Heterotrophic and autotrophic metabolism in Mediterranean streams

Anna M. Romaní i Cornet

Universitat de Barcelona
Facultat de Biologia
Departament d'Ecologia

HETEROTROPHIC AND AUTOTROPHIC METABOLISM IN MEDITERRANEAN STREAMS

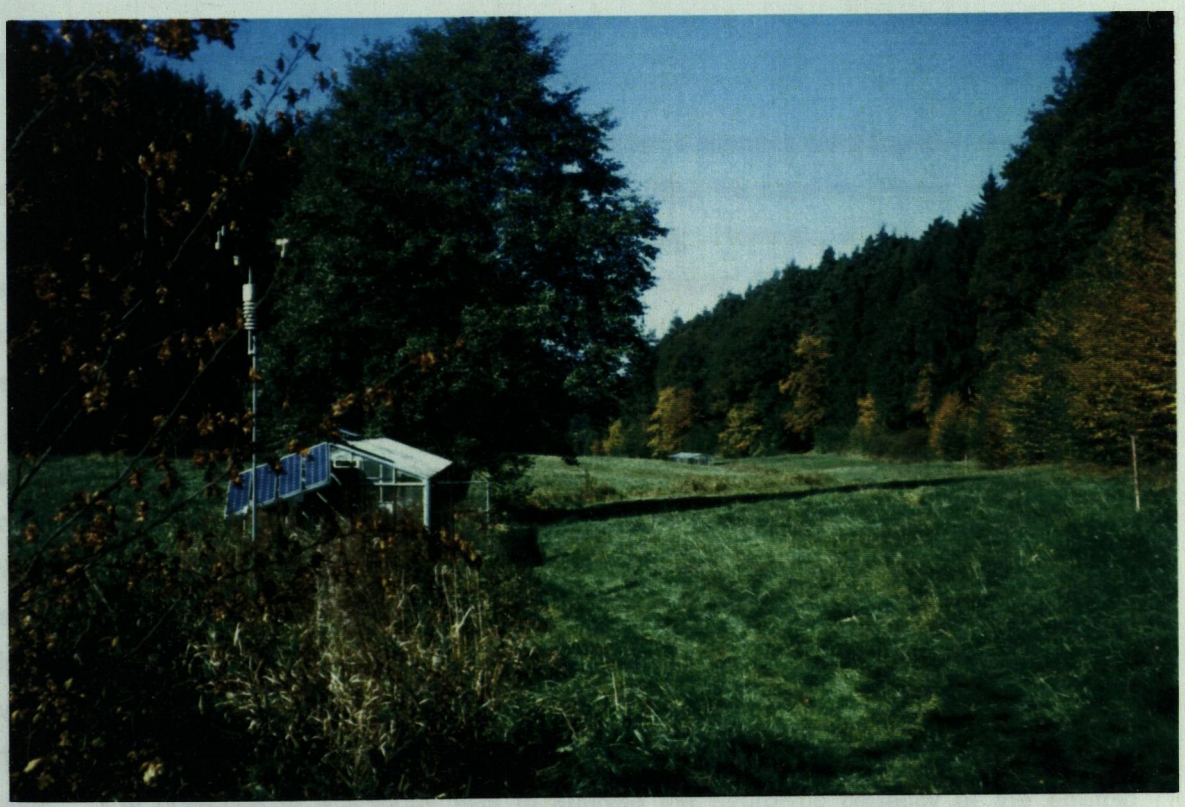
Ph. D. Thesis

Anna M. Romaní i Cornet

7. THE BREITENBACH: A CENTRAL EUROPEAN MOUNTAIN STREAM

Study site

The Breitenbach is an unpolluted first-order stream in Hesse (West Germany), 100 km north-east of Frankfurt (51°N) which rises about 350 m a.s.l. in woodland (see Fig. 7 in chapter 1). The stream is 4.2 Km long and flows into the river Fulda. The streambed surface area is 3173 m^2. The mid to lower reaches (studied here) flow predominantly through grassland with short shadier reaches flanked by alder (*Alnus glutinosa*), willow (*Salix*) and hazel (*Corylus avellana*) (Cox, 1990a). The stream catchment is forested by *Fagus sylvatica* and *Pinus sylvestris.* The catchment geology is dominated by bunter sandstone resulting in low ion concentration in stream water (conductivity 140-190 $\mu S\ cm^{-1}$, Ca^{2+} 16-18 mg L^{-1}, Mg^{2+} 2-5 mg L^{-1}) (Marxsen et al. 1997). Mean discharge was 26 L s^{-1}. The average pH of the stream was 7.1 with low levels of dissolved nutrients (SRP 20-45 $\mu g\ L^{-1}$, NO_3-N 600-1300 $\mu g\ L^{-1}$) (Marxsen et al. 1997).



Study site in the Breitenbach.

Ectoenzymatic activities in epilithic biofilms of the Breitenbach

Abstract

The activity of the extracellular enzymes β-glucosidase, β-xylosidase, phosphatase, and aminopeptidase were measured on the epilithic biofilms of the Breitenbach. Vmax values for the four enzymes were higher in the open, higher water velocity site (A), than in the open, low water velocity site (B) and the forested site (C). The higher algal biomass accumulated at site A might provide a higher substrate availability enhancing the hydrolytic capacity of the heterotrophs. At site C the high polysaccharide degradation capacity might result from the important input of leaf fall during the study period (October-November 1995). On average, turnover times of substrate hydrolysis (Tt) for the four enzymes were from highest to lowest: 80 hours (β-xylosidase), 19 hours (phosphatase), 17 hours (β-glucosidase) and 5 hours (aminopeptidase) expressing the slow recycling of hemicellulosic compounds in contrast to the fast utilization of proteinaceous compounds. The Tt for the phosphatase activity was higher than that measured for Mediterranean streams.

Introduction

High extracellular enzymatic activity and bacterial biomass were found in sediments of the Breitenbach (Marxsen 1988, Marxsen and Witzel 1990, Marxsen and Fiebig 1993), since it is an important habitat for organic matter processing. However, in some stretches the streambed of the Breitenbach is covered with small stones. Extracellular enzymatic activity may also occur on the stones also contributing to the degradation of the organic matter of the stream. This study focus on the epilithic ectoenzymatic activities of the Breitenbach.

Several environmental parameters affect the biomass accrual and activity of epilithic stream biofilms. Variations in the water velocity can influence biomass accumulation as well as nutrient uptake rate by the epilithic organisms especially in oligotrophic streams (Horner and Welch 1981, Stevenson 1984). Epilithic biomass and growth could also be influenced by light availability (Hill 1996), which would determine the availability of autochthonous organic matter for the heterotrophs. The input of allochthonous organic matter in low-order forest streams varies with the season becoming maximum during the leaf fall period (McDowell and Fisher 1976).

The objective of this study was to analyse the hydrolytic capacity of the epilithic community in this small unpolluted central European stream. To cover the different habitats found in this stream, and the possible diversity in organic matter input, three different sites, which differ in light, current velocity and allochthonous input, were investigated. The study period (autumn) involved a great input of leaves, especially at the forest site.

The extracellular enzymes β-glucosidase, β-xylosidase, leucine-aminopeptidase, and phosphatase which are involved respectively in the degradation of polysaccharides, proteins

and organic phosphorus compounds were measured during autumn 1995. Vmax (maximal velocity of the enzyme), Km (apparent Michaelis constant) and Tt (turnover time of substrate hydrolysis) were obtained for each enzyme by the kinetic approach (chapter 5). The epilithic community was studied by the use of artificial substrates (clay tiles, Sabater and Romaní 1996).

Materials and Methods

Sampling

Epilithic biofilm samples from the Breitenbach at its mid reach were collected in October 1995. Artificial substrates (clay tiles, 0.64 cm^2 surface area, 1 cm high), 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. Three sites (A, B and C) located in the same stream stretch (Fig.1) which differ in water velocity (Schiltknecht currentmeter, Table 1) and light availability were considered.

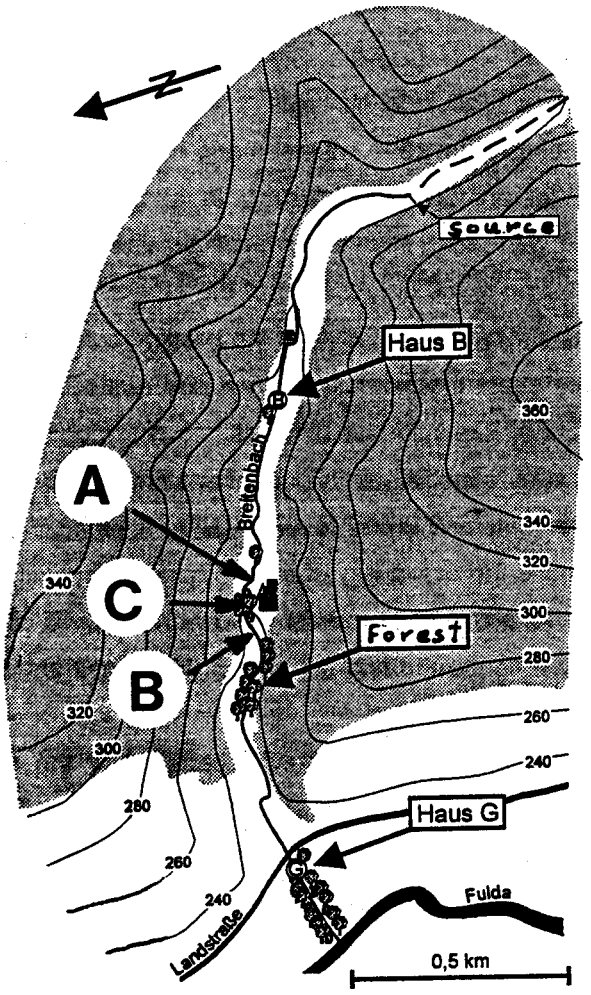


Fig. 1. Map of the Breitenbach and the sampling sites.

Site A was open and with a mean water velocity of 0.219 m s^{-1} (Table 1). Site B was also open but water velocity was the lowest (Table 1). Site C was covered by riparian canopy; current velocity was between that of site A and B but highly variable (Table 1). The experiment was carried out in autumn 1995 and therefore there was a considerable input of leaves to the streambed. Tiles from the three sampling sites were collected for enzymatic activity, chlorophyll-a analysis and bacterial counting. All activity measurements were performed 15-30 minutes after sampling.

TABLE 1. Current velocity and canopy of the Breitenbach at each sample site. Current values are means and standard deviations (in brackets) (n=10).

Site	Current (m s^{-1})	Canopy
A	0.219 (0.055)	open
B	0.125 (0.035)	open
C	0.191 (0.091)	forested

Enzyme assays

Enzymatic activities were determined using MUF (4-methyl-umbelliferyl)-substrate analogues (from Sigma) for the measurement of β-glucosidase, β-xylosidase, and phosphatase, and Leucine-MCA (L-leucine-4-methyl-coumarinyl-7-amide from Calbiochem) for the measurement of leucine-aminopeptidase. Tiles were incubated in a shaking bath at natural stream temperature (8°C) in the dark for two hours. For each enzyme determination, 4 ml of fluorogenic substrate at 0.01, 0.025, 0.05, 0.1, 0.2, 0.3, 0.5 mM concentration was added to each tile. A longer incubation period (2 h) and higher volume of incubated MUF-substrate solution (4 ml) were used here than for the Mediterranean streams, since a lower activity level was suspected in these biofilms from such a small, oligotrophic, central European stream. Four replicates and one blank were considered for each concentration and each site. Filtered sterilized stream water (0.2 μm, Sartorius) was used for dilutions of fluorogenic substrates. Reaction was stopped by adding 1 ml of 0.05 glycine buffer pH 10.5 to each tube. The fluorescent product (MUF or MCA) released by enzyme activity was measured using a Kontron SFM25 spectrofluorometer at 455 nm emission under 365 nm excitation. Quantification was achieved by calibrating the spectrofluorometer with a standard alkaline solution of MUF or AMC. Enzymatic kinetic parameters, Vmax (maximal reaction velocity) and Km (apparent Michaelis constant), were calculated by non-linear regression analysis using the Enzfitter program for the PC, version 1.05 (Leatherbarrow 1987). The turnover time (Km/Vmax ratio) was also calculated after transformation of the parameters to the same units.

Algal biomass, bacterial density, and SEM observations

Chlorophyll-a was extracted from the tiles (10 replicates for each site) following the procedures described in chapter 2. Absorbance was measured in a Kontron spectrophotometer (Uvikon 810). The ratio of chlorophyll to carotenoids and/or chlorophyll degradation products (OD430/OD665 ratio, Margalef 1983) was also calculated. The algal composition was determined under optical microscope after sonication of the tiles (120 sec).

