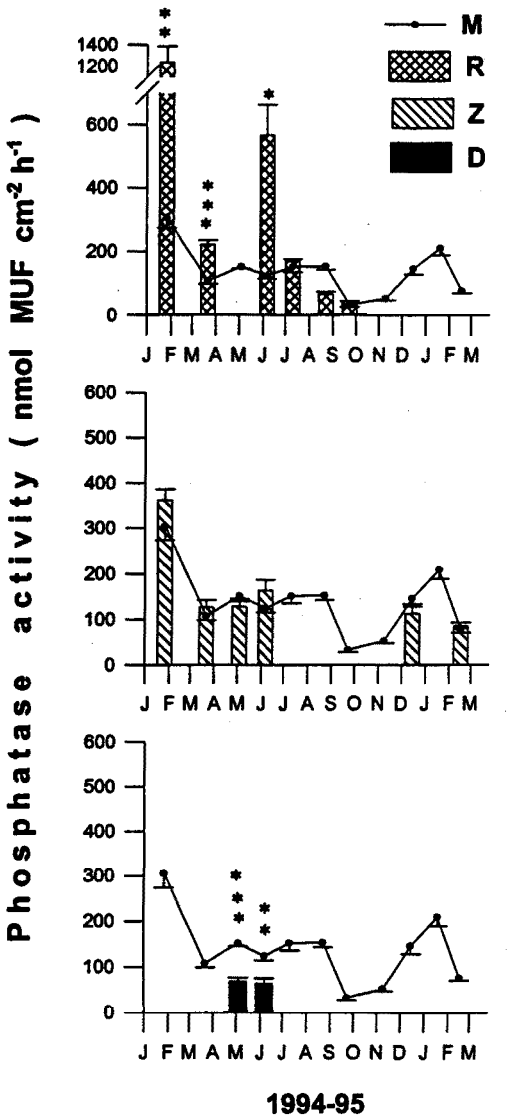


Fig. 9. Temporal variation of the phosphatase activity in the different algal patches. M= mixed community, R= *Rivularia* community, Z= *Zygnema-Spirogyra* community, D= Diatom bloom. Error bars represent standard errors of the mean, n=5. Significant differences between the R, Z or D community and the M community on a given sampling date are indicated: * $p<0.05$, ** $p<0.01$, *** $p<0.001$ (two-tailed *t*-test).

Discussion

Ectoenzymatic activities measured in La Solana stromatolitic patches were much higher than in epilithic stream biofilms studied elsewere (Marxsen and Witzel 1990, Sinsabaugh et al. 1991, Jones and Lock 1993, Chapell and Goulder 1994a, chapter 3). However, values were similar than in biofilms growing on wood surfaces (Sinsabaugh et al. 1991a). The high enzymatic

activities observed in La Solana may be related to the accumulation of organic matter within this thick stromatolite, as indicated by the high organic carbon and nitrogen content.

The differences in ectoenzymatic activities indicates that there is a particular use of organic matter in each patch. Not only were annual averages different but also the seasonal behaviour, and thus the relationship between the different enzymes.

Algal excretion might regulate the lowest ectoenzymatic activities in the diatom bloom (Fig. 5). The monomeric compounds released by the diatoms, easily assimilable by bacteria, can repress extracellular enzymatic activities (Chróst 1990). The diatom bloom showed a high chlorophyll-a content with the lowest OD430/OD665 ratio (Fig. 5), suggesting the ability for a high photosynthetic activity (Gotschalk and Alldredge 1989), that would lead to an important algal excretion. The accumulation of mucilage, mainly from the diatom stalks (Hoagland et al. 1993), and abundant algal excretion is revealed by the high organic carbon content (Fig. 5). On the other hand, the availability of DOC within the mucilagenous material (Decho and Herndl 1995, Smith et al. 1995) might be responsible for the high respiratory activity found in this patch (Norrman et al. 1995).

The enzymatic activities were not very variable throughout the duration of the diatom bloom, except for β-glucosidase which increased from May to June coinciding with the ageing of the mucilage (Herndl 1992, Middelboe et al. 1995) (Fig. 8a). This increase in β-glucosidase activity could be related to higher proportion of substrate available for this enzyme after other enzymes (such as endoglycosidases) have degraded the mucilage to lower molecular-weight molecules (Decho and Herndl 1995).

In some patches there was a distinctive shift in the organic matter source used by the heterotrophs. The *Zygnema-Spirogyra* community showed a shift in the β-xylosidase:β-glucosidase ratio between winter 1994 and spring 1994 (Table 3). The lower ratio in spring suggests the utilization of polysaccharides coming from algal release which mainly enhance β-glucosidase activity (Deshpande and Eriksson 1988, Jones and Lock 1993). The high biomass and photosynthetic activity in spring is characteristic of La Solana, especially for the Zygnematales community (Guasch and Sabater 1994, 1995). It is likely that autotrophic activity does affect the organic matter source, leading to an increase in the β-glucosidase activity. The increase in the β-xylosidase:β-glucosidase ratio in winter and autumn indicates that heterotrophs are also cleaving xylobiose molecules usually found in decaying plant material (Lachke 1988).

The *Rivularia* community was more constant in the use of a given organic matter source. Few variations in the β-xylosidase:β-glucosidase ratio were observed over the study period in this patch (Table 3). The low photosynthetic activity in this community (Guasch and Sabater 1995) and the high OD430/OD665 ratio that indicates a large amount of carotenoids, suggest that there is a low autotrophic input in this patch. Therefore this community may mainly use the organic matter accumulated in the stromatolite, such as extracellular polysaccharides and cyanobacterial sheaths (Lange 1976, Decho 1990). Glycolate excreted by the cyanobacteria (Bateston and Ward 1988, Fründ and Cohen 1992) is suggested to be the major

source of organic carbon in microbial mats together with organic compounds from fermentation (Stal 1995).

The extremely high phosphatase activity in the *Rivularia* community (Fig. 5) confers a resistance capacity to oligotrophic conditions, using organic phosphorus sources to obtain inorganic phosphorus when it is scarce in stream water (Jansson et al. 1988, Whitton 1991, Boavida 1991). The highest phosphatase activity was measured in winter 1994, coinciding with minimum values of SRP in stream water. The genus *Rivularia* has a special ability to hydrolyse organic phosphates (Livingstone and Whitton 1984). This gives a competitive advantage to this patch over the other patches living in the phosphorus limited La Solana (Guasch et al. 1995).

In annual averages, the highest β-glucosidase and β-xylosidase activities were measured in the mixed community, indicating a high cleavage of polysaccharide molecules in this patch. The few algae and cyanobacteria constituting the overstorey of this patch, indicate that the understorey of this stromatolitic crust is highly active. Possibly, the activity in the permanent crust (understorey) along with the organic matter accumulated within it, is the basis for the persistence of this stromatolitic crust. The lower importance of autotrophy in the overstorey of this patch and the accumulation of allochthonous organic matter such as leaf fall and decaying plant material could enhance the β-xylosidase activity, especially in autumn and winter (Table 3).

The different regulation of the ectoenzymatic activities in each patch and the thick structure of this stromatolitic biofilm underlie its ability to live in an oligotrophic stream, as well as its resistance capacity to the drastic changes in temperature, discharge and light. Drought caused a drastic decrease in bacterial density, photosynthetic activity and respiratory activity in the mixed community, but the activities recovered in two to three hours (Romaní and Sabater 1997, chapter 4.2). Also, the appearance of the different algal patches during the year acts as a mechanism to exploit all energy entrance using the adaptative capacity of each algal species. As has been oberved in marine intertidal mats (Paerl et al. 1993a and b), this behaviour optimizes carbon utilization in the system, and is a good strategy when living under oligotrophic conditions.

4.2. Metabolism recovery of a stromatolitic biofilm after drought in a Mediterranean stream

Abstract

Organisms dwelling in Mediterranean streams need to be adapted to dry periods. La Solana stream dried out completely during summer 1994. The recovery of the biofilm was studied in a laboratory experiment by immersing it in stream water. La Solana has a thick stromatolitic biofilm composed of the cyanobacteria *Rivularia* sp. and *Schizothrix* spp. Ectoenzymatic microbial activities (β-glucosidase, β-xylosidase and phosphatase), $H^{14}CO_3$ incorporation and respiration (Electron Transport System- ETS- activity) were measured immediately after rewetting and then every hour for 5 h. Both ETS and photosynthetic activities were low at the beginning of the rewetting but increased significantly after two hours. Recovery of enzymatic activities in the biofilm was immediate, since values measured at 0 h were similar to those obtained in the stream before drought. Enzymatic activities increased markedly after three hours to higher values than those reported before drought. All activities stabilized to initial values at the end of the experiment. It is suggested that the rapid recovery of the biofilm metabolism could be supported by the stromatolitic structure of La Solana biofilm acting as a reserve of organic matter, as well as the rapid rehydration provided by the cyanobacteria sheaths. This implies that the biofilm can exploit short rainfall periods in dry summers.

Introduction

Most rivers subject to the Mediterranean climate are defined by their irregular pattern of water discharge (Sabater et al. 1995). Low-order streams are usually affected by drought (especially in summer), which may even convert them to discontinuous streams. Drought can affect most of the organisms dwelling in the system (Williams and Hynes 1976) even though algae and cyanobacteria recover rapidly. Recovery of biofilm metabolism after drought has been observed in extreme habitats such as Antarctic streams, where microbial mats (usually composed by a cyanobacterial community) recover rapidly during watermelt (Vincent and Howard-Williams 1986). Metabolism recovery after a dry period has also been described for some cyanobacteria in sediments (Dubois and Kapustka 1983, Lüttge et al. 1995) and in subaerial environments (Coxson and Kershaw 1983). Cyanobacteria are extremely adaptable organisms (Stal 1995). Their cell structure is able to support desiccation stress due to its mucilagenous accumulation, photosynthetic pigments adaptation and stability of proteins (Fogg et al. 1973).

Little is known about the effect of dry periods in Mediterranean streams, where desiccation may occur in summer for relatively long periods (20-40 days). In this study, the recovery capacity of algal and bacterial metabolism in a thick, stromatolitic biofilm after a dry period (1 month) that occurred in La Solana stream was investigated, especially focussed on the short-term response of the autotrophic and heterotrophic metabolisms. For this purpose,

dry biofilm samples were rewetted in the laboratory and photosynthetic activity ($H^{14}CO_3$ incorporation), ectoenzymatic activities (β-glucosidase, β-xylosidase and phosphatase), and respiration (Electron Transport System activity) were measured. Experimental values are compared with those measured in the same stream biofilm before the drought.