Tiles for bacterial counting were preserved in 2% formalin until the enumeration assay. Bacterial enumeration was achieved by direct counting of each tile (5 replicates for each site) following the procedures described in chapter 2. Samples were counted on a fluorescence microscope (Polyvar) under 1250 magnification. Samples for SEM observations were fixed with 2.5% gluteraldehyde in phosphate buffer pH 7.5 and stored in the dark until observations were made. Sample preparation for the SEM is also described in chapter 2.

The possible differences in chlorophyll-a and bacterial cell density between the three sampling sites were analysed using an analysis of variance (ANOVA).

Results

Mainly diatoms of the genera *Navicula*, *Achnanthes*, *Gomphonema*, *Cymbella* and *Diatoma* composed the algal community of the epilithic biofilms growing on the artificial clay tiles in the Breitenbach (Fig. 2). The epilithic bacteria were diverse in their morphologies (Fig. 3). 80-85% of the bacterial cells were small cocci and coccobacilli (0.4-1.2 μm diameter), 9-13% were rod-shaped bacteria (2-3 μm length) and 1.5-4% were filaments (4-8 μm length). Filamentous cyanobacteria were also observed under the fluorescence microscope.

Chlorophyll-a density was significantly higher for the epilithic biofilms from site A than from sites B and C (ANOVA, $p<0.00001$, Fig. 4a), being not significantly different between sites B and C (ANOVA, $p=0.62$). The OD430/OD665 ratio was around 2 for the three sites (Fig. 4a).

Bacterial density was on average 6.5 10^7 cell cm^{-2} (Fig. 4b) and no significant differences were found between the three sampling sites (ANOVA, $p=0.27$).

β-glucosidase activity was similar at sites A and C but a higher affinity for the substrate (lower Km) was observed at site C (Fig. 5a). At site B, the lowest Km (highest affinity for the substrate) and lowest Vmax were measured for β-glucosidase. The turnover time of substrate hydrolysis was 17.4 hours on average being highest at site A (32.1 h, Fig. 6).

For the β-xylosidase activity, the highest Vmax and Km values were measured at site A. The values at sites B and C were lower and similar to each other (Fig. 5b). The Tt was 79.9 hours on average being higher at sites A (116.3 h) and C (80.72 h) than at site B (Fig.6). Tt for this enzyme was the highest (the slowest in recycling).

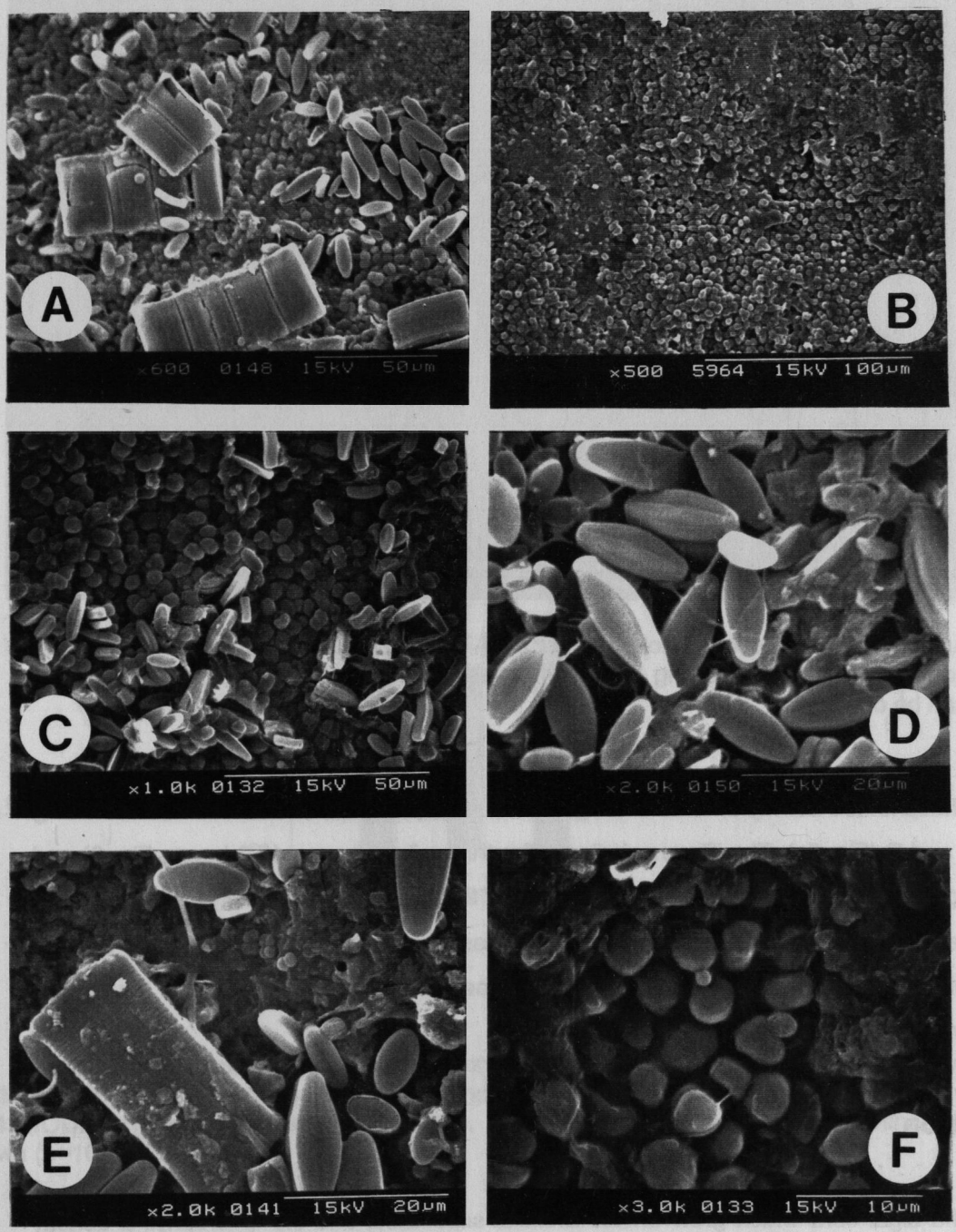


Fig. 2. SEM photographs of the colonized ceramic tiles in the Breitenbach. a) Diatom cells (*Diatoma*, *Achnanthes*) and bacteria which composed the epilithic biofilm, b) Some areas of the tiles were totally covered by bacteria cells, c) General view of bacterial and algal community. Pennate diatoms (genera *Cymbella*, *Gomphonema*, *Navicula*, *Achnanthes*) were the majority of the diatoms observed, d) and e) The diatoms were covered with filaments and mucilagenous material, f) Approach to the bacterial community.

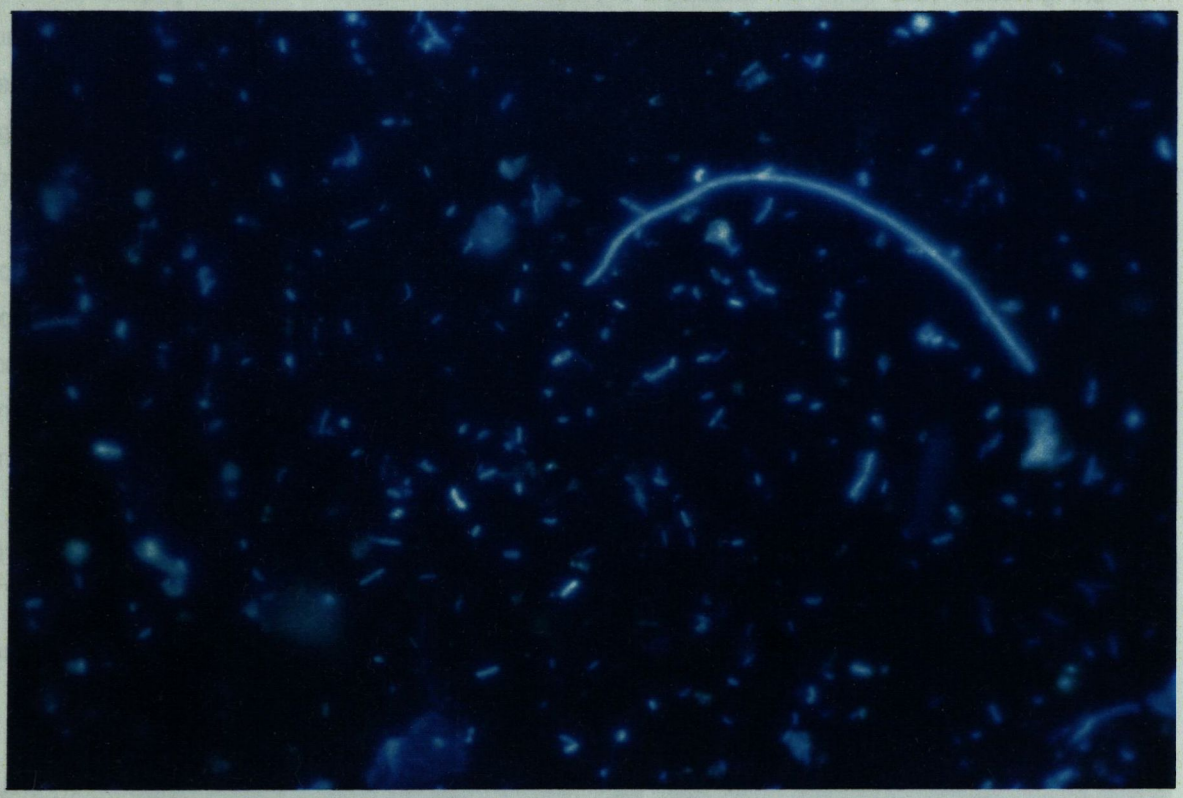


Fig. 3. Fluorescence microscope photograph of a DAPI stained bacterial preparation from the Breitenbach epilithic biofilm (growing on tiles). A great diversity in shapes of the rod-shaped bacteria can be observed.

Phosphatase activity showed the highest values of Vmax and Km at site A, decreasing to sites B and C (Fig. 5c). The Tt was similar at the three sites (mean= 19.3 hours, Fig. 6) and slightly higher (slower recycling) than the mean Tt for the β-glucosidase activity.

Leucine-aminopeptidase activity (Fig. 5d) was higher at site A than B or C, the Km being similar at the three sites. The Tt for this enzyme was the lowest (mean= 4.9 hours, Fig. 6) and similar at the three sites.

Discussion

The algal flora which grew on the artificial substrates, and the chlorophyll-a density accumulated, were similar to those observed on natural stones of the Breitenbach (Cox 1990a and b). The bacterial density on the epilithic biofilms was also similar to values obtained from this stream (Freeman et al. 1993). Therefore, the clay tiles were shown to be reliable for allowing the colonization of the epilithic biofilm in the Breitenbach.

The epilithic ectoenzymes in the Breitenbach were similar to values reported from epilithic biofilms in low-order streams (Chapell and Goulder 1994a and 1994b) but slightly higher than those obtained from dark grown biofilms (Freeman et al. 1993).

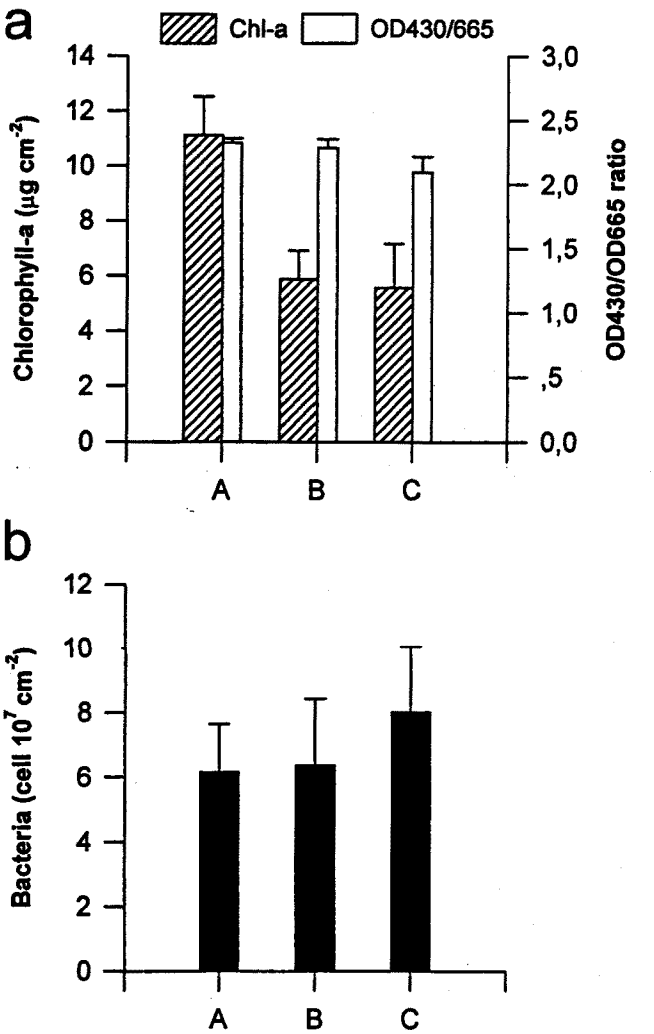


Fig. 4. Algal biomass and bacterial density on the epilithic biofilms of the Breitenbach growing at sites A, B and C (described in the text and in Fig. 1). a) Chlorophyll-a density and the OD430/OD665 ratio, b) Bacterial cell density. Means + standard errors (vertical lines) are shown (n=10 in a, n=5 in b).

The three habitats (sites A, B, C) under consideration differ in their epilithic ectoenzymatic activities (Fig. 5) indicating that there are differences in organic matter input. The specific microenvironment found at each site could be reponsible for such differences in heterotrophic metabolism. The higher water velocity as well as the lack of riparian vegetation (high incident light) at site A could provide favourable conditions for growing algae at this site (higher chlorophyll-a density, Fig 4a) in contrast to sites B and C, enhancing the ectoenzymatic activities at site A (Fig. 5). Although less biomass accumulation has been observed as a result

of shear stress (Lau and Liu 1993), water velocity may increase growth by increasing the transport of nutrient from the stream water to the biofilm, through biotic and abiotic uptake processes (Whitford and Schumacher 1961, Pfeifer and McDiffett 1975, Lock and John 1979), which could be important for biofilms living at low nutrient concentrations (Horner and Welch 1981). The positive effect of light on growing algae has been described elsewhere (Sumner and Fischer 1979, Hill 1996), especially for those sites where light may be limiting the primary production (Guasch and Sabater 1994). The higher autochthonous organic matter input at site A might provide the heterotrophs with "high quality" organic matter (Haack and McFeters 1982b, Kaplan and Bott 1989) such as polymeric substrates for ectoenzyme hydrolysis (Jones and Lock 1993). An increase in polysaccharidic ectoenzymatic activities along with chlorophyll-a and photosynthetic activity has been observed in epilithic biofilms (chapter 8).

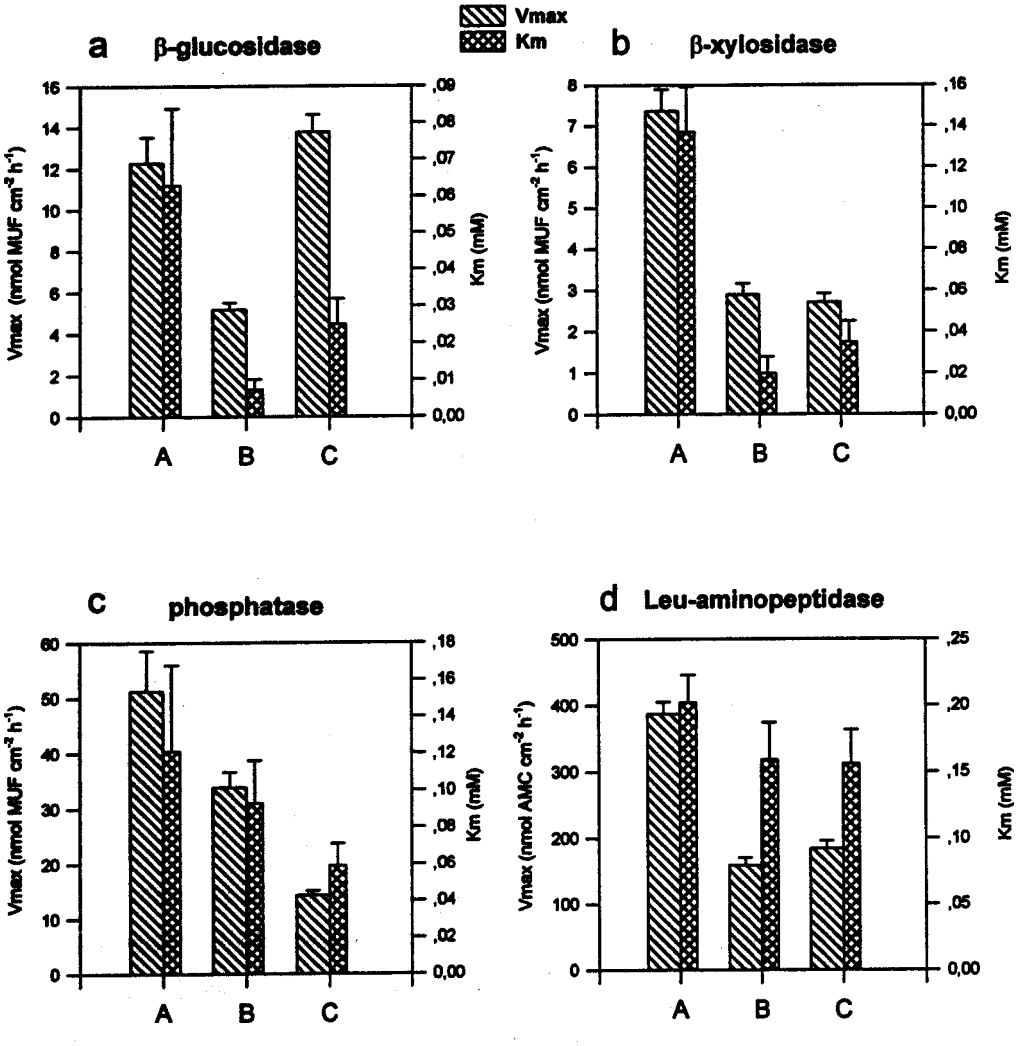


Fig. 5. Ectoenzymatic activities on the epilithic biofilms of the Breitenbach growing at sites A, B and C. Vmax (left bars) and Km (right, thinner bars) are shown. Values are means + standard errors (vertical lines). a) β-glucosidase activity, b) β-xylosidase activity, c) Phosphatase activity, d) Leucine-aminopeptidase activity.

Aminopeptidase activity might be also enhanced by the release of proteinaceous substances by algal cells (Hoch et al. 1996). Degradation of senescent algal cells can also be a source of proteinaceous compounds (Jorgensen 1987) as has been observed in the photic zone of a lake during phytoplankton bloom and breakdown (Halemejko and Chróst 1986, Middelboe et al. 1995), and in sea water (Hollibaugh and Azam 1983). The higher phosphatase activity at site A may also be affected by the contribution of algal phosphatases (Jansson et al. 1988).

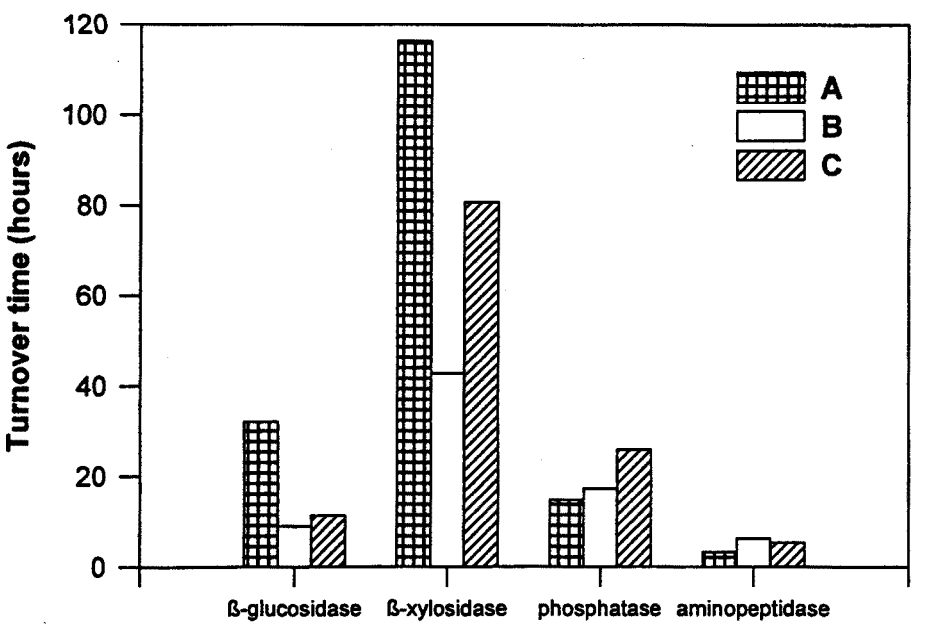


Fig. 6. Turnover time of substrate hydrolysis for the four enzymes analysed on the epilithic biofilms of the Breitenbach growing at sites A, B and C.

The lower ectoenzymatic activities at site B (open, low current) in comparison to site A might be a result of having a lower algal biomass (Fig. 4a). As explained above, the lower current velocity in such an oligotrophic stream could affect the algal growth, however, the lower chlorophyll-a density at site B could be further affected by macroinvertebrate grazing which was observed only on the tiles at site B and could diminish algal biomass (Hart 1992, Wellnitz et al. 1996). At this site a similar organic matter source to be used by the heterotrophs than at site A is suggested, since a similar value for the β-xylosidase:β-glucosidase ratio was obtained

(Table 2). However, the lower polysaccharide degradation capacity (Table 2) and lower Km values (higher affinity) expressed the lower amount of autochthonous input.

TABLE 2. Polysaccharide degradation capacity (β-glucosidase plus β-xylosidase Vmax) and β-xylosidase:β-glucosidase ratio in the three sample sites of the Breitenbach.

	Polysaccharide degradation capacity (nmol MUF cm^{-2} h^{-1})	**β-xylosidase:β-glucosidase** ratio
Site A	19.64	0.599
Site B	8.07	0.559
Site C	16.48	0.196

In contrast, at site C (forested) the lower ectoenzymatic activities (Fig. 5) might be a result of the low incident light due to the canopy cover which caused lower chlorophyll-a density (Fig. 4a) and therefore lower autochthonous input. However, β-glucosidase activity at site C was as high as at site A (Fig. 5a). Although a lower algal biomass was accumulated at site C, the utilization of organic compounds from the leaves accumulated on the streambed is suggested since the collecting of the epilithic biofilm samples was made just after the leaf fall. The higher β-glucosidase activity at site C could be a result of the use of leaf leachates (e.g. polysaccharides) (Sinsabaugh and Linkins 1988) which are released as water-soluble compounds during the initial stages of leaf decay (Lock and Hynes 1976, Boulton and Boon 1991). The lower Km (higher affinity) for the β-glucosidase at site C than at site A could indicate that the input of substrates for this enzymatic activity is a pulse source at site C but a constant source at site A (algal material). The polysaccharide degradation capacity at sites A and C was similar (Table 2) but the difference in the β-xylosidase:β-glucosidase ratio (Table 2) indicates the use of a different organic matter source. Further experiments should be planned at the forested site during other periods of the year.

Turnover time of substrate hydrolysis was on average 80:19:17:5 hours for β-xylosidase:phosphatase:β-glucosidase:aminopeptidase (Fig. 6) indicating slower recycling for the polysaccharides than for the proteinaceous compounds. Values for the three sites were similar except for the higher Tt (slower recycling) for β-glucosidase at site A and for β-xylosidase at sites A and C which might result from a greater availability of substrates for these enzymes at these sites. These average turnover times were similar to those measured for the epilithic biofilms in Riera Major stream (chapter 5) but significantly higher for the phosphatase activity in the Breitenbach. The slower recycling of organic phosphorus materials in contrast to the fast recycling of the proteinaceous materials (low Tt) could indicate that nitrogen is a more limiting compound than phosphorus for the heterotrophs living in the Breitenbach stream biofilms.

8. ALGAL-BACTERIAL RELATIONSHIPS

8.1. Metabolic changes associated with biofilm formation in an undisturbed Mediterranean stream

Abstract

Respiratory activity (ETS), ectoenzymatic activity (β-glucosidase and β-xylosidase) and photosynthetic activity ($H^{14}CO_3$ incorporation) in the biofilm were measured in a shaded stream during a colonization sequence (43 days) on artificial substrates (unglazed clay tiles) and compared with older (aged) tiles. In the first five days bacterial densities and ectoenzyme activities showed a sharp increase. After two weeks, algal density, chlorophyll-a and productivity increased moderately. Chlorophyll-a did not reach similar values to those of the aged biofilms until the last day of colonization. Photosynthetic activity seemed to be relevant to the heterotrophs metabolism during substrate colonization, as could be deduced from the significant correlation between β-glucosidase and $H^{14}CO_3$ incorporation, algal cell densities, and chlorophyll-a. Respiratory activity (ETS) decreased in the older biofilms, accordingly to their higher algal and bacterial density. Younger biofilms (up to 8 days old) showed higher ETS activity per cell, indicating a fast response of microorganisms to substrate availability.