Materials and methods

Experimental procedure

Crusts of the stromatolitic biofilm were collected from La Solana in July 1994. Some of the measurements ($H^{14}CO_3$ incorporation, respiratory activity) were repeated in August 1994 after a meagre rain reached the streambed. Pieces of the crust of approx. 15x15 cm surface area, 1 cm thick were carefully collected from the streambed using a spatule. Such large pieces were used in order to minimize edge effects when rewetting. The crusts were wrapped in aluminium foil and maintained at low temperature for its transport to the laboratory. At the begining of the experiment, the crusts were unwrapped and immersed in a plexiglass tank (20 liters capacity) filled with La Solana stream water (collected one month before and preserved at 4°C). To reproduce stream water flow in the tank, a pump was installed to recirculate water and the tank was placed under light (150 μmol photons m^{-2} s^{-1}). Mean water temperature was kept at 20°C. Samples were taken from the tank at the time of immersion and 1, 2, 3, and 5 hours thereafter. At each sampling time, biofilm samples (ca. 1.1 cm^2 surface area) were cut from the biofilm crust, and placed in sterile glass vials and activity measurements were performed. Samples for chlorophyll-a analysis were frozen and kept in the dark until pigment extraction. Samples for bacteria cell counting were placed in sterile glass vials with 2% formalin.

Algal biomass and bacterial density

Chlorophyll-a was measured in triplicate, the ratio of chlorophyll to carotenoids and/or chlorophyll degradation products (OD430/OD665) also being determined (see procedures in chapter 2). The composition and vertical distribution of the algal and cyanobacterial taxa in the stromatolitic structure was observed under optical microscopy. Bacterial density (DAPI stain, epifluorescence microscopy) was estimated in triplicate (see procedures in chapter 2). The different bacteria morphotypes were also distinguished.

Organic Carbon and Nitrogen biofilm content

Organic carbon and nitrogen content in the biofilm crust was obtained after drying (two days at 110°C) and grinding of the material. HCl 2N was added to each sample to remove inorganic carbon. Elemental analysis of carbon and nitrogen was performed with a C/N Analyzer 1500 Carlo Erba using vanadium pentoxide as the oxidation catalizer.

Metabolism measurements

Primary production was measured in two replicate samples, one killed-control sample and one darkened incubated sample by ^{14}C incorporation. Electron Transport System (ETS) activity was assayed in three replicates and one killed control. Extracellular β-D-glucosidase, β-D-xylosidase and phosphatase potential activities were determined in three replicates and one killed-control. Two blanks of filter-sterilized stream water were also incubated for each enzyme. All measurements were determined following the procedures described in chapter 2.

All activities and biomass were expressed per surface area of stromatolitic crust used for each measurement by image analysis (as in chapter 4.1).

Data analyses

Correlation between the different activities during the experiment was perforemd by product-moment Pearson correlation. To compare activities at different rewetting times, one-way analysis of variance (ANOVA) was performed.

Results

Observation of the stromatolitic biofilm under light microscopy showed two distinct layers. The first layer (0-3 mm), clearly identified by its greenish colour, was dominated by *Rivularia biasoletiana* also with *Schizotrix* spp. and occasionally *Homeothrix* sp. In the second deeper layer (3-6 mm), characterized by a yellow-brown colour, *Schizotrix* spp. was the most abundant cyanobacteria but some *Rivularia* filaments were also found. The difference in colour between layers was due to the predominance of chlorophylleous pigments in the first layer and carotenoid pigments in the second (Guasch and Sabater 1995). This structure coincides with what was described as the mixed community in section 4.1 with an even poorer overstorey, and occupied nearly 90% of the streambed during drought (the rest being spots of *Rivularia* community, chapter 4.1).

Bacterial density in the samples after drought (Table 1) was lower than before the drought period (Table 2). The organic carbon content in the drought biofilm (Table 1) was slightly but not significantly lower than that observed before the drought period (ANOVA, $p= 0.22$) (Table 2).

Chlorophyll-a per surface area and OD430/OD665 in the drought biofilm (Table 1) were higher than those observed before drought (Table 2). Higher OD430/OD665 indicated an increase in carotenoids or degradation products (pheophitins).

TABLE 1. Biomass and elemental composition in La Solana biofilm after 30 days of drought.

	Average	SD	n
Chlorophyll-a ($\mu g\ cm^{-2}$)	22.457	2.306	3
OD430/OD665	6.339	0.566	3
Bacteria (cell $10^9\ cm^{-2}$)	1.786	0.56	3
% Organic Carbon	4.892	1.557	5
% Organic Nitrogen	0.359	0.034	5
C/N ratio	16.036	5.43	5

TABLE 2. Algal and bacterial activities and biomass in La Solana biofilm just before the drougth period (Spring 1994).

(n=3)	Average	SD
β-glucosidase activity ($nmol\ cm^{-2}\ h^{-1}$)	54.31	17.35
β-xylosidase activity ($nmol\ cm^{-2}\ h^{-1}$)	26.64	4.99
Phosphatase activity ($nmol\ cm^{-2}\ h^{-1}$)	127.19	22.53
ETS activity ($\mu g\ cm^{-2}\ h^{-1}$)	2.90	0.35
^{14}C incorporation ($\mu g\ C\ cm^{-2}\ h^{-1}$)	12.72	17.22
Chlorophyll-a ($\mu g\ cm^{-2}$)	13.65	2.08
DO430/DO665	5.60	0.63
Bacteria (cell $10^9\ cm^{-2}$)	7.30	0.65
% Organic Carbon	5.76	0.67
% Organic Nitrogen	0.36	0.02
C/N ratio	18.73	0.65

Photosynthetic activity in the biofilm crust appeared after one hour of rewetting and a peak of activity was observed at two hours, decreasing again at the end of the experiment (Fig. 1). $H^{14}CO3$ incorporation after rewetting was lower than before drought (Table 2). In contrast, in August (after some rain occured) instantaneous photosynthetic activity (12.37 $\mu gC\ cm^{-2}\ h^{-1}$) was similar to that recorded before drought (Table 2). $H^{14}CO3$ incorporation referred to chlorophyll-a per surface area (photosynthetic efficiency) was lower after the desiccation stress.

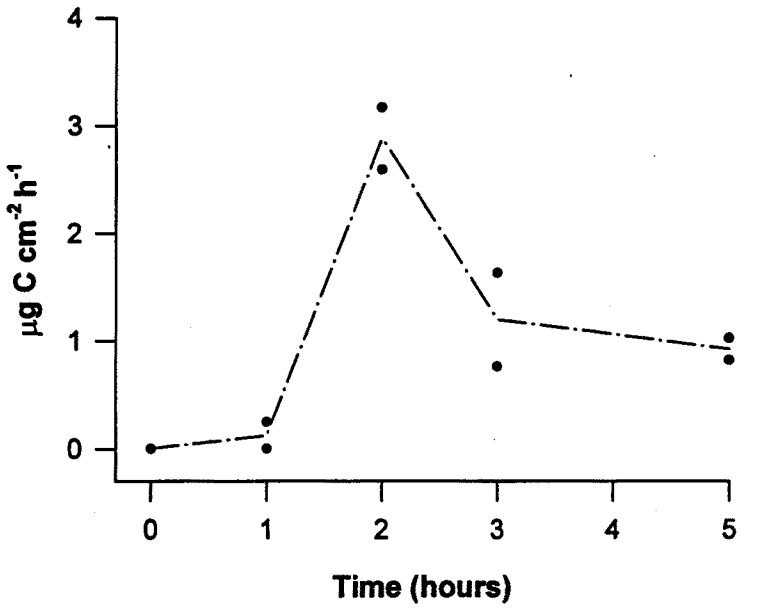


Fig. 1. Photosynthetic activity ($H^{14}CO_3$ incorporation) in La Solana drought stromatolitic biofilm as a function of rewetting time. Points indicate single results (n=2) and the line is drawn through mean values.

Respiratory activity showed a similar trend to ^{14}C incorporation but it was already significant at the beginning of the rewetting (Fig. 2). A peak of activity was also observed at two hours, decreasing later to initial values. No significant differences between activities at 0 h and 5 h were found (ANOVA, p=0.690). However, the ETS activity of the biofilm community did not reach the values obtained before drought (Table 2). In August 1994 an ETS activity of 3.12 µg formazan cm^{-2} h^{-1} was recorded, close to that found before the drought period (Table2).

Ectoenzymatic activities were highly significant immediately after rewetting (Fig. 3), reaching values similar to those obtained before drought (Table 2). Phosphatase and β-glucosidase activities followed the same pattern throughout the rewetting experiment (Fig. 3a and b), with low variation in activities the first 2 hours and a peak at 3 hours, decreasing to initial values at 5 hours. Significant correlation was found between β-glucosidase and phosphatase activities (r=0.93, p=0.021, n=5). The pattern for β-xylosidase was slightly different, but a peak at three hours after rewetting was also observed (Fig. 3c). No significant differences between initial activities and activities after five hours of rewetting were found in any of the three enzymes (ANOVA, p>0.1).

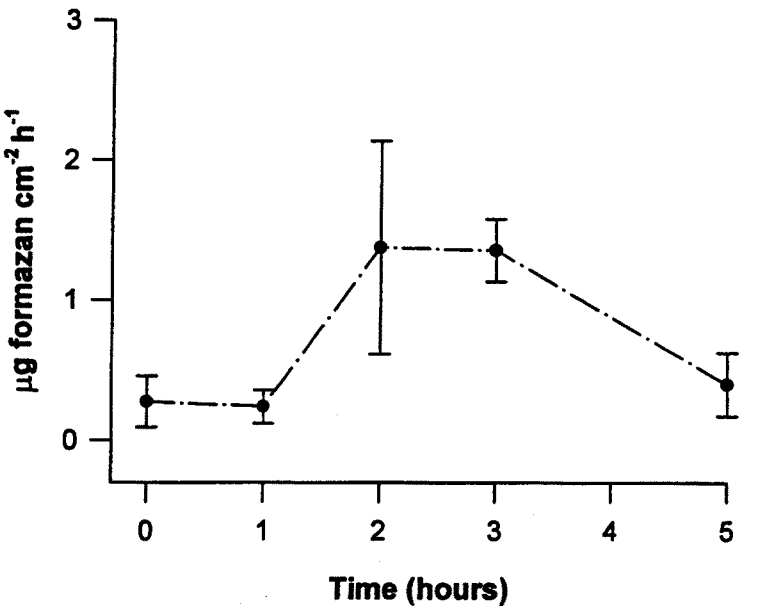


Fig. 2. Respiratory activity (ETS) in La Solana drought stromatolitic biofilm as a function of rewetting time. Means ± standard errors are shown, n=3.

Discussion

Biofilm metabolism in La Solana stream showed a rapid recovery when rewetted after one month under drought and high incident light conditions. However, only some of the processes measured during the experiment recovered immmediately to pre-drought conditions.

Photosynthesis had a fast but not immediate recovery after the rewetting. Recovery time of photosynthetic activity was similar to those reported elsewere in cyanobacterial mats (Coxson and Kershaw 1983, Vincent and Howard-Williams 1986, Lüttge et al. 1995) and in dessicated cyanobacteria (Scherer et al. 1984). In five hours, photosynthetic activity does not seem to be recovered completely since values were lower than before drought. The need of a certain lag period to recover after drought is also suggested by the immediate, higher $H^{14}CO_3$ incorporation recorded in August 1994 in a weakly humidified biofilm.