Introduction

Some of the biofilm properties (polysaccharide matrix development, organic matter retention, ion-exchange mechanisms, nutrient diffusion) can change depending on its type or age. It has been shown that the biomass accrual related to biofilm age affects photosynthesis (Boston and Hill 1990, Guasch et al. 1995), as well as gas and nutrient diffusion (Mulholland et al. 1991) that occurs inside the biofilm. Other studies have highlighted that river biofilms appear remarkably resilient to organic matter depletion from the overlying waters, in part because of the function of carbon reserve of the polysaccharide matrix (Freeman and Lock 1995). Colonization of biofilm can be described as a process of several overlapping stages (Stock and Ward 1989) resulting from the progressive response of the organisms to imposing physical factors (light, temperature, water current) and nutrient availability. The evolution of biofilm metabolism have been monitored throughout the colonization of a bare substrate to determine whether a simple biofilm has a differential metabolism than another older, more complex biofilm. This study has been conducted in an oligotrophic, undisturbed stream during a period of low light availability (Guasch and Sabater 1994) to highlight the possible role of the primary producers in such an unfavourable situation for the algae. The main objective was to show that a progressive ageing and complexity of the biofilm would result in the respective metabolic variations in algae and bacteria.

Materials and methods

Study site

The experiment was carried out in Riera Major, an undisturbed second-order Mediterranean forest stream (chapter 3). The physical and chemical characteristics during the period of study (13 June to 26 July 1994) are summarized in Table 1. Light irradiance reaching the river bottom was very low, on the average ca. 20 μmol photons m^{-2} s^{-1}. Very little precipitation was recorded over the sampling period, as is usual in Mediterranean streams during summer (Sabater et al. 1995). In this period water flow reached its minimum (12 L s^{-1} during mid July 1994).

TABLE 1. Physical and chemical characteristics of Riera Major stream during the study period.

	Mean (n=11)	SD
Temperature (°C)	14.04	1.26
Incident light (μmol m^{-2} sec^{-1})	21.83	13.73
pH	8.01	0.20
Alkalinity (meq l^{-1})	2.23	0.09
Conductivity (μS cm^{-1})	224.25	17.07
Oxygen (mg l^{-1})	8.81	0.85
DOC (mg l^{-1})	0.77	0.65
Nitrate (μg l^{-1})	276.6	50.65
Ammonia (μg l^{-1})	10.78	4.48
SRP (μg l^{-1})	7.44	4.25

Sample collection

Small, unglazed clay tiles (0.64 cm^2 of surface area and 1cm height) were glued using colourless silicone onto flat surfaces of natural boulders, and immersed in a stream riffle stretch to allow colonization. Tiles were randomly collected at 0, 1, 2, 3, 4, 7, 9, 14, 21, 30 and 43 days of colonization. Tiles were gently rinsed of coarse debris and placed in sterile glass tubes with stream water and maintained cold (on ice), in the dark, during transport. Tiles for bacterial counts were fixed with 2% formalin. Samples for SEM observations were fixed with 2.5% glutaraldehyde in phosphate buffer pH 7.5 and stored in the dark until SEM observations. Cell densities (bacteria and algae), enzymatic and respiratory activities, and primary production and chlorophyll-a were measured on the colonizing tiles. The same measurements (except cell densities) were also performed using six to twelve week old tiles located at the same site. From previous tests, it was established that six weeks was the minimum time to allow the development

of an aged community in Riera Major, i.e. one that strongly resembled the natural ones in species density and community structure.

Light was measured with a LiCor underwater cell immediately above the colonizing surfaces. Water temperature, pH, alkalinity, conductivity and dissolved oxygen were also measured at each sampling date. Water dissolved inorganic carbon (DIC) was calculated from measures of alkalinity, temperature, pH and conductivity using the computer program WATEQX (Van Gaans 1989). Water was filtered with precombusted Whatman GF/F filters to analyse dissolved inorganic nutrients (nitrate, ammonia and soluble reactive phosphorus), as well as DOC. Three replicates were performed for each analysis following the procedures described in chapter 2.

Bacterial density, algal biomass, and SEM observations

Bacterial densities (DAPI stain, epifluorescence microscopy) and algal densities (inverted microscope) were estimated in triplicate. Chlorophyll-a was measured separately in triplicate. SEM was used to follow the colonization sequence on the tiles. All measurements were determined following the procedures described in chapter 2.

Metabolism measurements

Extracellular β-D-glucosidase and β-D-xylosidase potential activities were determined in tiles (3 replicates) and one formaldehyde-killed control. Two blanks of filter-sterilized stream water were also incubated for each enzyme. The Electron Transport System (ETS) activity was measured using three replicate tiles and one killed-control tile. ETS activity was also expressed in a cell basis by summing bacteria and algal cells (chapter 2.2). Primary production was measured using three replicate tiles, one killed-control tile and one dark-incubated tile. All measurements were determined following the procedures described in chapter 2. Even though ambient light was below saturation, the saturated light conditions (150 μmol photons m^{-2} s^{-1}) was used for the ^{14}C incorporation assay since this is sometimes experienced by the summer algal communities in Riera Major, since sunflecks through the forest canopy (Guasch and Sabater 1995) provide pulsing light energy.

Data analyses

Significant differences between the colonization and the aged tiles were analyzed through a one-way analysis of variance (ANOVA). Correlation analysis of the colonization and environmental data set was performed using product-moment Pearson coefficient.

Results

Bacteria were the earliest colonizers of the bare tiles (Fig. 1a, b, and c). Bacteria showed a first phase of rapid occupation (up to day 5), followed by a slower increase, although cell densities continued to rise (Fig. 2a). No clear succession of bacterial forms was apparent during

the colonization period. Rod-shaped and small coccoid bacteria constituted the characteristic population throughout. Algal colonization followed a similar two period-pattern to the bacteria, but the magnitude and respective time length differed when compared with results observed for bacterial density. The density of algal cells remained very low until day 22, and reached the highest values during the last three weeks of the experiment (Fig. 2b). Algal remains (such as empty diatom frustules and dead cells) were common in the earlier days, but the density of pioneer taxa increased from day 14. These were mainly diatoms of the genus *Achnanthes* (Fig. 1d) and small cyanobacteria filaments (Fig. 1a). Progressively some green algae (*Ulothrix*) as well as other diatom taxa (mainly from the genera *Amphora*, *Gomphonema* and *Cymbella*) were increasingly frequent (Fig. 1e and f). Chlorophyll-a increased from day 8 onwards, but it approached similar concentrations to those of the aged tiles only on day 43 (Fig. 2c).

Low levels of C uptake were observed until the last days of the experiment (Fig. 2d). $H^{14}CO_3$ incorporation on the colonizing tiles was significantly different to that recorded in aged tiles, which however, was highly irregular. Incorporation of $H^{14}CO_3$ on the colonizing tiles was correlated with chlorophyll-a content ($r=0.87$, $p<0.05$, $n=11$) and density of algal cells ($r=0.79$, $p<0.05$, $n=11$).

Ectoenzymatic activities are shown in Fig. 2e and f. The comparison between aged and colonizing tiles shows that the activity of both β-glucosidase and β-xylosidase increased steeply during the first eight and five days of the colonization respectively. Thereafter, differences between the two kinds of tiles were not significant ($p= 0.0699$ and $p= 0.1798$ respectively). From day 8 onwards the ectoenzyme activities fluctuated markedly both in the aged and in the colonizing tiles. Their trends of change remained very similar, except at the end of the experiment, when β-glucosidase tended to increase while β-xylosidase decreased. Correlation analysis revealed that β-glucosidase and β-xylosidase activities increased with bacterial density in the colonizing tiles ($r= 0.84$ and 0.6 respectively, $p<0.05$, $n=11$). β-glucosidase shows a positive correlation with algal density, $H^{14}CO_3$ incorporation and chlorophyll-a concentration ($r=0.66$, 0.64 and 0.86, respectively $p<0.05$, $n=11$). In contrast, enzyme activities in the aged substrata did not show any significant correlation either with the environmental or with the biological variables measured.

ETS activity per unit area showed a steep increase at day 4, but from day 5 significant differences with the aged tiles were not found (Fig. 2g). ETS fluctuated both in the colonizing and in the aged tiles. ETS was significantly correlated only to β-xylosidase activity ($r=0.8$, $p<0.05$, $n=11$). When ETS activity per cell was considered (Fig. 2h) two periods of different activity were apparent. Up to day 8 there was a distinctly higher respiratory activity (5.19 +/- 3.64 10^{-10} μg $cell^{-1}$ h^{-1} on average), which decreased during the last part of the colonization (average value of 1.29 +/- 1.16 10^{-10} μg $cell^{-1}$ h^{-1}).

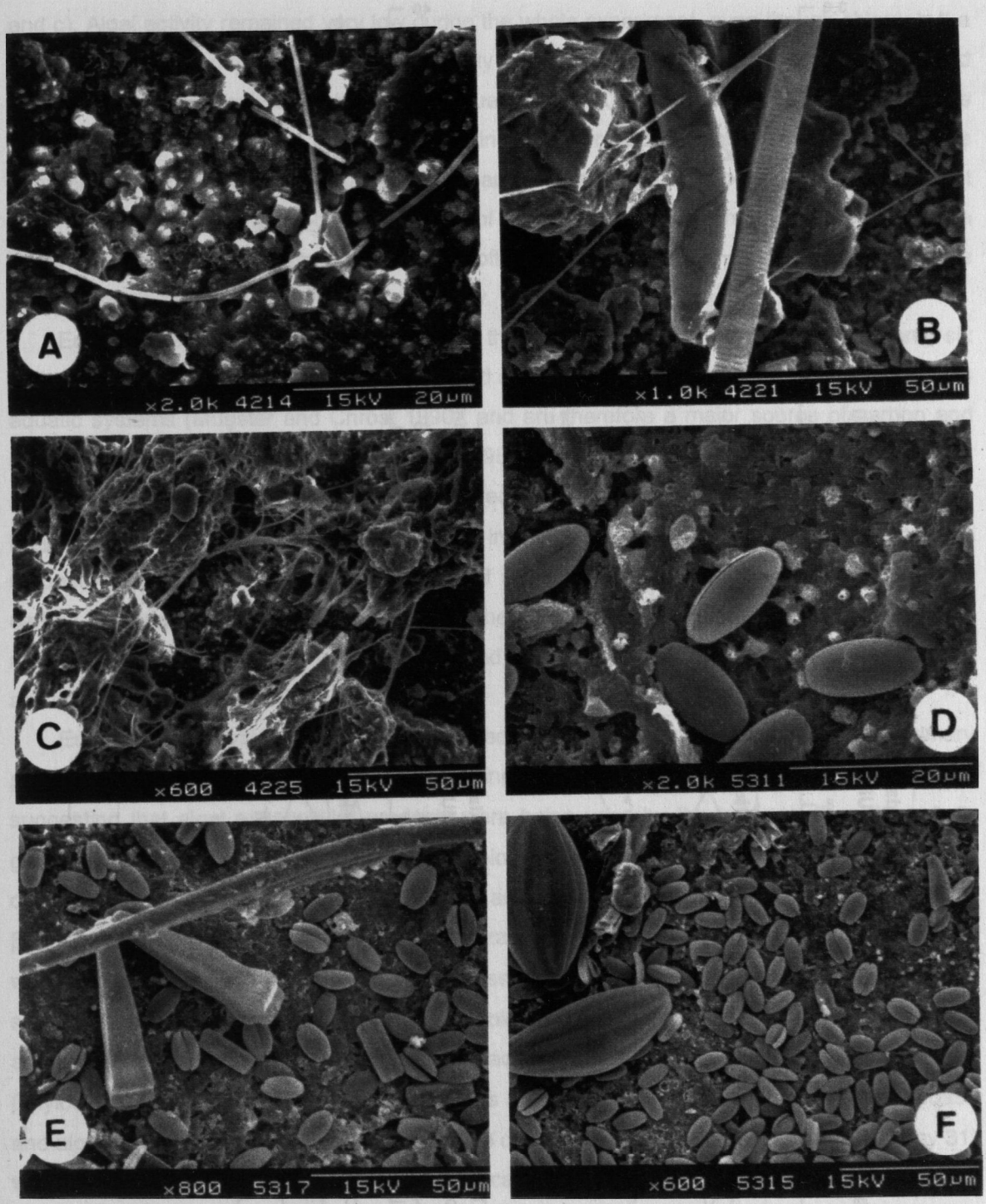


Fig. 1. Sequence of SEM photographs of the colonizing tiles. (a) Bacteria and filamentous cyanobacteria scattered on the substrate of a young biofilm (day 2). (b) *Epithemia* (left) and *Synedra* (right) diatom cells partially covered by mucopolysacharide strips in day 3. (c) Web of mucopolysacharide strips covering the tile in the day 4. (d) Some cells of the diatom *Achnanthes minutissima* are apparent over the day 21 tile. (e) Abundance of *A. minutissima* and *Amphora ovalis* (on the left) in day 30. (f) Green algae (upper filament) and rare diatoms (*Gomphonema acuminatum*) occurred scarcely at day 30.