The higher OD430/OD665 in the drought biofilm suggests that the drought stress stimulated the carotenoid accumulation. A chromatic adaptation has been described for cyanobacteria, increasing carotenoid concentration with increasing light intensities (Fogg et al. 1973). Carotenoid accumulation has been observed in Antarctic cyanobacterial mats (Hawes 1993, Quesada et al. 1995) and in terrrestrial cyanobacteria (Scherer et al. 1988) as

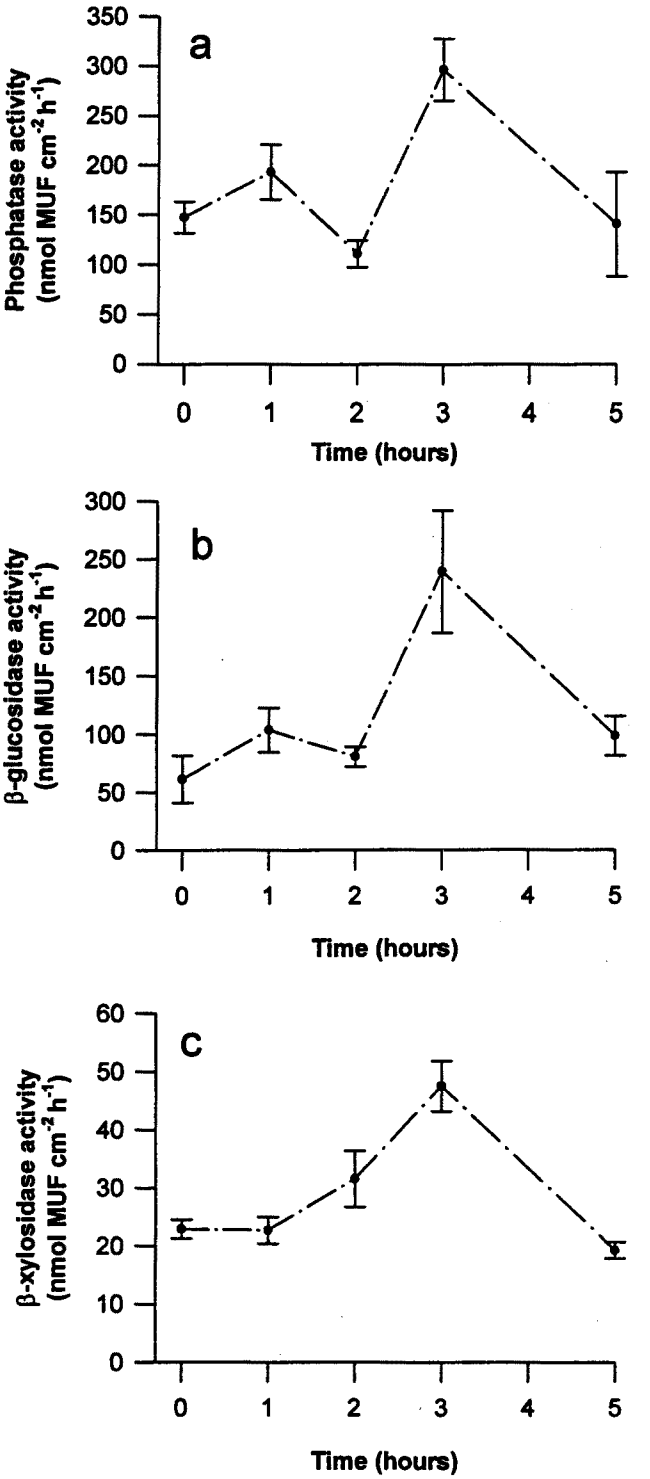


Fig. 3. Ectoenzymatic activities in La Solana stromatolitic biofilm as a function of rewetting time. Means ± standard errors (vertical bars) are shown, n=3.

a protection against photooxidation. Moreover, important carotenoid concentration in La Solana biofilm prevents photoinhibition at summer ambient irradiances (Guasch and Sabater 1995). The lower photosynthetic efficiency during drought can be due either to the carotenoid

accumulation (Millie et al. 1990) or to the self-shading effect of biomass accumulated in the biofilm (Hawes 1993, Guasch and Sabater 1995).

A lag period to recover after drought is also suggested for respiratory activity since ETS after 5 h of rewetting did not reach the values obtained before drought, in contrast to the immediate response of ETS in August 1994, when the biofilm was humidified.

On the other hand, ectoenzymatic activities in the drought biofilm responded immediately after rewetting. The recovery of β-glucosidase and β-xylosidase could be explained by an inducing mechanism (Chróst 1990) due to the accumulation of substrates for these enzymes within the biofilm. β-glucosidase and β-xylosidase are microbial enzymes (mainly bacterial and fungal) involved in cellulose and hemicellulose degradation respectively (Desphande and Eriksson 1988, Lachke 1988). Polysaccharides accumulating in the biofilm (as mucilagenous material from cyanobacterial sheaths, decaying cell walls, bacterial envelopes) could be an important carbon source for the microbial heterotrophs (Lock 1990) as well as a place to retain water and thus enhancing survival of desiccation (Roberson and Firestone 1992). In this way, the high photosynthetic activity and the diatom bloom recorded in La Solana biofilm just before drought (chapter 4.1) were a high quality organic matter input probably utilized over the drought period. The slightly lower β-xylosidase activity may be due to the higher recalcitrancy of substrates for its decomposition (Boschner et al. 1995). Moreover, phosphatase activity could be induced by organic phosphate compounds accumulating in the biofilm from dead cells. High phosphatase activity in La Solana stream can be related to the low phosphorus concentration and the high ratio of inorganic N:P (Martí et al. 1994), as has been observed in other *Rivularia*-dominated communities (Livingstone and Whitton 1984). Recovery of metabolism, and thus protein synthesis may enhance the peak of ectoenzyme activity observed after 3 h of rewetting. However, enzymatic activities measured at the begining of rewetting may be due to free enzymes accumulated within the polysaccharide matrix (Lock 1993).

The thickness and composition of this stromatolitic biofilm are likely to be important features for its recovery after such extreme conditions. Its role as a reserve pool of organic matter is indicated by the high organic C and N content and the fairly steady proportion of C/N between the drought and normal water flow periods. Cyanobacterial material and other mucilagenous substances interspersed in the biofilm can contribute to its fast rehydration (Verrechia et al. 1995). Moreover, cyanobaceria have been described as resistant to drought, probably related to the highly stable structure of their proteins (Fogg et al. 1973). The ability of the biofilm to recover its metabolism rapidly could imply the exploitation of short rainfall periods that can occur during summer when streambed is dry, even though when they are not sufficient to produce runoff. Its ability to store and use organic matter could ensure its survival.

5. ECTOENZYME KINETICS IN RIERA MAJOR AND LA SOLANA

5. Characterization of ectoenzyme kinetics in Mediterranean streams

Abstract

The enzyme kinetics were analyzed in the benthic biofilm at two second-order Mediterranean streams in each of the four seasons. The Vmax (maximal velocity), Km (apparent Michaelis constant) and Tt (turnover time of substrate hydrolysis) were obtained for the β-glucosidase, β-xylosidase and phosphatase activities by the Michaelis-Menten approach. Vmax values were always higher for the cyanobacterial crust in La Solana (an open stream) than for the sandy and epilithic biofilms in Riera Major (a forest stream). Km values were in the same range indicating that affinity and thus substrate availability might be similar between streams possibly because of their being the same size (second-order), with similar dissolved organic carbon concentration in stream water and similar environmental conditions. However, the turnover time was much lower in La Solana than in the epipsammic and epilithic biofilms of Riera Major. The rapid recycling of the organic matter in La Solana might be related to the more labile substrates for the heterotrophs (organic compounds from the primary producers), while the slower turnover time in Riera Major might be a result of it receiving an input of more recalcitrant material (leaf fall from the riparian vegetation).

Introduction

Ectoenzymatic activities have been widely studied in planktonic environments (e.g. Hoppe 1983, Chróst 1990, Münster et al. 1992), sediments (Sinsabaugh and Findlay 1995, Marxsen and Schmidt 1993) and stream biofilms (Sinsabaugh and Linkins 1988, Jones and Lock 1993, Chapell and Goulder 1994a, chapter 3, chapter 4). The majority of these studies have been carried out by measuring potential enzymatic activities when adding an artificial substrate at substrate-saturation concentration which permits intensive sampling in ecological studies. However, a much more realistic approach to the ectoenzymatic activities is achieved when the kinetic behaviour of each enzyme is measured, therefore providing the kinetic parameters (Vmax and Km) which are related to natural substrate availability (Chróst and Rai 1993, Chróst and Riemann 1994, Button 1994) as well as the turnover time, related to the efficiency of substrate utilization (Gocke 1977, Hoppe et al. 1988). Moreover, the difficulty of measuring enzymatic velocicites of natural microbial assemblages at low substrate concentration (Lewis et al. 1988) together with the usually unknown natural substrate concentrations (Hoppe et al. 1988, Marxsen and Fiebig 1993, Chróst and Rai 1993) make the kinetic approach very useful in ecological studies.

This study focuses on the kinetic behaviour of ectoenzymes in the biofilms of two Mediterranean streams, Riera Major and La Solana, which differ in watershed lithology and vegetation and in their benthic primary production (Guasch and Sabater 1994, Martí and Sabater 1996). Located close to each other, the two streams have similar environmental conditions and

similar stream size (second-order) which make these streams easily comparable. In Riera Major, a forest stream, the sandy biofilm is an important carbon cycling place especially for the accumulation of organic matter (plant material, leaf fall) while the epilithon has a lower hydrolytic potential capacity (chapter 3.1). In La Solana, an open stream, the benthic community is composed of a thick cyanobacterial crust with major microbial activity in its understorey layer (chapter 4.1).

Previous studies in both streams showed great differences in the potential activity of the enzymes β-glucosidase, β-xylosidase and phosphatase on the various benthic substrates (chapter 3.1 and chapter 4.1). It was suspected that such differences were the result of varying substrate availability and/or varying efficiency in its utilization, due to differences in the organic matter input for each benthic substrate in each stream. This was tested by the kinetic approach, which permits the calculation of the efficiency in the organic matter cycling (turnover time) and the affinity of each enzyme for the substrate (Km).

Materials and Methods

Sampling

Streambed biofilm samples were collected from the Riera Major and La Solana. Samples were collected in late-May (spring), mid-July (summer), late-September (autumn) and mid-January (winter) in each stream during 1995-96. The physico-chemical parameters during the study period are summarized in Table 1.

TABLE 1. Physical and chemical characteristics of Riera Major and La Solana streams. Values are means and standard errors for the study period (n=4).

	Riera Major		La Solana	
	Mean	SE	Mean	SE
Temperature (°C)	11.3	1.4	11.2	1.7
Light (μmol m^{-2} s^{-1})	122.4	113.12	420	247.89
Conductivity (μS cm^{-1})	237.5	10.25	443.0	1.32
pH	8.1	0.1	7.9	0.1
Oxygen (mg L^{-1})	10.1	1.12	9.77	1.56
DOC (mg L^{-1})	2.2	0.31	4.0	0.4
DIC (mg L^{-1})	22.5	2.0	45.7	2.1
NO_3-N (μg l^{-1})	461.5	91.8	137.7	63.4
NH_4-N (μg l^{-1})	23.8	8.13	19.2	6.2
SRP (μg l^{-1})	10.8	4.2	4.1	1.8

In Riera Major, both epipsammic and epilithic biofilms were considered. Sand samples from the top 2 cm were collected with a PVC sand-corer sampler (5 cm diameter). Artificial colonized substrates (clay tiles placed in the stream six-to-eight weeks before sampling, Sabater and Romaní 1996) were collected as epilithic biofilm samples. Tiles were only collected in summer and autumn. In La Solana, pieces of the thick biofilm (cyanobacterial crust) were carefully collected. Riera Major sand samples (ca. 2 ml in volume) and tiles (0.64 cm^2, 1 cm high), and La Solana cyanobacterial crust (ca. 1.1 cm^2 in surface area) were placed in sterile glass vials with stream water and maintained in cold (on ice) dark conditions and transported to the laboratory. The extracellular enzyme assays were performed in the laboratory, two to three hours after sampling.

At each sampling date, light, temperature, pH, dissolved oxygen and conductivity were measured in the field. Filtered (precombusted Whatman GF/F filters) water samples (three replicates for each analysis) were taken in order to analyse inorganic nutrients (nitrate, ammonium and soluble reactive phosphorus) as well as dissolved organic carbon (DOC) and dissolved inorganic carbon (DIC) following the procedures described in chapter 2.

Extracellular enzyme assays

Extracellular β-D-glucosidase, β-D-xylosidase, and phosphatase activities were determined spectrofluorometrically using MUF (methylumbelliferyl)-substrate analogues. MUF substrates were dissolved in methylether and diluted with filter-sterilized river water (Gelman, 0.2 μm) to 0.025, 0.05, 0.075, 0.1, 0.2, 0.3, 0.4, and 0.5 mM immediately before assay. For all enzyme assays, 2 ml of the substrate solutions were added to triplicate biofilm samples and formaldehyde-killed controls for each sample type (Riera Major sand and tiles and La Solana cyanobacterial crust). Samples were incubated in the dark under continuous shaking for 1h at ambient stream temperature. Blanks for each substrate concentration were also incubated. After the addition of 0.05 M Glycine buffer pH 10.4, fluorescence was measured at 455 nm under an excitation wavelengh of 365 nm (Kontron, SFM25). Activities were related to biofilm surface area. Sand grain surface area was measured following the procedures of Marxsen and Witzel (1991) as described in chapter 3.1 and cyanobacterial crust surface area was measured by image analysis as described in chapter 4.1.

Enzyme kinetic data analysis

MUF-substrates were added in progressive concentrations to the samples to establish enzyme-substrate saturation curves and to enable the calculation of the kinetic parameters of these enzymes by the Michaelis-Menten approach. Although heterogeneous microbial communities can deviate from Michaelis-Menten kinetics (Lewis et al. 1988), natural microbial communities often fit the Michaelis-Menten equation (e.g. Marxsen and Schmidt 1993), especially if a narrow concentration range is used (Wright and Burnison 1979, Azam and Hodson 1981). The Michaelis-Menten approach is recommended as a practical working model when studying natural populations (Williams 1973).

Most enzymatic reactions followed Michaelis-Menten kinetics, giving the rectangular hyperbola when plotting the velocity of reaction (v) against increased concentrations of substrate ([S]) according to the formula:

$$v = (Vmax\ [S])/(Km + [S]) \qquad (1)$$

where Vmax is the maximum velocity of the enzyme and Km the apparent Michaelis constant, which is equal to the concentration of substrate for the half-maxima velocity and expresses the affinity of the enzyme for the substrate. However, when the enzymatic reaction measured fell in the initial values of the Michaelis-Menten curve, values followed the linear formula (Vrba et al. 1993):

$$v = [S]\ Vmax/Km = [S]/Tt \qquad (2)$$

where Tt (= Km/Vmax) is the turnover time of substrate hydrolysis. Fig. 1 shows an example of such a data set for both models.

A non-linear regression analysis of the experimental data was applied to determine their best fit to the Michaelis-Menten model (1) and compared to the linear model (2), using the IBM PC computer software program "Enzfitter", version 1.05 (Leatherbarrow 1987). This routine employs the Marquart algorithm (Marquart 1963). To determine the model which gave the best fit to the experimental data, the sums of squares of residuals were calculated and divided by the corresponding degrees of freedom (df=n-2 for model 1, df=n-1 for model 2, n=27). The model which showed the lowest values of sums of squares of residuals was chosen. The mean squared related error (MSRE= $\sum(e_i^2 / y_i)/n$, the mean of the quotient of the squared residuals to the estimates) was calculated for each regression analysis. Furthermore, a test of randomness (median runs test) was used to test the independence of the errors for each model applied (Lai 1996). A substrate inhibition was observed for the phosphatase activity in La Solana at substrate concentrations over 0.2 mM, possibly due to an acidification of the solution when increasing the MUF-phosphate concentration (J. Nedoma pers. comm.). Since the phosphatase activity in La Solana was substrate saturated at 0.2 mM, calculations of the kinetic parameters by the Michaelis-Menten approaches where performed with data from 0 to 0.2 mM substrate concentrations (n=18).

The "Enzfitter" program was also used to obtain the Vmax and Km values. The turnover time (Tt) of substrate hydrolysis was calculated by the quotient Km/Vmax after transformation of the data to the same units to express the Tt in hours.

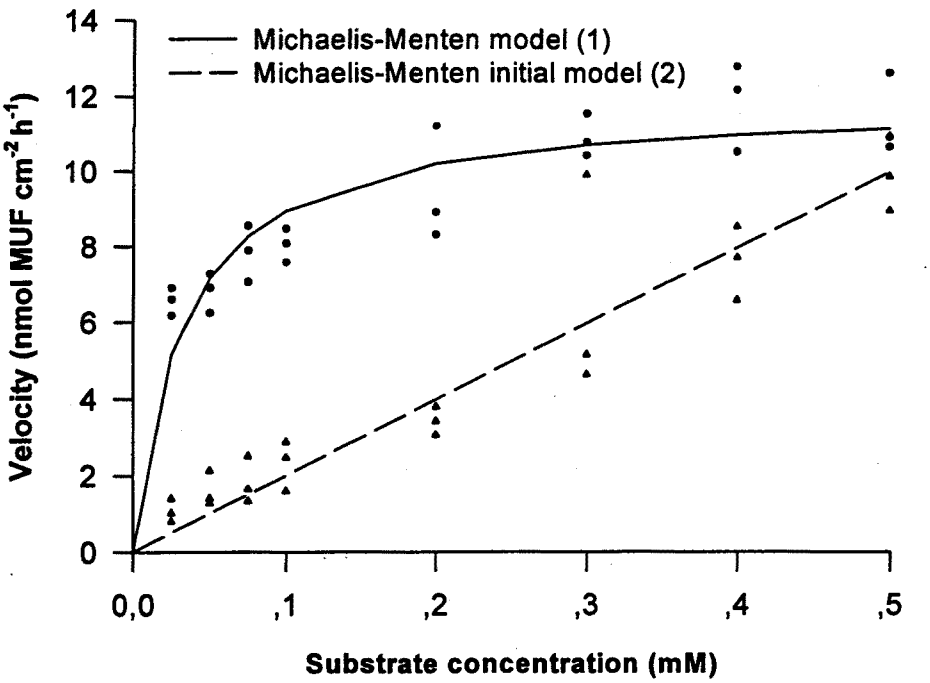


Fig. 1. Kinetic data analysis fits different models (Michaelis-Menten approaches): β-glucosidase activity in Riera Major sand in 22-09-95 (dark circles) fits model 1, β-xylosidase activity in Riera Major tiles in 13-07-95 (dark triangles) fits model 2. Models are described in the text.

Results

For the three ectoenzymes and the three substrates (sand, tiles and cyanobacterial crust), the experimental data fitted the Michaelis-menten approach (model 1) with a mean squared related error, (MSRE)<4 for La Solana and the phosphatase activity in Riera Major tiles (n=27, except n=18 for phosphatase activity in La Solana) and MSRE<0.5 for Riera Major sand (n=27). The curve obtained with the artificial tiles from Riera Major for the β-xylosidase activity in summer was an exception and followed a straight line (MSRE=0.235, Fig. 1, model 2). The test of randomness of the errors was not significant for any of the models obtained ($p>0.1$), indicating that the errors were independent. Therefore, Vmax, Km and Tt were obtained but only the Tt was obtained for the β-xylosidase on tiles in summer (Fig. 2, 3 and 4).

For the β-glucosidase activity, Vmax were higher in La Solana than in Riera Major sand and tiles in all the seasons (Fig. 2) and the lowest values were measured in winter for the two streams. Km values for this enzyme were more similar between streams, ranging between 0.03-0.20 mM, although values were always slightly higher for La Solana than for Riera Major sand. A

higher Km was observed for the tiles in autumn. The low turnover time for β-glucosidase was characteristic of La Solana, with a mean value of 7.5 hours, in contrast to the ca. 24 hours for Riera Major (sand and tiles).

The Vmax for the β-xylosidase activity were higher in La Solana than in Riera Major sand and tiles (Fig. 3). Vmax reached low values in winter for Riera Major sand and La Solana and also in autumn in La Solana. Smaller differences were observed for the β-xylosidase Km values (ranging between 0.08-0.60 mM), which were slightly higher for La Solana. The higher Km were measured in winter in both streams. The turnover time was extremely high in Riera Major sand in winter (390 hours), and was also high on tiles in summer (183 hours). In contrast, the lowest β-xylosidase Tt was measured in La Solana (ca. 24 hours).

Kinetic parameters for phosphatase activity showed greater differences between streams (Fig. 4). The Vmax was clearly higher in La Solana. As observed for the other ectoenzymes, the Km were more similar between streams (ranging between 0.03-0.11mM), but the values were higher for Riera Major sand and tiles than for La Solana. Vmax and Km were lowest in winter in both streams. The turnover time was extremely fast in La Solana (low Tt, ca. 1.1 hours), while very high phosphatase Tt values were measured in Riera Major tiles (ca. 4.6 hours), and even higher in Riera Major sand (ca. 14.5 hours).

Discussion

The Vmax values of β-glucosidase, β-xylosidase, and phosphatase activities in Riera Major sand and tiles and La Solana cyanobacterial crust were similar to the potential activities measured in previous studies (Table 2). However, differences between Vmax for sand and tiles were less evident than those observed in the annual study for the potential enzymatic activities (chapter 3.1).

In spite of the huge differences in Vmax values between streams, less drastic differences between the Km values were observed, indicating that the affinity of these enzymes for the substrate may be rather similar. A relationship between Km values and substrate availability has been described for extracellular enzymes (Fontigny et al. 1987, Chróst and Riemann 1994), indicating that there are fewer differences in substrate availability between streams than in maximal reaction velocities. Possibly, the similar stream size (second-order) and similar DOC concentration in stream water determine the substrate availability in these Mediterranean streams and thus a similar range in the Km values is obtained. Slightly higher Km values (lower affinity) for the polysaccharidic enzymes were characteristic of Riera Major, especially in the sandy substrate, suggesting a major input of cellulosic and hemicellulosic polysaccharides in this stream habitat.