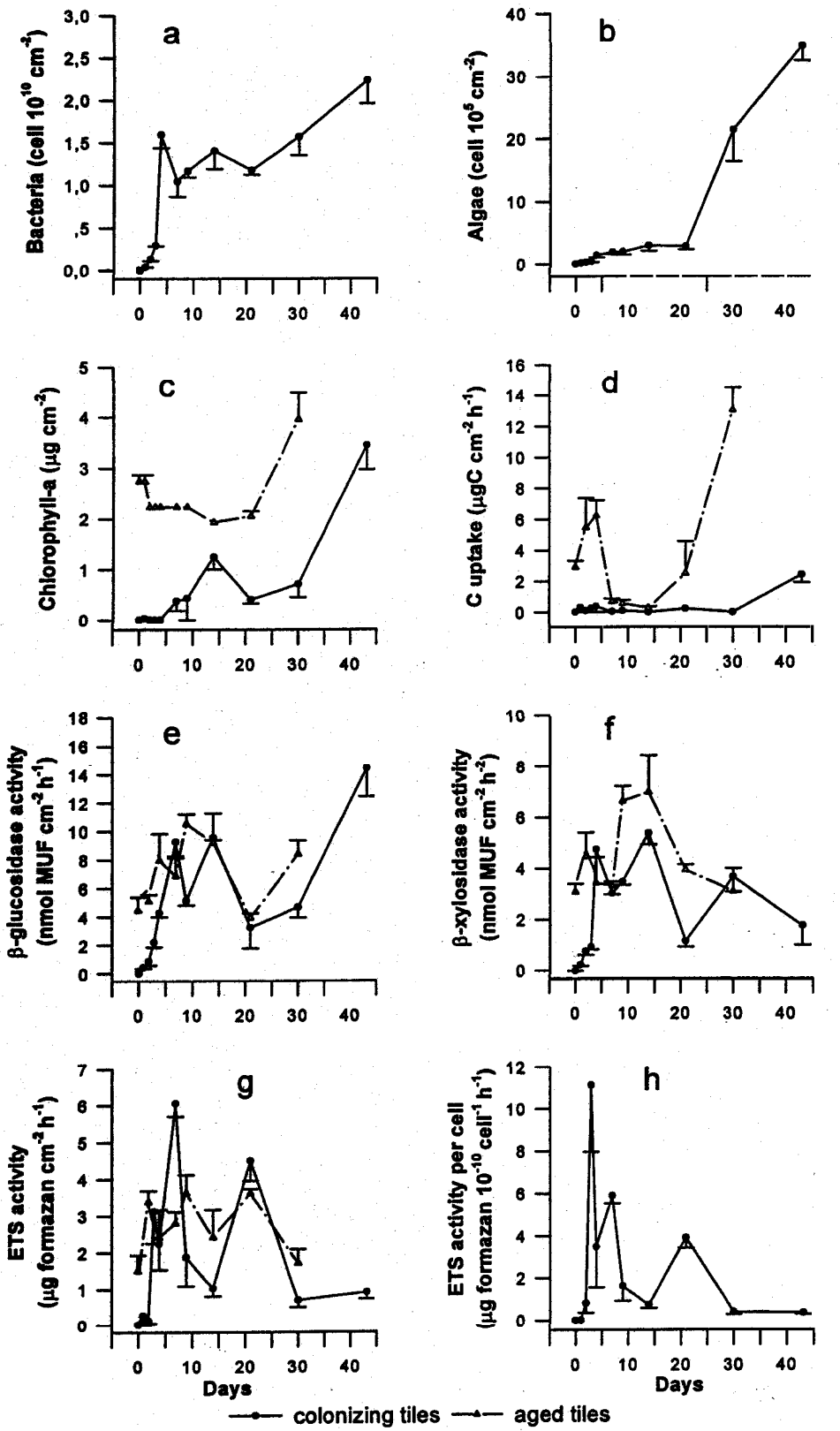


Fig. 2. Evolution of the structural and functional variables measured throughout the 43-day-experiment in the colonizing (black circles) and aged (empty triangles) tiles. Means ± standard errors (vertical bars) are shown (n= 3).

Discussion

Bacteria colonized the bare tiles rapidly but algae were far slower to colonize (Fig. 1a, b, and c). Algal activity remained very low during the whole experiment, even though chlorophyll-a was noticeable on day 9. Low light availability clearly slows algal colonization (e.g. Hill and Knight 1988). In fact only the chlorophyll-a values of day 43 (Fig. 2c) were of the same order as those regularly occurring in Riera Major during summer (Guasch and Sabater 1994). The apparent predominance of heterotrophs over autotrophs during the colonization period is not uncommon in low order, light-limited streams of temperate regions (Minshall *et* al. 1983). The initially high heterotrophic character of the biofilm is evidenced by a quicker increase in the ectoenzymatic activities than in the algal activity.

Ectoenzymatic activity became similar in the colonizing and in the aged tiles in only six days. Polysaccharides (both from plant litter and algae) constitute a significant portion of DOM in aquatic systems (Münster and Chróst 1990), and are therefore a major source of carbon and energy for epilithic bacteria (Kaplan and Bott 1989). The predominance of cellulose rather than hemicellulose as a carbon source can be inferred from the higher activity of β-glucosidase than β-xylosidase. This was the characteristic both in the colonizing and aged tiles. Their activities respectively reflect, at least in part, the use of autochthonous (algal) or allochthonous (plant) carbon material by heterotrophic bacteria. The possibility of a direct relationship with the allochthonous organic carbon was not confirmed by any correlation between the ectoenzymatic activities and the stream DOC concentration.

Ectoenzyme activities were highly correlated with microbial and algal biomass and primary production. β-glucosidase activity in the colonizing tiles was correlated to algal-related variables, suggesting that algal extracellular products were being used by the bacteria. Even though β-glucosidase mediates the hydrolysis of cellobiose both from allochthonous plant and algal celluloses, a preeminence in the degradation of autochthonous algal material could be assumed. β-glucosidase activity increased during colonization, as well as bacterial and algal biomass, while β-xylosidase activity was seen to decrease. β-glucosidase and β-xylosidase followed the same pattern during the first 5 to 8 days of colonization, when the biofilm was still scarce in algae. Only when the autotrophic biomass and activity became apparent, did β-glucosidase and β-xylosidase follow different patterns. There was a significant correlation between β-glucosidase and algal chlorophyll-a when the last days of the colonization experiment (from day 15 to day 31) were considered separately. These results indicate the existence of a trophic link between the algae and the bacteria in an unfavourable situation for the photosynthetic activity. Similar connections were observed during the decay of a phytoplankton bloom, when β-glucosidase had the maximum activity (Chróst 1989). Also in light- incubated biofilms β-glucosidase activity was strongly correlated with photosynthetic communities (Jones and Lock 1993).

The higher ETS activity per cell was distinctive of the earlier colonized biofilms. Higher activities per cell have also been observed in disturbed biofilms, such as those affected by grazing or by a storm-flow. A higher ETS activity per cell at the first days of colonization of bare

grazing or by a storm-flow. A higher ETS activity per cell at the first days of colonization of bare substrates has been also calculated for dark and light-incubated tiles in a similar experiment performed in spring (chapter 8.2). Blenkinsopp and Lock (1992) found that biofilms disrupted by flooding had higher respiratory activities than intact biofilms, concluding that nutrient diffusion and ectoenzymatic activity increased after disruption. The ETS activity per cell in the biofilms decreased when the algal chlorophyll-a became more important, and the associated biofilm complexity increased. This is possibly related to the resistance to diffusion exerted by the polysaccharides matrix (Burkholder et al. 1990), which is capable to determine oxygen gradients in aerobic biofilms (Ramsing et al. 1993) and to modify nutrient transport through the biofilm (Stevenson and Glover 1993).

8.2. Effect of primary producers on the heterotrophic metabolism of a stream biofilm

Abstract

Algal biomass (chlorophyll-a) and metabolism ($H^{14}CO_3$ incorporation) were significantly correlated to heterotrophic metabolism (ectoenzymatic activities) in stream biofilms. Regression lines obtained using dark-grown biofilms and light-grown biofilms suggest that the response of the heterotrophs is faster in biofilms with low algal biomass accrual, and slows down when algae increase in their biomass. In light-grown biofilms a steeper slope of the regression lines was observed for the β-glucosidase activity than for the β-xylosidase activity. Also, β-glucosidase activity per cell was higher in the light-grown biofilms. These observations indicated the preferential use for algal-related (cellobiosic polysaccharides) products by the heterotrophs, when they are available, than others allochthonous (xylobiosic polysaccharides). This was confirmed by the similar slopes between β-glucosidase and β-xylosidase in dark-grown biofilms.

Introduction

Microbial sessile communities exposed to light in natural stream environments are associated with phototrophic organisms in a dense polymeric matrix (chapter 1). In epilithic biofilms, biomass accumulation, polysaccharide matrix development, difussion properties, and the input of allocthonous organic matter play an important role in biofilm metabolism (Lock 1993), hindering the identification of bacterial-algal relationships. However, in marine and freshwater planktonic environments bacterial-algal relationships have been widely described (Cole 1982, Bird and Kalff 1984, Le et al. 1994) and it is generally accepted that heterotrophic bacteria directly utilize products excreted by algae (Chróst 1981, Brock and Clyne 1984, Siuda et al. 1991). Only in especific conditions, an uncoupling of bacteria and phytoplankton has been observed such as in strong tidal mixing environments (Cho et al. 1994) and in a highly heterotrophic estuary (Findlay et al. 1991). In some oligotrophic streams a strong bacterial-algal link has been found (Stock and Ward 1989), while in others it was evidenced that allochthonous input was the main support for bacteria, thus masking a possible bacterial-algal relationship (Findlay et al. 1993). Several studies of epilithic biofilms conclude that algal exudates are a major carbon source for bacteria (Haack and McFeters 1982b, Geesey et al. 1978, Kaplan and Bott 1989). Furthermore, the development of algal biomass and the polysaccharide matrix increase the surface area which is available for bacterial colonization (Geesey et al. 1978). The link between bacteria and algae in the biofilm is possibly dependent on algal accrual (Sobczak 1996).

This study seeks to determine whether the algal growth affects the microbial metabolism in an epilithic biofilm, and whether biomass accrual might modulate this effect. Therefore, biofilm biomass and metabolism was analyzed during colonization (in dark and light conditions) to

monitor biomass intensively, from the bare substrate to a mature biofilm. The evolution in the use of organic material of algal origin by the heterotrophs was investigated through the variations in the activities of the ectoenzymes β-glucosidase and β-xylosidase (Chróst 1990), as well as in bacterial cell density, respiration activity (ETS) and photosynthetic biomass and activitiy. The experiment was performed in spring (maximum primary production of the epilithic community, Guasch and Sabater 1994) to maximize the possible effect of algae on the heterotrophic component of the biofilm.

Materials and Methods

Study site

The experiment was carried out in Riera Major, an undisturbed second-order Mediterranean forest stream (chapter 3). The physical and chemical characteristics during the period of study (13 March to 11 May 1995) are summarized in Table 1. Incident light was high (Table 1), and did not, therefore limit primary production (Guasch and Sabater 1995). Flow averaged 40 L s^{-1}.

TABLE 1. Physical and chemical characteristics of Riera Major stream during the study period.

	Mean (n=12)	**SD**
Temperature (°C)	7.48	1.398
Incident light (μmol m^{-2} sec^{-1})	812.9	217.2
pH	8.18	0.12
Conductivity (μS cm^{-1})	193.6	9.68
Oxygen (mg l^{-1})	10.63	0.76
DOC (mg l^{-1})	2.34	1.86
DIC (mg l^{-1})	21.24	0.79
Nitrate (μg l^{-1})	256.76	98.7
Ammonia (μg l^{-1})	17.36	27.82
Phosphate (μg l^{-1})	2.48	3.28

Sample collection

Small, unglazed ceramic tiles (0.64 cm^2 of surface area and 1cm height) were glued onto the flat surfaces of natural boulders using colourless silicone, and immersed in a stream stretch where they were to be colonized. Half of the boulders were left in the stream stretch in natural conditions (light-incubated), while the rest were placed inside an immersed dark tube

(dark-incubated). The plastic (PVC) tube (1.20 m long, 25 cm diameter) was partially buried in the streambed so as to ensure similar hydrological conditions to those in the stream. Light irradiance inside the tube was below 0.2 μmol photons m^{-2} s^{-1}. At the same site, six-to-eight week old tiles were used as control (Sabater and Romaní 1996). Tiles from light, dark, and control conditions, were randomly collected at 0, 1, 2, 3, 4, 7, 9, 14, 21, 29, 44, and 59 days of colonization, and dark tiles were also collected at 81 and 105 days of colonization to assure total colonization in the dark. Tiles were placed in sterile glass vials with stream water and kept cold (on ice) in the dark until their arrival in the laboratory. Samples for bacterial cell counting were fixed with 2% formalin. Cell numbers (bacteria and algae), ectoenzymatic activities, respiratory activities (ETS), chlorophyll-a, and primary production were measured in the light, dark and control tiles at each sampling date. Algal and bacterial cell numbers in the control tiles were only measured on the first and the last sampling dates. All activity measurements were performed in the laboratory, two to three hours after sampling.