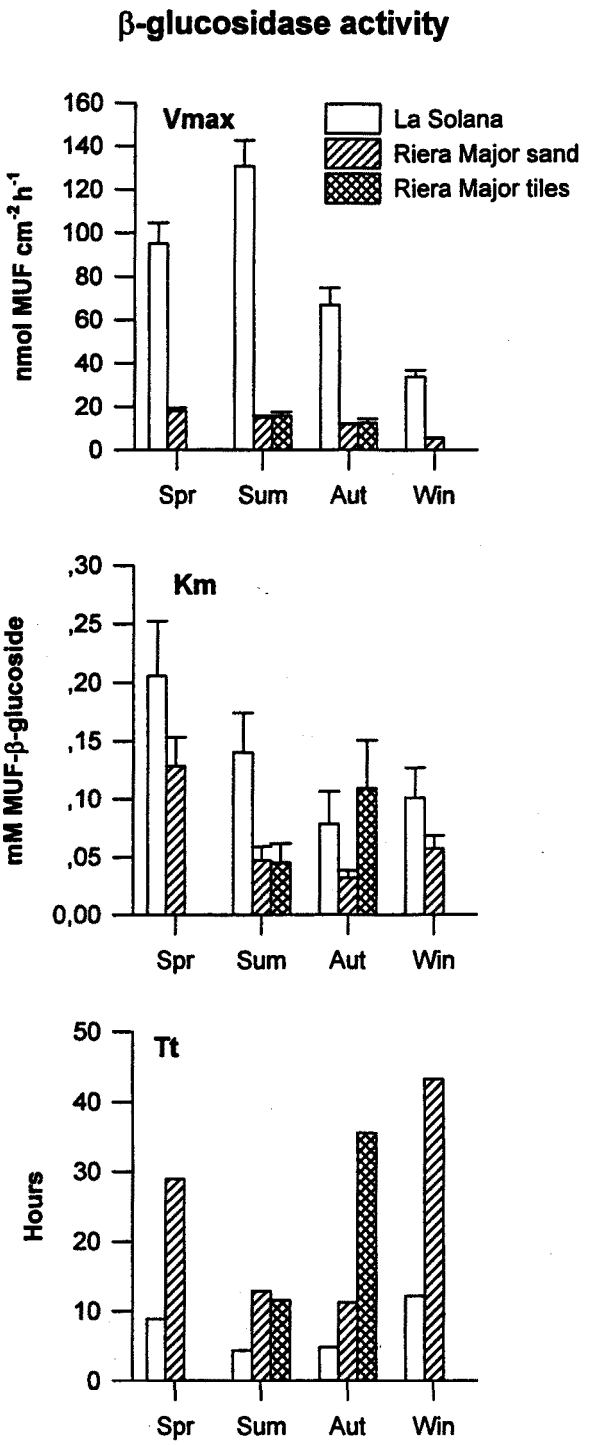


Fig. 2. Seasonal kinetic parameters of β-glucosidase activity in Riera Major sand, Riera Major tiles and La Solana cyanobacterial crust. Maximum velocity (Vmax), Apparent Michaelis constant (Km), Turnover time of substrate hydrolysis (Tt) = Km/Vmax. Mean ± standard errors (vertical bars) obtained after the non-linear regression analyses are shown for Vmax and Km.

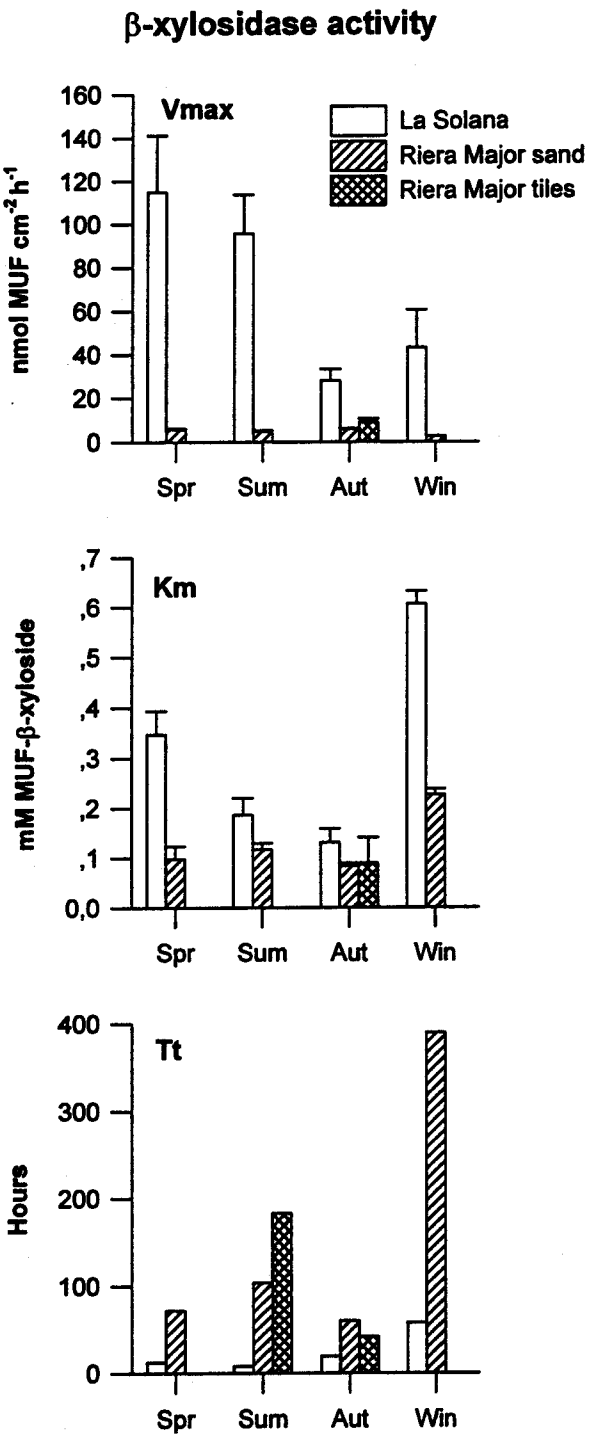


Fig. 3. Seasonal kinetic parameters of β-xylosidase activity in Riera Major sand, Riera Major tiles and La Solana cyanobacterial crust. Maximum velocity (Vmax), Apparent Michaelis constant (Km), Turnover time of substrate hydrolysis (Tt) = Km/Vmax. Mean ± standard errors (vertical bars) obtained after the non-linear regression analyses are shown for Vmax and Km.

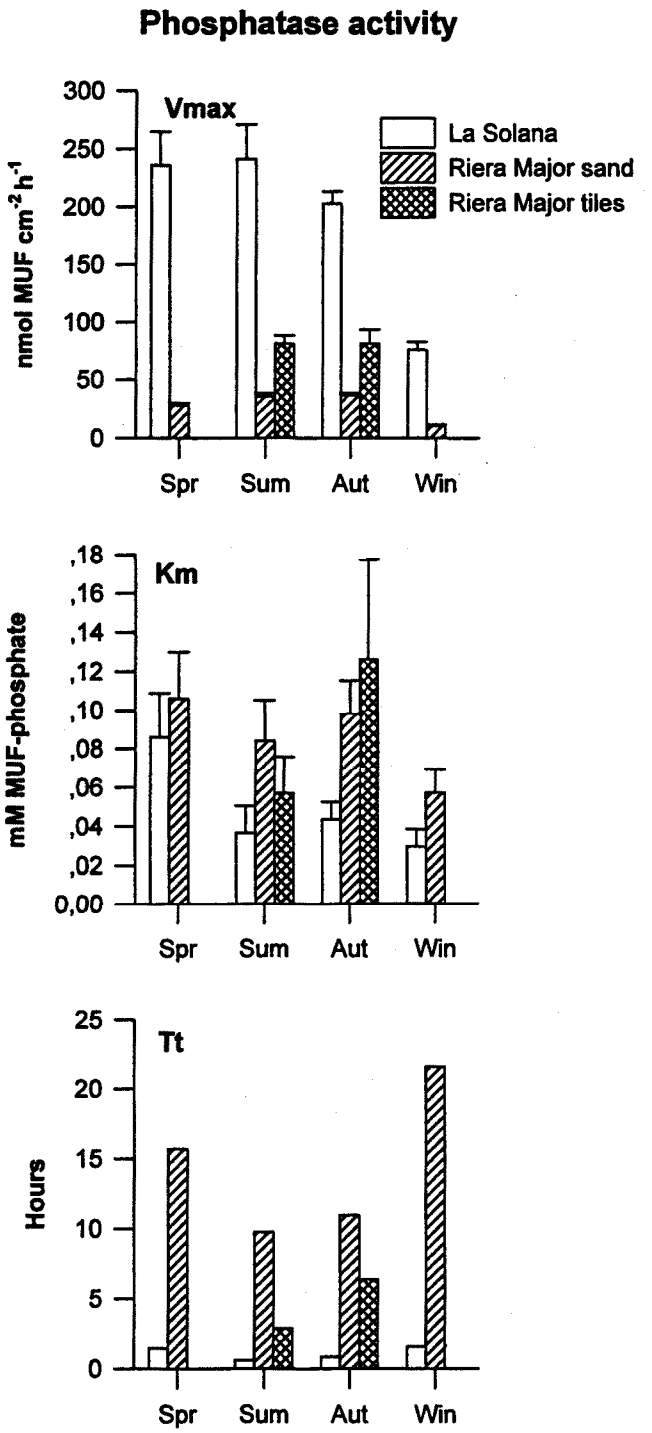


Fig. 4. Seasonal kinetic parameters of Phosphatase activity in Riera Major sand, Riera Major tiles and La Solana cyanobacterial crust. Maximum velocity (Vmax), Apparent Michaelis constant (Km), Turnover time of substrate hydrolysis (Tt) = Km/Vmax. Mean ± standard errors (vertical bars) obtained after the non-linear regression analyses are shown for Vmax and Km.

TABLE 2. Potential ectoenzymatic activities in Riera Major sand and tiles and La Solana cyanobacterial crust summarized from previous studies in both streams during the period January 1994-February 1995. Values are seasonal means (n=3), expressed in nmol MUF cm^{-2} h^{-1}, and standard deviations (in brackets).

	β-glucosidase activity		β-xylosidase activity		Phosphatase activity	
Site and season	Mean	SD	Mean	SD	Mean	SD
Riera Major sand						
Spring	22.7	(8.8)	10.6	(6.2)	26.2	(21.6)
Summmer	26.1	(0.7)	10.9	(2.9)	20.5	(18.8)
Autumn	13.5	(3.8)	6.3	(0.5)	13.3	(16.5)
Winter	6.2	(3.0)	1.9	(0.6)	11.4	(7.3)
Riera Major tiles						
Spring	4.79	(0.80)	1.69	(1.81)	50.48	(51.86)
Summmer	9.85	(7.41)	2.65	(1.35)	21.86	(9.91)
Autumn	5.44	(1.16)	3.63	(0.57)	46.29	(44.44)
Winter	5.18	(1.02)	2.42	(1.24)	45.50	(9.27)
La Solana						
Spring	54.3	(17.3)	26.6	(4.9)	127.2	(22.5)
Summer	91.2	(14.1)	40.9	(24.6)	152.1	(0.92)
Autumn	75.7	(8.6)	61.2	(6.5)	76.3	(60.3)
Winter	41.9	(9.2)	29.1	(11.5)	142.4	(94.1)

The especially low Vmax and high Tt values in the Riera Major sand and tiles indicate a slower cycling of organic matter than in La Solana and suggest that there are differences in substrate lability between streams. Substrates available for enzyme reaction might be more recalcitrant in the forested Riera Major (organic matter accumulated on the sediment along with plant material from the riparian vegetation) than in La Solana, where riparian vegetation is much lower. Cellulose in wood has a high degree of polymerization, therefore limiting the degradation rate by microbial extracellular enzymes (Burns 1983). Moreover, the possible lignin content together with the low nutrient content may also limit decomposition rates (Peters et al. 1987, Benner et al. 1988).

Differences in the kinetic behaviour between sand and tiles in Riera Major were not evident for the polysaccharidic enzymes (Fig. 2 and 3). Even though enzymatic dynamics on the tiles was restricted to only two seasons (summer and autumn), it was obvious that the cleavage of phosphomonoesters was faster and greater on the tiles than on sand (lower turnover time, higher Vmax for the phosphatase activity, Fig. 4). The greater cycling velocity of organic phosphorus compounds on the epilithon may be due to the higher substrate availability for this

enzyme (lower affinity, higher Km, Fig. 4) as well as to the algal contribution to this enzymatic activity (Jansson et al. 1988, Cotner and Wetzel 1992).

However, the lowest Tt and the highest Vmax for the three studied enzymes were recorded in La Solana. This faster cleavage of organic matter may indicate that the heterotrophic organisms utilize more labile compounds than in Riera Major. The relevant activity of primary producers (Guasch and Sabater 1994) in such an open stream possibly provides algal exudates (Fogg 1966) which are easily assimilated carbon sources requiring a much simpler extracellular processing than the structural polysaccharides (Atlas and Bartha 1987) probably available in Riera Major. Algae generally have lower C:N ratios than other primary food sources in streams (Naiman and Sedell 1979, Mann 1988), and are generally digested more efficiently.

A particular characteristic of La Solana ectoenzyme kinetics was the higher substrate affinity (lower Km) of the phosphatase activity, indicating that the enzyme has developed a high affinity for the substrate, especially at low substrate concentration (Button 1991, Cotner and Wetzel 1991). This behaviour may be extremely useful in this stream where concentrations of inorganic phosphorus are low and phosphorus limitation have been described (Guasch et al. 1995, Martí and Sabater 1996). Furthermore, the high Vmax and low Km results in extremely low turnover time (1.1 hours) and therefore a fast recycling for the organic phosphorus compounds in this stream. These results are in agreement with the high potential phosphatase activity measured on the different algal patches of the cyanobacterial crust, especially on the *Rivularia* community (chapter 4.1).

The comparison between ectoenzymatic dynamics in Riera Major and La Solana suggests that differences in the utilization of organic matter in both streams are due to substrate quality (more labile in La Solana, more recalcitrant in Riera Major), rather than to substrate availability (similar range in Km values). The DOC composition (and lability) is a key factor for its bacterial utilization (Middelboe and Sondergaard 1993, Meyer 1994). The major difference in the cycling velocity of the organic matter, faster in the open stream (La Solana) than in the forest stream (Riera Major), is clearly expressed by the turnover time. This parameter (Tt) is possibly the most interesting when studying enzymatic activities since it is the reverse of the initial slope of the Michaelis-Menten curve, reflecting the enzyme velocity at low substrate concentration which is in fact related to the real activity of the microorganisms. The turnover time can be useful for comparing enzymatic activities in different environments.

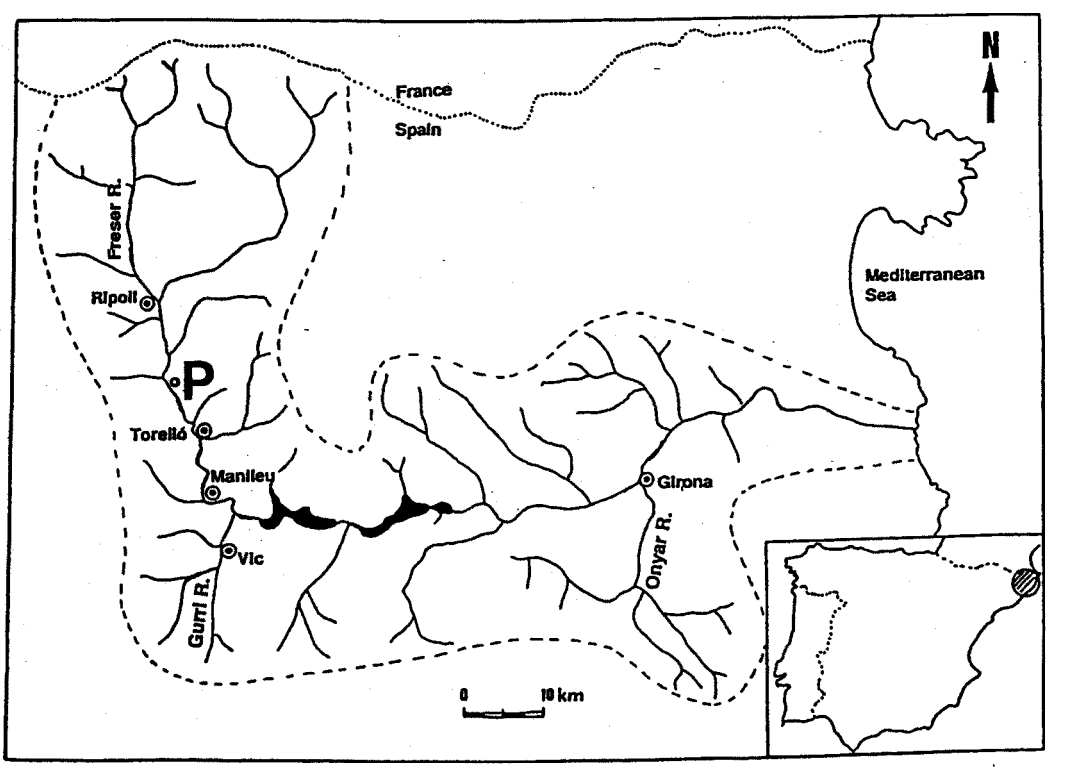
The turnover time was highest for β-xylosidase and lowest for phosphatase. On average Tt for β-xylosidase:β-glucosidase:phosphatase activities were 156:24:14.5 hours in Riera Major sand, 112:24:4.6 hours in tiles and 24:7.5:1.1 hours in La Solana. The highest Tt for the β-xylosidase expresses the slower degradation of hemicellulose which might be related to its high stability (Opsahl and Benner 1993) and complex structure (Atlas and Bartha 1987). The phosphomonoesters, the substrates for the phosphatase activity (Jansson et al. 1988), might be the most labile substrates of the three enzymes since they are rapidly recycled. Turnover times in these Mediterranean streams are extremely fast when compared to those reported and/or calculated from planktonic environments (Münster et al. 1989, Admiraal and Tubbing 1991,

Chróst 1994) which ranged from 10-100 weeks for β-glucosidase and 2-50 days for phosphatase. However, the Tt calculated from these enzymes in small European streams (Marxsen and Witzel 1991, Marxsen and Schimdt 1993, Scholtz and Marxsen 1996) and in periphytic biofilms (Scholtz and Boon 1993) were in the range of those obtained in Riera Major and La Solana. These low Tt express the fast organic matter cycling in the benthic communities of stream environments (Fisher and Likens 1973), which underlies the strategy of minimizing downstream losses (Newbold et al. 1982).

6. THE TER: A NUTRIENT-RICH MEDITERRANEAN RIVER

Study site

The river Ter rises in the eastern Pyrenees and flows 208 km to the Mediterranean sea (see map below). The watershed area is of 3010 Km^2. Its drainage area is mainly calcareous except for the headwaters, which are siliceous. The river Ter catchment is strongly influenced by human activity (agriculture, industry, hydraulic exploitation). Discharge shows an irregular pattern usually increasing in spring (snow melting) and autumn (rain and floods) and drastically decreasing in summer (Sabater et al. 1995). The benthic algal community is mainly composed by diatoms (Sabater and Sabater 1988, Sabater et al. 1992). The sampling point was a riffle zone located in the middle stretch of the river (Ter at Montesquiu, 4th order, see map and picture below) where it receives inputs from some populated areas (Ripoll) and agricultural lands. The altitude of its four order stretch ranges 980-500 m above sea level and has a mean discharge of 9600 L s^{-1}. Nutrient content of the river Ter water is high (709.2 µg L^{-1} nitrate, 3804 µg L^{-1} ammonia, 51.1 µg L^{-1} soluble reactive phosphorus in the study site) (Armengol et al. 1993).



Map of the river Ter and location of the study site (P). The main cities and tributaries are indicated. Inset, location of the river Ter with respect to the Iberian Peninsula.

Picture of the study site in the river Ter.

Temporal dynamics of epilithic ectoenzyme activity in the Ter river

Abstract

The ectoenzymatic activity in the epilithic biofilm of a fourth-order river, the Ter, followed a markedly seasonal pattern, most activities and biomass showing a peak in spring and autumn. Discharge and nutrients were the most important factors for the regulation of the biofilm metabolism. The activity of epilithic ectoenzymes was higher during high discharge periods, which were coupled with higher DOC content in the stream water. The biofilm was characterized by high algal biomass and activity and bacterial density. This was the most important site for the cycling of organic matter, while the water column had rather low activities. In spite of being a nutrient-rich system, the ectoenzymatic activities were not significantly higher in the river Ter than in other streams of lower nutrient content.

Introduction

In undisturbed, low-order streams, benthic bacteria directly utilize autochthonous organic matter compounds (Haack and McFeters 1982b), but allochthonous plant material can also be an important carbon source (Kaplan and Bott 1983). In higher-order streams, the water column can also be a place for organic matter processing and thus, for enzymatic activity (Admiraal and Tubbing 1991), increasing with nutrient content (Chapell and Goulder 1994b). However, for a similar stream order, Mediterranean rivers have lower water discharge and a much shorter water column, the epilithon still playing an important role in energy transfer (Sabater et al. 1995).

The extreme variations in the water regime is characteristic of Mediterranean rivers (Armengol et al. 1991) being strongly linked with the water chemistry (Sabater and Armengol 1986). The distribution of the benthic algal community of the Ter river is related to variations in the mineral content of the water and human influence (Sabater and Sabater 1988, Sabater 1989). Benthic algal biomass is tightly related with fluctuations in discharge (Sabater 1988).