Light was measured with a LiCor underwater cell situated immediately above the colonizing surfaces. Water temperature, pH, dissolved oxygen and conductivity were also measured on each sampling date. Filtered (precombusted Whatman GF/F filters) water samples (three replicates) were taken to analyse inorganic nutrients (nitrate, ammonia and soluble reactive phosphorus), as well as dissolved organic carbon (DOC) and dissolved inorganic carbon (DIC) following the procedures described in chapter 2.

Bacterial density, algal biomass, and SEM observations

Bacterial density (DAPI stain, epifluorescence microscopy) and algal density (inverted microscope) were estimated in triplicate following the procedures described in chapter 2. Chlorophyll-a was measured separately in triplicate after extraction in 90% acetone (chapter 2). The ratio of chlorophyll to carotenoids and/or chlorophyll degradation products was measured as the quotient of the optical densities at 430/665 nm (Margalef 1983). SEM was used to follow the colonization sequence on the tiles in light and dark conditions.

Metabolism measurements

Extracellular enzyme potential activities (β-D-glucosidase and β-D-xylosidase), community respiration (ETS), and primary production ($H^{14}CO_3$ incorporation) were assayed following the procedures described in chapter 2. For each assay, five replicates of each sample type (light-incubated, dark-incubated and control tiles) and two killed-control tiles (and two tiles in darkened tubes for primary production) were used. For the ectoenzymatic activities, two blanks of each MUF-substrate prepared with filter-sterilized stream water was also included.

Data analyses

Differences between control and light-grown biofims, and light and dark-grown biofilms were analyzed through an analysis of variance (ANOVA, two single factor with replicates).

Differences between control and light-grown biofilms, and light and dark-grown biofilms after sixty days of colonization were analyzed by a *t*-test. Values in dark incubated biofilms taken after 81 and 105 days of colonization were compared to values at day 59 by a *t*-test. Correlation analysis was performed using product-moment Pearson coefficient. Regression analyses were performed for ectoenzymatic activities to chlorophyll-a and $H^{14}CO_3$ incorporation in light-incubated, dark-incubated, and control tiles.

Results

Bacteria and diatoms (mainly the genera *Cocconeis*, *Achnanthes* and *Cymbella*) had accumulated on the light-incubated tiles after three weeks (Fig. 1a). This contrasted with the scarce amount of material accumulated on the dark-incubated tiles (Fig 1d). At week eight, other algae appeared on the light-incubated tiles (Fig. 1b) and a mucilagenous material developed among the bacterial community (Fig. 1c). On the dark-incubated tiles damaged diatoms and broken valves were observed (Fig. 1e and f).

Bacterial cell density increased during colonization on both light and dark-incubated tiles. Significant differences between the two were observed after 9 days of colonization (ANOVA, $p<0.0004$) (Fig. 2a). However, differences in algal cell density between light and dark-incubated tiles were significant from the first day of the experiment (ANOVA, $p<0.0001$, Fig. 2b). Both bacterial and algal cell density on light-incubated tiles were not significantly different to those of the control tiles at the end of the experiment (*t*-test, $p=0.66$, $p=0.64$, respectively).

Chlorophyll-a density increased drastically on light-incubated tiles after one week of colonization; whereas a very low chlorophyll-a accumulation was observed on dark-incubated tiles (Fig. 2c). Significant differences between light and dark were found after 5 days of colonization (ANOVA, $p<0.0001$). A significantly lower chlorophyll-a concentration and higher OD430/OD665 ratio were observed on dark-incubated tiles at the end of the experiment (Table 2). Chlorophyll-a densities on light-incubated tiles were not significantly different from those on control tiles after three weeks of colonization (ANOVA, $p>0.1$), but values diverged at the end of the experiment (ANOVA, $p=0.0006$) (Fig. 2c).

Photosynthetic activity on the light-incubated tiles increased steeply during the first week, and after 9 days it was not significantly different to that of the control tiles (ANOVA, $p=0.058$, Fig. 2d). $H^{14}CO_3$ incorporation was not detected in dark-incubated tiles.

Light and dark ETS activity were significantly different after 9 days of colonization (ANOVA, $p<0.0001$, Fig. 2g). Differences were not significant between light and control tiles after 29 days of colonization (ANOVA, $p=0.053$).

Light and dark ETS per cell was also calculated and a peak was observed after 3 days of colonization. At the end of the experiment activity per cell was higher in light-incubated than in dark-incubated tiles (Fig. 2h).

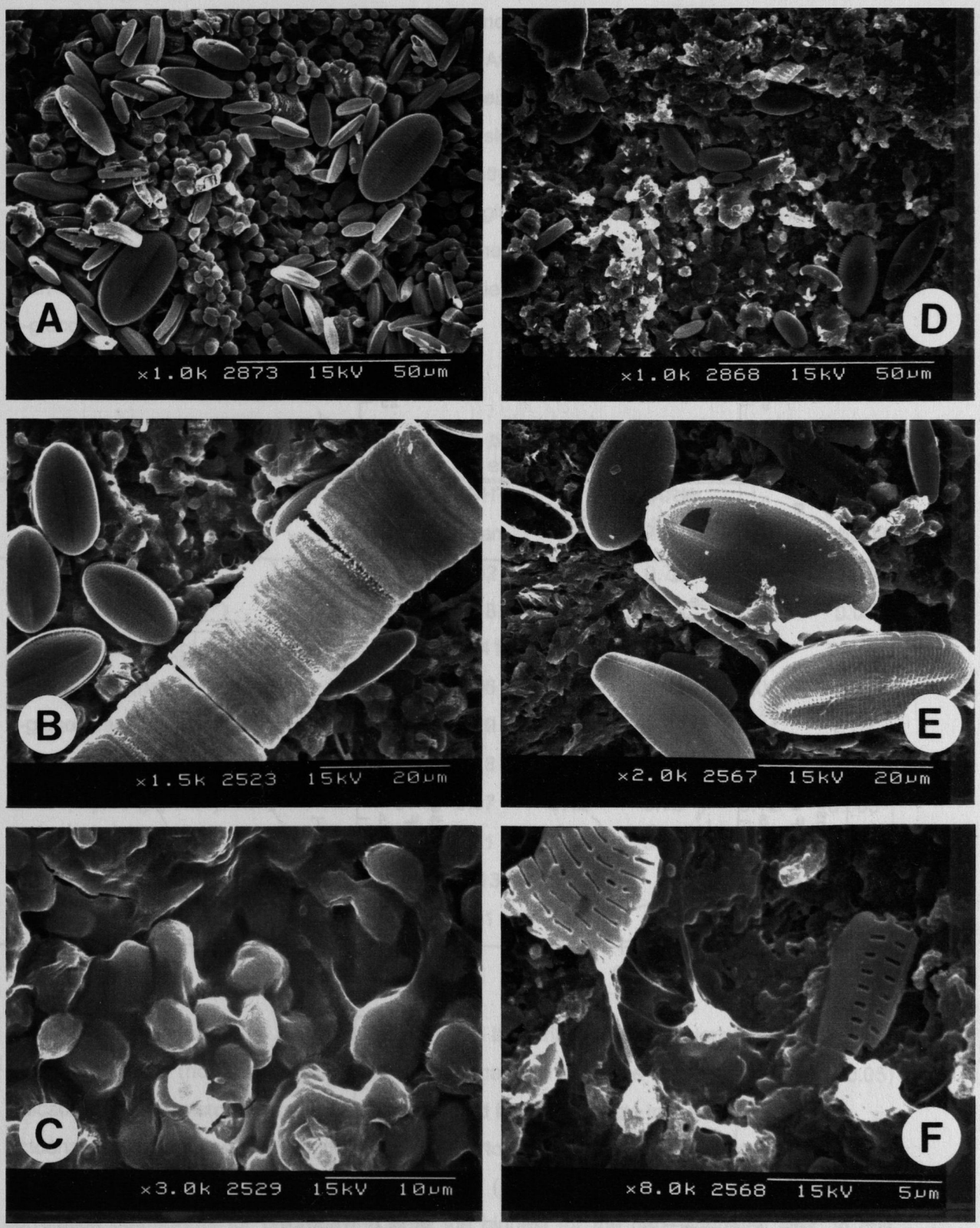


Fig. 1. Sequence of SEM photographs on the light-incubated (a, b, c) and dark-incubated (d, e, f) tiles. (a) Bacteria and diatom cells (*Achnanthes*, *Cocconeis*) totally covered the light-incubated tiles by day 21. (b) Larger diatoms (*Diatoma*) appeared by day 59. (c) Bacterial cells were covered by mucopolysaccharide (day 59). (d) Few baterial cells and diatoms were observed on the dark-incubated tiles by day 21. (e) Empty diatoms and broken frustules on day 59. (f) Mucilagenous material and detritus accumulated on the dark-incubated tiles (day 59).

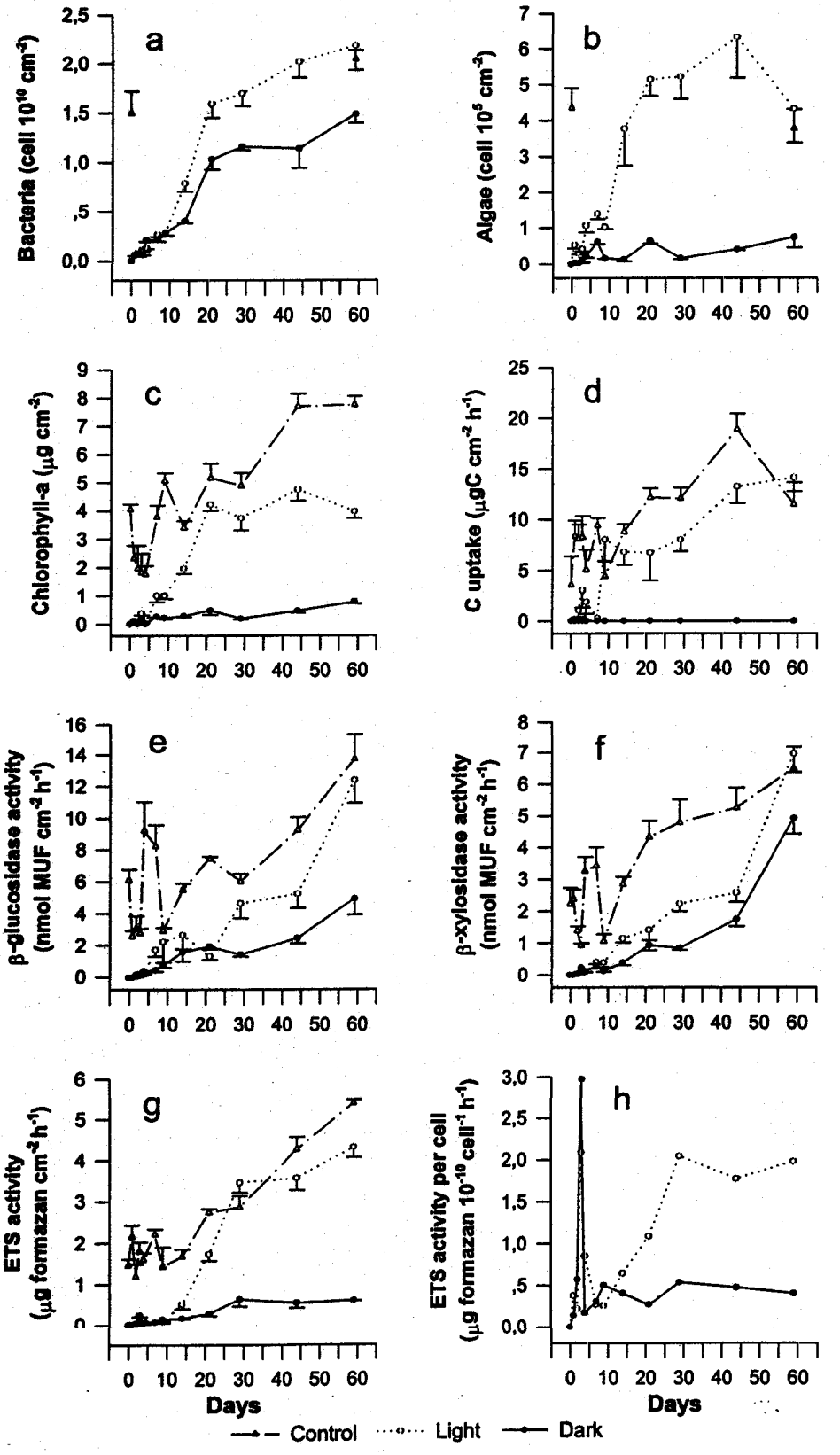


Fig. 2. Evolution of the biofilm metabolism on light-incubated (empty circles), dark-incubated (dark circles) and control (empty triangles) tiles throughout the colonization experiment. Means ± standard errors (vertical bars) are shown (n =3 in a, b and c, n= 5 in d, e, f and g).