In this chapter, the factors which control the hydrolysis of organic compounds by the epilithic biofilm in a large (4th order) nutrient-rich Mediterranean river are investigated. For this purpose, the activity of the ectoenzymes β-glucosidase, β-xylosidase and phosphatase were analysed monthly in the river Ter during an annual cycle together with the respiratory activity, bacterial density, and the algal activity and biomass. The study was planned in order to cover the annual variation of the physical and chemical parameters. Related to the chemical variability and nutrient input in this Mediterranean river, two questions were formulated: first, do the seasonal variations affect the epilithic ectoenzyme dynamics?, and second, does such a nutrient-rich environment impose especially high epilithic ectoenzyme activities? Results are

also compared to other Mediterranean streams of lower order and nutrient-poor waters, Riera Major and La Solana (chapter 3.1 and chapter 4.1).

Materials and methods

Sampling

Field samples were taken at monthly intervals from March 1994 to February 1995, excepting November 1994 and January 1995, when the access to the sampling point was difficulted because of high floods. Artificial substrates (clay tiles, 0.64 cm^2 surface area), which were glued on stream boulders and placed in the streambed six-to-eight weeks before sampling (chapter 3.1), were collected as epilithic biofilm samples. Tiles were placed in sterile glass vials with stream water and maintained cold (on ice), in the dark, during transport. Artificial substrates for bacterial counts were collected in sterile glass vials and fixed with 2% formalin. River water samples were collected on December 1994 to evaluate the ectoenzymatic activity in the water column. All measurements of ectoenzymatic, respiratory and photosynthetic activity were performed in the laboratory, two to three hours after sampling.

At each sampling date, incident light, temperature, pH, dissolved oxygen and conductivity were measured in the field. Filtered water samples were taken in order to analyse inorganic nutrients (nitrate, ammonium and soluble reactive phosphorus), as well as dissolved organic carbon (DOC) and dissolved inorganic carbon (DIC) following the procedures described in chapter 2.

Metabolism measurements

Extracellular β-D-glucosidase, β-D-xylosidase, and phosphatase potential activities were determined in tiles (five replicates) and formaldehyde-killed controls (two replicates). To analyse the ectoenzymatic activities in the river water (December 1994), 10 ml of river water was incubated with each MUF-substrate to a final concentration of 0.3 mM. Two blanks of filter-sterilized stream water were also incubated for each enzyme. The Electron Transport System (ETS) activity was measured using five replicate tiles and two controls. Primary production was measured in tiles using five replicates, two killed-control tiles and two dark-incubated samples. All measurements were determined following the procedures described in chapter 2.

Algal biomass and bacterial density

Chlorophyll-a was extracted with 90% acetone using three replicates (chapter 2). The ratio of chlorophyll to carotenoids and/or chlorophyll degradation products (OD430/OD665 ratio) was also measured. Algal composition and community structure was determined under light microscopy. Bacterial density was estimated in triplicate after sonication (90 s), dilution and DAPI stain (chapter 2). The different bacterial morphotypes were also observed.

Data analyses

Differences between sampling dates for each variable were analyzed using a one-way analysis of variance (ANOVA). Correlation analyses (Pearson coefficient) were performed with biological and environmental variables.

Results

The physical and chemical characteristics of the river Ter at Montesquiu changed remarkably between seasons (Table 1). Incident light was maximum in spring but water temperature was maximum in summer. In this period conductivity and dissolved inorganic nitrogen were also maximal while discharge and DOC were very low. DOC concentration was maximum in autumn. The lowest water temperature and highest oxygen content were measured in winter. Discharge and DOC were significantly correlated when all measurements were considered ($r=0.66$, $p=0.048$, $n=10$).

TABLE 1. Physical and chemical characteristics of the river Ter. Values are seasonal means (n=3) and standard deviations for the study period (1994-1995), except discharge values obtained from daily measurements. The annual mean and standard deviation is also shown.

	Spring 1994	Summer 1994	Autumn 1994	Winter 1995	mean (SD)
Temperature (°C)	13.6 (4.4)	21.1 (0.1)	9.6 (3.7)	5.9 (2.6)	11.57 (6.34)
Light ($\mu mol\ m^{-2}s^{-1}$)	1951 (499)	953 (145)	277.5 (84.9)	665 (177)	1071 (749.8)
Discharge (Ls^{-1})	3942 (1786)	932 (1156)	12840 (21607)	2524 (1906)	9569 (10070)
Cond. ($\mu S\ cm^{-1}$)	291.3 (27.3)	333.5 (4.9)	294.0 (25.9)	321.0 (9.9)	310.3 (27.5)
pH	7.9 (0.5)	8.2 (0.1)	8.1 (0.4)	8.5 (0.04)	8.16 (0.36)
Oxygen ($mg\ L^{-1}$)	9.4 (1.3)	6.5 (0.1)	9.2 (1.8)	11.4 (1.9)	9.20 (1.95)
NO_3-N ($\mu g\ L^{-1}$)	205.9 (244)	1396.1 (287)	824.2 (93)	604.4 (115)	709.2 (471)
NH_4-N ($\mu g\ L^{-1}$)	92.4 (123.7)	17711 (7101)	140 (58.7)	102.4 (60.4)	3804 (7768)
SRP ($\mu g\ L^{-1}$)	89.1 (32.8)	77.3 (49.7)	12.9 (4.2)	25.0 (29.6)	51.04 (43.03)
DIN:SRP	7.7 (8.9)	5607.8 (1569)	154.2 (50.4)	238.8 (297)	1218 (2376)
DOC ($mg\ L^{-1}$)	4.9 (5.4)	2.5 (0.8)	9.7 (5.6)	5.9 (2.0)	6.26 (6.31)
DIC ($mg\ L^{-1}$)	27.9 (7.5)	25.3 (0.8)	26.3 (2.2)	28.3 (6.3)	26.6 (3.7)

The epilithic bacterial community was characterized by different bacterial morphotypes (Fig. 1). In spring and summer ca. 90% were small cocci (0.2-0.4 µm diameter) and coccobacilli (0.5-0.8 µm long), the rest being larger rod-shaped bacteria and chains. The proportion of small cocci diminishes to ca. 80% in autumn and winter, because of the increase of large rod-shaped bacteria (2.5 µm long) and thin filaments (0.3-0.4 µm width, 6 µm long). Bacterial density was

higher in spring and autumn than in summer and winter (ANOVA, $p<0.0001$, Fig. 2a). Maximum bacterial density was measured in May ($5.5\ 10^{10}$ cell cm^{-2}).

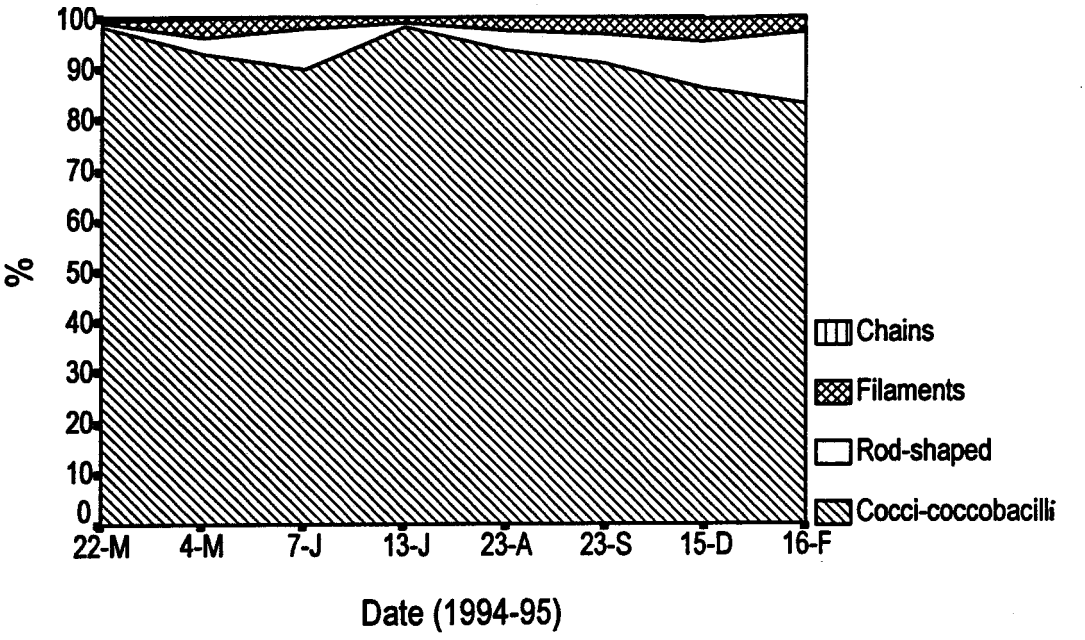


Fig. 1. Relative abundance of the different bacterial morphotypes in the river Ter epilithic biofilm during the study period.

The benthic algal community was mainly composed by diatoms (*Nitzschia* sp., *Cymbella* sp., *Achnanthes* sp., *Navicula* sp., *Diatoma* sp., *Gomphonema* sp., *Cocconeis pediculus* Ehr., *Rhoicosphenia curvata* (Kütz) Grun. and by the green alga *Cladophora glomerata* (Linn.) Kütz. Cyanobacterial chains, occasionally covered by mucilagenous material, were characteristic of the summer benthic community. Benthic chlorophyll-a density was 30 μg cm^{-2} in annual average. Drastic changes of chlorophyll-a were observed during spring and summer being significantly higher in March and July than in June and August (ANOVA, $p=0.0035$, Fig. 2b).

The seasonal pattern was a characteristic of the epilithic biofilm activities (Fig. 2c, d, e, f). Respiratory activity (ETS, Fig. 2c) showed significant differences between sampling dates (ANOVA, $p=0.0063$), in spite of being highly variable. ETS values in March were higher than in July and August. ETS activity was positively correlated to bacterial density, and negatively to nitrate concentration (Table 2).

β-glucosidase and β-xylosidase activities were also higher in spring and autumn than in summer and winter (Fig. 2d and e) (ANOVA, $p<0.0001$, $p=0.0001$, respectively). β-glucosidase was significantly correlated with ETS and discharge, while β-xylosidase was only significantly correlated with the OD430/665 ratio (Table 2).