β-glucosidase and β-xylosidase activities increased slowly during the first week of the experiment on both light and dark-incubated tiles. Afterwards, a higher increase was observed on light-incubated tiles (Fig. 2e and f), differences being significant after 7 days for β-glucosidase (ANOVA, $P<0.0001$) and after 9 days for β-xylosidase (ANOVA, $p<0.0001$). Ectoenzymatic activities on light-incubated tiles were not significantly diferent to those of the control tiles at the end of the experiment (ANOVA, $p=0.45$ for β-glucosidase and $p=0.62$ for β-xylosidase). However, light-incubated tiles had significantly higher β-glucosidase and β-xylosidase activities than dark-incubated tiles, but ectoenzymatic activities per cell were only significantly higher for β-glucosidase (Table 2).

TABLE 2. Mean bacterial and algal activities and biomass on dark-incubated and light-incubated tiles after sixty days of colonization. Differences are expressed by the *t*-test probability.

		LIGHT		DARK		*t*-test
	n	Mean	SD	Mean	SD	probability
Algae (cell 10^5 cm^{-2})	3	4.32	1.62	0.72	0.28	0.03
Bacteria (cell 10^{10} cm^{-2})	3	2.18	0.43	1.49	0.16	0.04
β-glucosidase (nmol MUF cm^{-2} h^{-1})	5	12.41	3.23	4.93	2.26	0.002
β-xylosidase (nmol MUF cm^{-2} h^{-1})	5	6.96	1.31	4.91	1.08	0.01
β-glucosidase/cell (10^{-10} nmol $cell^{-1}$ h^{-1})	5	6.19	0.72	3.82	0.44	0.02
β-xylosidase/cell (10^{-10} nmol $cell^{-1}$ h^{-1})	5	3.18	0.02	3.31	0.96	0.42
Chlorophyll-a (μg cm^{-2})	3	3.97	0.42	0.78	0.12	0.003
OD430/OD665	3	2.61	0.73	3.93	0.94	0.006
$H^{14}CO_3$ incorporation (μgC cm^{-2} h^{-1})	5	14.13	3.04	0	0	0.0002
ETS (μg formazan cm^{-2} h^{-1})	5	4.31	0.53	0.60	0.02	0.00005

Ectoenzymatic, ETS activities and algal and bacterial densities in dark-incubated tiles at days 81 and 105 (not shown) did not differ significantly from those at day 59 (*t*-test, $p>0.05$) indicating that the microbial populations had reached the steady state in the dark.

Ectoenzymatic activities were significantly related to chlorophyll-a and photosynthetic activity throughout colonization, fitting linear regressions (Fig. 3 and Fig. 4). Slopes and square correlation coefficients for chlorophyll-a were the highest for the dark-incubated tiles. In this case, the regression lines were similar for β-glucosidase and β-xylosidase. For light-incubated and control tiles, regressions were not as significant, and a gentler slope was characteristic of the β-xylosidase regression line. For $H^{14}CO_3$ incorporation, the regression coefficients were significant for both enzymes for light-incubated tiles, but only for β-xylosidase on the control tiles (Fig. 4). A lower slope for β-xylosidase was also observed for light-incubated and control tiles.

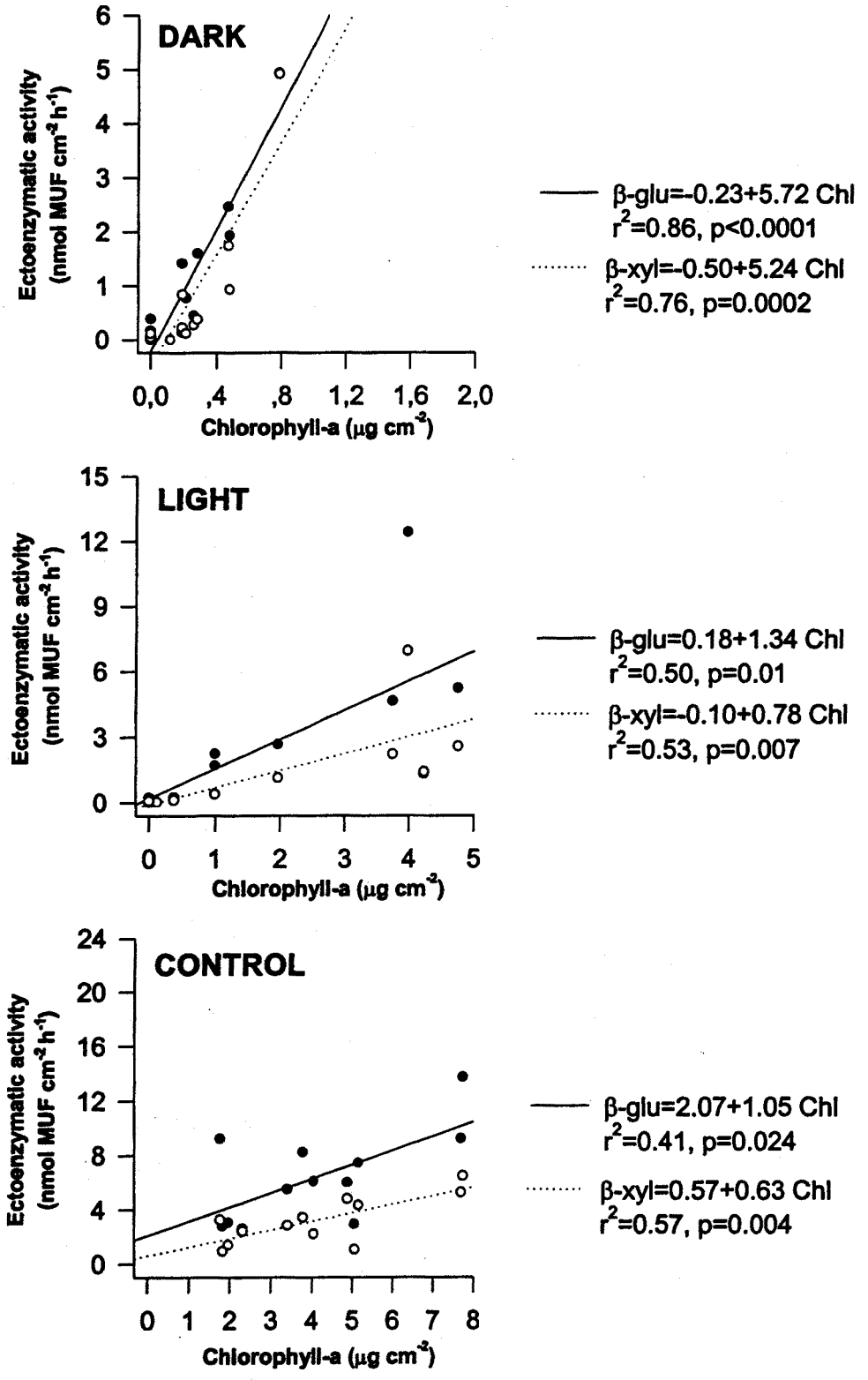


Fig. 3. Linear regressions between the chlorophyll-a concentration and the ectoenzymatic activities on dark-incubated, light-incubated and control tiles. The single points indicate the mean values obtained at each sample time (dark circles for β-glucosidase and empty circles for β-xylosidase). The regression lines, square correlation and the probability of F-Fischer after the ANOVA analysis are shown for both β-glucosidase activity (β-glu) and β-xylosidase activity (β-xyl).

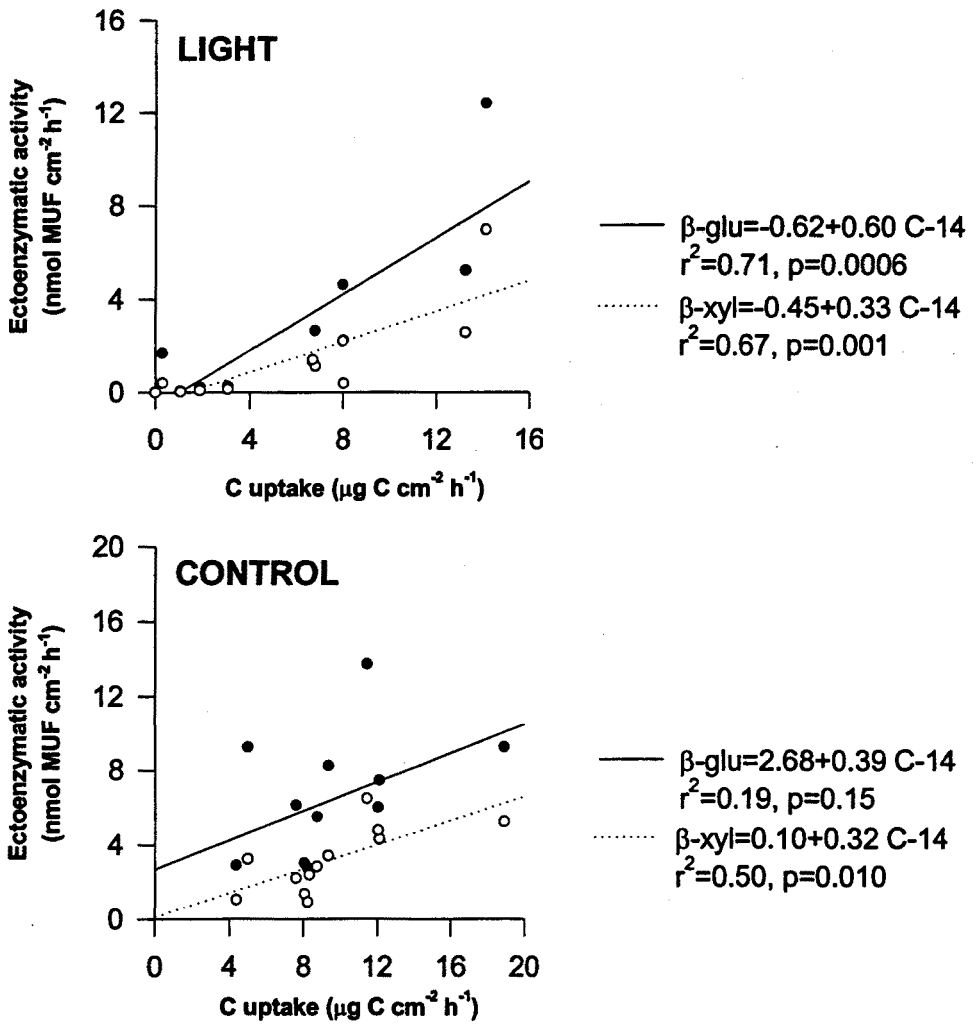


Fig. 4. Linear regressions between the photosynthetic activity (as $H^{14}CO_3$ incorporation) and the ectoenzymatic activities on light-incubated and control tiles. The single points indicate the mean values obtained at each sample time (dark circles for β-glucosidase and empty circles for β-xylosidase). The regression lines, square correlation and the probability of F-Fischer after the ANOVA analysis are shown for both β-glucosidase activity (β-glu) and β-xylosidase activity (β-xyl).

Ectoenzymatic activities were significantly related to chlorophyll-a and photosynthetic activity throughout colonization, fitting linear regressions (Fig. 3 and Fig. 4). Slopes and square correlation coefficients for chlorophyll-a were the highest for the dark-incubated tiles. In this case, the regression lines were similar for β-glucosidase and β-xylosidase. For light-incubated and control tiles, regressions were not as significant, and a gentler slope was characteristic of the β-xylosidase regression line. For $H^{14}CO_3$ incorporation, the regression coefficients were

significant for both enzymes for light-incubated tiles, but only for β-xylosidase on the control tiles (Fig. 4). A lower slope for β-xylosidase was also observed for light-incubated and control tiles.