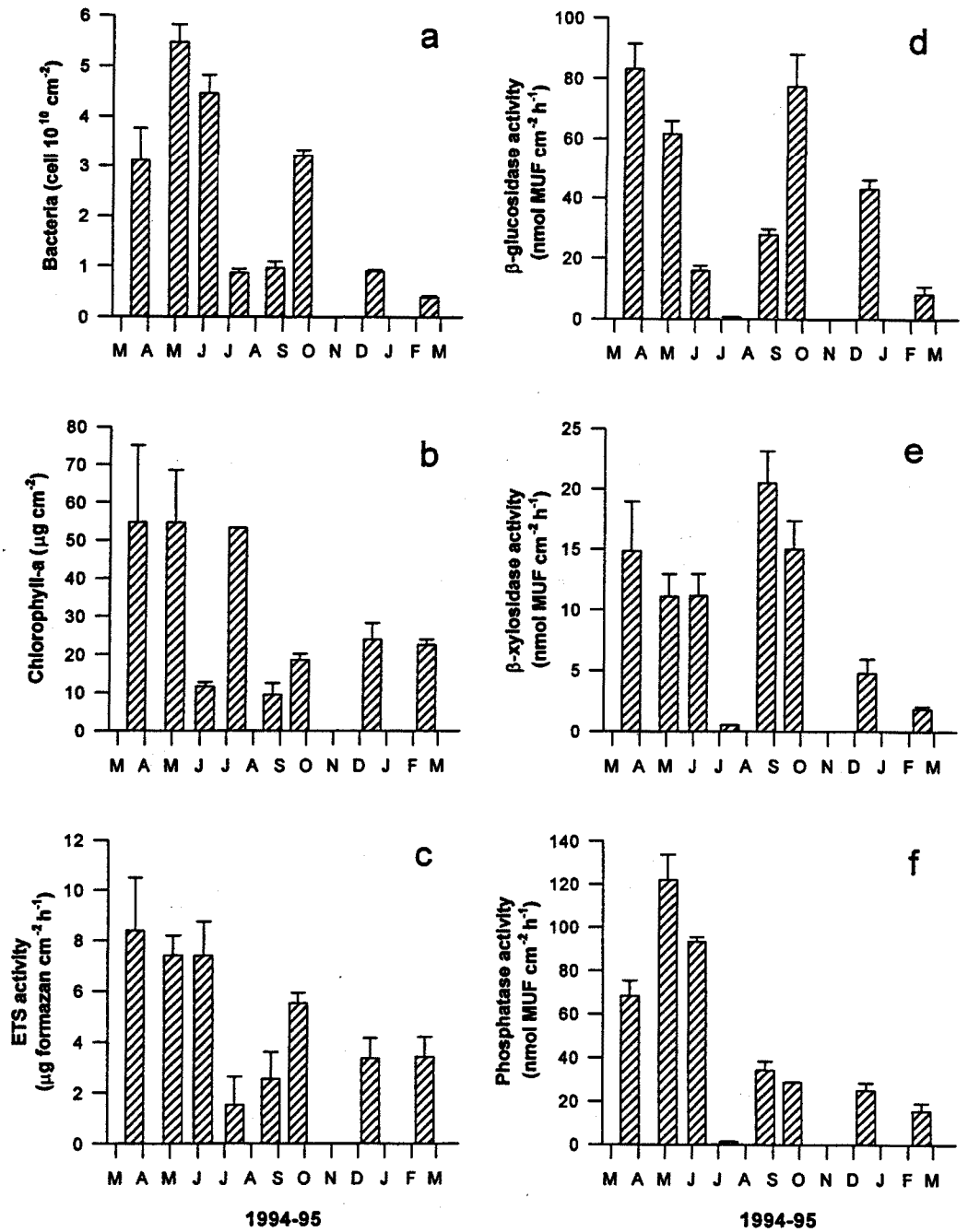


Fig. 2. Temporal variation of epilithic activities and biomass in the river Ter, March 1994-February 1995: (a) bacterial cell density, (b) chlorophyll-a density, (c) respiratory (ETS) activity, (d) β-glucosidase activity, (e) β-xylosidase activity, and (f) phosphatase activity. Means ± standard errors (vertical lines) are shown, n=3 in a and b, n=5 in c, d, e and f.

Phosphatase activity was higher in spring than in the rest of the study period (Fig. 2f) (ANOVA, $p<0.0001$). The activity of this enzyme was significantly correlated to bacterial density, ETS and ambient irradiance, and negatively correlated to nitrate concentration and conductivity (Table 2).

TABLE 2. Significant Pearson coefficents of correlation between epilithic activities and environmental variables in the Ter river. Significance level is indicated as: *<0.05, **<0.01.

	Bacteria	ETS	OD430/665	NO_3	Cond.	Disch.	Light
β-glucosidase						0.89**	
β-xylosidase			0.88**				
Phosphatase	0.91**	0.83**		-0.75*	-0.72*		0.78*
ETS	0.86**			-0.89**			

Photosynthetic activity was ca. 3.7 μgC cm^{-2} h^{-1} over the study period, except for a peak in September (Fig. 3) (ANOVA, $p<0.0001$). Dark ^{14}C incorporation was rather steady (no significant differences, ANOVA, $p=0.41$) being 1.35 μgC cm^{-2} h^{-1} in annual average. Dark incorporation in August was slightly higher than light incorporation.

Discussion

Epilithic bacterial density and algal chlorophyll-a were high in the river Ter at Montesquiu, indicating the structural importance of the epilithon (Sabater and Sabater 1992). The epilithic bacterial density was higher than in other stream biofilms of similar order (Blenkinsopp et al. 1991, Jones and Lock 1993, Chapell and Goulder 1994a). Chlorophyll-a density and photosynthetic activity on the epilithon were also high when compared to other stream biofilms (Lock 1981, Bott et al. 1985, Sabater S 1988). The role of the benthic habitat as the main site for energy transducing in this Mediterranean river is indicated by the low ectoenzymatic activity measured in the water column (1.5 nmol l^{-1} h^{-1} for β-glucosidase, 43.4 nmol l^{-1} h^{-1} for β-xylosidase and 51 nmol $l^{-1}h^{-1}$ for phosphatase, mean values, n=5, in December 1995). These values are lower than those reported for the water column of rivers of a similar order but of different water regime (Admiraal and Tubbing 1991, Chapell and Goulder 1995).

The epilithic ectoenzymatic activities in the Ter were similar to those observed in other oligotrophic, low-order tributaries of this river (chapter 3 and 4) and to other analogous streams (Chapell and Goulder 1994a). This is in apparent contradiction with the hypothesis that

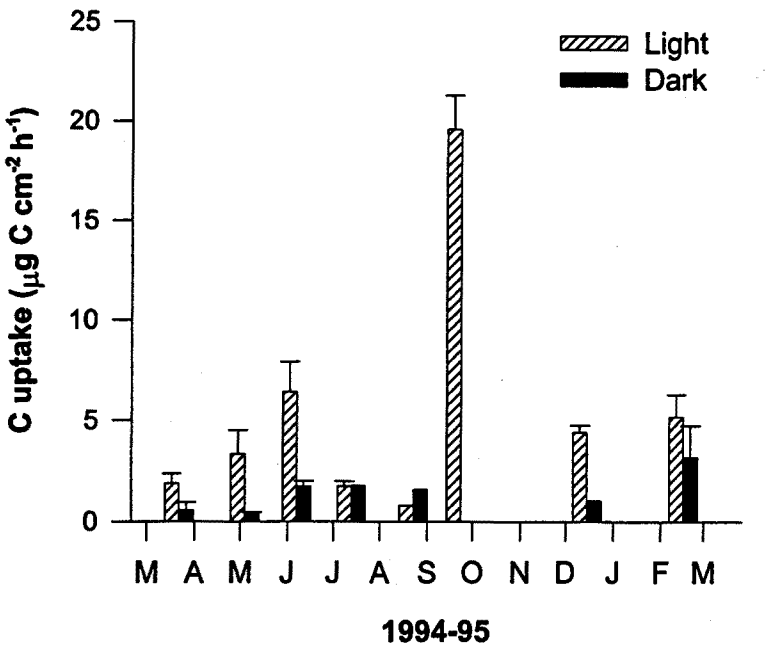


Fig. 3. Temporal variation of light and dark ^{14}C-HCO_3 incorporation in the epilithic biofilm in the river Ter, March 1994-February 1995. Means ± standard errors (vertical lines) are shown, n=5 (n=2 in the dark).

activity would be higher in this river of higher dissolved organic matter and nutrient content (Table 1). It is likely that a higher availability of readily assimilable organic substrates would make unnecessary higher rates of organic matter cleavage by microbial ectoenzymes (Chróst 1991b), this being related to the higher benthic algal biomass and/or to the nature of the organic matter transported according to the higher river size (Naiman and Sedell 1981, Naiman et al. 1987). In a higher-order river, the importance for the allochthonous input (leaves, plant material) decreases in relation to autochthonous production (Naiman and Sedell 1979, Naiman et al. 1987), which may provide easily assimilable polysaccharides for bacteria. Higher-order rivers have a higher processing efficiency of organic matter (Newbold et al. 1982). Although a higher proportion of fine particulate organic matter content has been postulated for higher-order rivers (Vannote 1980, Minshall et al. 1983, González and Pozo 1997), readily metabolized compound would be less abundant (Minshall et al. 1983, Amon and Benner 1996) and therefore may play a less relevant role in ectoenzymatic regulation than epilithic algae. Nevertheless, ectoenzymatic epilithic activities in the river Ter were higher than those measured in epilithic biofilms from other fourth-order streams (Sinsabaugh and Linkins 1988, Jones and Lock 1993), which were possibly inhibited by the presence of humic materials (Freeman and Lock 1992).

A clear seasonal pattern was characteristic in all activities and biomass measurements in the river Ter epilithon. Maxima occurred in April-May and September-October and minima in July (Fig. 2 and 3). These variations seem to be strongly influenced by the physico-chemical parameters of the river water. Physical and chemical factors, such as discharge, nitrate content, conductivity and light do follow seasonal variations which are analogous with those of β-glucosidase, phosphatase and respiratory activities. The higher β-glucosidase activity in the river biofilm (spring and autumn) coincided with the high discharge periods of the river Ter (Table 2). These periods could be favourable for the biofilm metabolism due to the greater transport of DOC. A similar positive relationship to discharge is suggested for phosphatase activity. The activity of this ectoenzyme is negatively correlated to conductivity (which, in turn, is negatively correlated with discharge). A significant correlation between β-glucosidase and phosphatase activities with discharge was observed in the water column of the Danube river and the utilization of the introduced allochthonous organic and inorganic nutrients was suggested (Hoch et al. 1996). Although no significant correlation between β-xylosidase activity and discharge (or any related variable) was found (Table 2), it followed a similar time pattern to the β-glucosidase activity (Fig. 2) therefore suggesting that this enzyme is also influenced by the changes in water flow and in general by the variations of the physico-chemical parameters. However, β-xylosidase activity could be also related to the input of compounds from decaying algae since it shows a positive correlation to the OD430/OD665 ratio (indicative of the presence of chlorophyll degradation products, Margalef 1983, Table 2).

The drastic decrease in the biofilm activity and biomass in July could be related to the extremely dry conditions during that month. The scarcity of rainfall (25.5 mm in total) was responsible for the drying out of some tributaries of the river Ter (e.g. chapter 4.2) and caused a descent of water flow in the Ter (reaching ca. 400 L s^{-1}). Variations in discharge influence the water chemistry and the dynamics of the benthic community (Armengol et al. 1991) and algal biomass (Sabater 1989, Sabater and Sabater 1992) in the river Ter. The results of this study indicate that physico-chemical parameters, especially discharge, also influences the activities of epilithic ectoenzymes. Therefore, discharge should be taken as a key parameter when studying the organic matter degradation capacity of the biofilm in larger rivers. These observations indicate that an increase in the potential enzymatic activity should be expected when increasing the water discharge. However, a possible exception to this behaviour would be during the high flood periods. Because there is a decrease in benthic biomass in these periods (Biggs and Close 1989, Biggs and Thomsen 1995), which could be remarkable in Mediterranean rivers (Sabater and Sabater 1992), it is likely that ectoenzymatic activities would be minimized rather than enhanced. This response is, however, hypothetical and specific research should be planned to clarify its validity.