Discussion

Light-grown biofilms accumulated higher algal and bacterial densities than were accumulated by dark-grown biofilms. Algae provide a greater surface area for colonization (Gessey et al. 1978, Hamilton 1987) than is provided by a bare substrate deprived of light. Bacteria feeding on algal material have favourable growth conditions (Gessey et al. 1978, Haack and McFeters 1982a and 1982b, Stock and Ward 1989). The development of primary producers in light-grown and control biofilms might also be responsible for the higher respiratory activity here, in contrast to that recorded in dark-grown biofilms (Blenkinsopp et al. 1991). Light-grown biofilms (both light-incubated and control tiles in this experiment) show higher degradation activities because of the higher availability of substrates (Blenkinsopp and Lock 1992).

The increase in the ectoenzymatic activities along with chlorophyll-a and photosynthetic activity in dark, light and control biofilms (Fig. 3 and Fig. 4) would seem to confirm that algal material is used by the heterotrophic community (chapter 8.1). However, autotrophic biofilms showed a different response to increasing algal biomass and activity to that shown by heterotrophic biofilms (Fig. 3 and 4). Dark-grown biofilms respond rapidly to chlorophyll-a accumulation (higher slope of the regression line), while in light-grown and control biofilms, a higher increase of chlorophyll-a density is necessary to observe an analogous increase in the ectoenzymatic activities. Therefore, the more algae accumulated on the biofilm, the slower is the ectoenzymatic response of the microbial community. In light-grown biofilms, algae, bacteria and polysaccharide accumulation might act as an organic matter reservoir (Freeman and Lock 1995) toning down the response to increasing algal biomass. It could be argued that such differences between light-grown and dark-grown biofilms are an effect of differences in nutrient diffusion being limited in the thicker biofilms (Hamilton 1987). However, light and dark-grown biofilms were similar in their thickness, being both rather thin. Furthermore, the shaking procedure used during the incubation was designed to eliminate any diffusion barrier.

The non-limiting light conditions for primary producers during the experimental period (Guasch and Sabater 1995) could be thought to be the only scenario where this heterotrophic behaviour in response to algae could be detected. But, the validity of this heterotrophic behaviour in Riera Major stream is stressed when analyzing other data from an analogous experiment performed in the summer (when the canopy limits light availability, chapter 8.1). In that case, a significant linear regression was found between β-glucosidase activity and chlorophyll-a (β-glu=2.62+3.79 Chl, r^2=0.73, p=0.0008). The slope under that conditions was in between those found for light and dark-grown biofilms in the current study. This was not unexpected, since the chlorophyll-a concentration also ranged between that of the light and dark-grown biofilms.

The different response of β-glucosidase and β-xylosidase activities to chlorophyll-a accrual and $H^{14}CO_3$ incorporation was observed in both light-grown and control biofilms (Fig. 3 and Fig. 4). The steeper slope found for β-glucosidase than for β-xylosidase reflects the preferential use of cellulose compounds, which are more easily and more rapidly degraded than hemicellulose compounds (Opsahl and Benner 1993, Boschkner et al. 1995, Lachke 1988). When available, bacteria preferentially use metabolites of phototrophs (Haack and McFeters 1982a), such as cellobiosic molecules, cleaved by β-glucosidase (Deshpande and Eriksson 1988). In contrast, in dark-grown biofilms there would seem to be little choice between the two enzymes (similar response) since high quality organic matter input (such as polysaccharides released by algae) is scarce. The (low) chlorophyll-a accrual in dark-grown biofilms might be due to passive settlement of colonists (Steinman and Parker 1990).

The highest values for β-glucosidase activity per cell (Table 2) underlie the greater utilization of cellobiose molecules by the heterotrophic community in light-grown biofilms. Algal released polysaccharides in light-grown biofilms might enhance β-glucosidase activity (Somville 1984, Jones and Lock 1993). The ratio β-xylosidase:β-glucosidase in light incubated biofilms was ca. 0.5, a value commonly quoted for stream biofilms (Sinsabaugh and Linkins 1988, Chapell and Goulder 1994a). In contrast, in dark incubated biofilms, this ratio was nearly 1, which might be related to the higher input of hemicellulose polysaccharides with respect to cellulose in dark conditions. The accumulation of decaying algae in dark-grown biofilms is indicated by the high values of the OD430/OD665 ratio (Table 2) (Margalef 1983).

We conclude that algal accumulation on the epilithic biofilm modulates the utilization of the organic matter by the heterotrophic community in three aspects: a) it increases the amount of organic substrate available for bacteria and therefore leads to a higher cleavage of macromolecules, b) it favours the use of cellobiosic as opposed to xylobiosic polysaccharides, probably due to the presence of high quality organic matter such as algal exudates, and c) it confers a slower response to the microbial community in relation to its own accrual. The low nutrient and DOC concentration in Riera Major stream water probably reinforces the capacity of the bacterial community to respond to changes in algal density and activity as has been observed in a laboratory prepared biofilm under low DOC conditions (Murray et al. 1986). Additional research needs to be conducted so as to validate the applicability of this model to different environmental situations.

9. MEASURING ECTOENZYMATIC ACTIVITIES IN MEDITERRANEAN STREAM BIOFILMS

9. Measuring ectoenzymatic activities in Mediterranean stream biofilms: general trends and relationship to the bacteria/algae biomass ratio

Abstract

Seasonal studies of biofilm ectoenzymatic activities performed in Mediterranean streams provided enough data to investigate the existence of any general behaviour for such activities. Statistical analyses of the data (Principal components, Pearson Correlation, Canonical Correlation) were used. From the physico-chemical parameters, conductivity and DIC (dissolved inorganic matter) were mostly responsible for ectoenzyme variability, while biofilm chlorophyll-a density was the most relevant biological parameter. β-xylosidase was more closely related to the allochthonous than the autochthonous organic matter sources. High phosphatase activities were found with low values of SRP in stream water. The ectoenzymatic activities measured in Mediterranean streams were higher than those from European streams but similar to those from North American and Australian streams. However, the ratio of β-xylosidase:β-glucosidase activity was similar for the streams of the different regions considered, being ca. 0.5. A negative relationship was found between the ectoenzymatic activities and the bacteria/algae biomass ratio of each studied stream biofilm (the Mediterranean and several European streams), stressing the importance of algae for the heterotrophs. It is concluded that autotrophs play a more relevant role as an organic matter source for the heterotrophs and therefore for carbon cycling than has generally been postulated for stream biofilms.

Introduction

The ectoenzymatic activities measured in stream biofilms have been related to environmental parameters such as nutrient concentration (chapter 3.1, chapter 6), water discharge (chapter 6, chapter 7), light and temperature (Sinsabaugh and Linkins 1988, chapter 3.3, chapter 5, chapter 6), and to biological parameters (Chapell and Goulder 1994a, chapter 3, chapter 4, chapter 8). In several studies ectoenzymatic activities have also been related to substrate availability in preference to environmental changes (e.g. Meyer-Reil 1987). In this chapter the relationship of ectoenzymatic activities to the environmental parameters and physiographic features was investigated for the three Mediterranean streams studied. The main objective was to find out whether there is any general trend for these heterotrophic activities for the Mediterranean streams and whether they differ from other studied streams in other regions (Europe, America, Australia). For this first purpose, ectoenzymatic data from the studied Mediterranean streams and from bibliographic sources for the European, American and Australian streams were analysed.

The second main objective was to investigate whether there is a relationship between the biomass of bacteria and algae of a given stream biofilm and its capacity to cleave organic

substrates. Few studies have focused on the effect of biofilm related parameters (i.e. bacteria and algal biomass, the main organisms which compose the biofilm structure) on the enzymatic activitiy in a given stream biofilm. In some stream biofilms, a significant correlation between ectoenzyme activity and bacterial cell density and/or chlorophyll or biomass has been observed (Sinsabaugh and Linkins 1988, Chapell and Goulder 1994a, chapter 8), but in other studies this relationship is weak or non-existent (Sinsabaugh et al. 1991a, Chapell and Goulder 1992, Jones and Lock 1993). Although the accrual of bacteria and algae might be important for the regulation of organic matter degradation capacity in a given stream biofilm, the relative amount of algal and bacterial biomass (bacteria/algae ratio) is probably more important in regulating the ectoenzymatic activity, since there are structural and functional relationships between them (chapter 8). Depending on the relative contribution of bacteria and algae on the biofilm biomass, the biofilm metabolism would be more autrotrophic or heterotrophic, which may determine a higher or lower level of enzymatic activity.

For this second purpose, data from the four study sites considered in this project (Riera Major, La Solana, Ter and Breitenbach) and their different benthic substrates (epilithic, epipsammic, cyanobacterial crust) were analyzed together with results from other studied streams (European, American, Australian) also considering different substrates (wood, leaves, natural stones, glass beads). The collection of data from such different substrates and sites provided us with results from different stream biofilms, which differed in the density of bacteria and algae, giving us the possibility of investigating a wide range of biofilm compositions. Results of the β-glucosidase, β-xylosidase and phosphatase activities and the bacterial and chlorophyll-a densities and biomass have been analyzed together.

Stream comparison

Comparison of the studied streams

The data from the seasonal studies in the Riera Major (chapters 3.1 and 3.2), La Solana (chapter 4.1), and river Ter (chapter 6), and from the study in the Breitenbach (chapter 7), were collected (Table 1). For the Riera Major the three substrate types were considered: stream-edge sand (n=10), mid-channel sand (chapter 3.1 together with the surface sand results from 3.2, n=21), tiles (n=10) and subsurface sand (n=9). For La Solana, the four stromatolitic algal patches of the cyanobacterial crust were considered: the mixed community (n=11), the *Rivularia* community (n=6), the *Zygnema-Spirogyra* community (n=6) and the diatom bloom (n=2). For the Ter (n=8) and the Breitenbach (n=3) data from the artificial clay tiles were considered (chapters 6 and 7, respectively). Values of β-glucosidase activity, β-xylosidase activity, phosphatase activity, chlorophyll-a density and bacterial cell density at all sites and sampling times (n=84) were used in the Principal Components Analysis (PCA) so as to visualize the stream differences and the seasonal distribution of the samples.

TABLE 1. Ectoenzymatic activities, respiratory activity (ETS), bacterial and chlorophyll-a density in the different stream biofilms from the four study sites (Riera Major, La Solana, river Ter and Breitenbach). Values are means of monthly values from the study period in each stream (Riera Major mid-channel sand: January 1994-August 1995, stream-edge sand and tiles: January 1994-February 1995, subsurface sand: October 1994-August 1995, La Solana: January 1994-February 1995, river Ter: February 1994-February 1995, Breitenbach: October-December 1995). The standard deviation of the mean is also shown except for the Diatom bloom (n=2) were the range is shown.

		β-glucosidase activity nmol cm^2 h^{-1}		β-xylosidase activity nmol cm^2 h^{-1}		Phosphatase activity nmol cm^2 h^{-1}		Respiratory activity (ETS) µg cm^{-2} h^{-1}		Bacterial cell density cell 10^{10} cm^{-2}		Chlorophyll-a density µg cm^{-2}	
Site and substrate	n	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD
Riera Major													
Mid-channel sand	21	14.62	7.21	6.53	3.62	20.43	14.51	0.81	0.61	0.97	0.89	1.79	2.11
Stream-edge sand	10	15.59	9.38	7.20	7.19	14.7	9.30	0.74	0.67	0.87	0.63	2.02	1.66
Subsurface sand	9	6.63	1.41	2.54	0.72	15.22	8.43	0.35	0.07	0.20	0.14	0.29	0.21
Artificial substrates (tiles)	10	5.68	3.56	2.38	1.32	39.11	32.3	1.55	1.70	2.41	0.62	4.06	2.17
La Solana													
Patches of the cyanobacterial crust:													
Mixed community	11	64.79	20.58	44.54	22.17	136.77	75.6	3.59	1.91	0.46	0.24	13.30	4.45
Rivularia community	6	49.31	37.98	22.33	19.85	383.77	456.9	8.53	5.92	0.98	0.42	4.44	14.41
Zygnema-Spirogyra community	6	50.28	28.80	20.91	9.51	163.15	100.7	3.89	0.45	0.60	0.23	20.05	9.25
Diatom bloom	2	15.74	4.2-34	6.71	3.1-12	66.34	28-90	12.95	3-34	0.41	0.3-0.6	32.66	19-43
Ter													
Artificial substrates (tiles)	8	39.72	31.7	9.97	7.02	48.56	41.8	4.95	2.58	2.47	1.92	31.07	19.76
Breitenbach													
Artificial substrates (tiles)	3	10.40	4.59	4.32	2.63	33.09	18.5			0.007	0.001	7.53	3.12

The ectoenzymatic activities, respiratory activity, and chlorophyll-a and bacterial density in the different study sites and substrates are summarized in Table 1. Since these variables were considered for the PCA, the results will be discussed together.

The results of the PCA showed an arrangement of β-glucosidase, β-xylosidase and chlorophyll-a by the first component which explains 42.3% of the variance (Table 2). The second component arranges with bacterial density and chlorophyll-a and negatively with β-xylosidase activity, explaining 24.4 % of the variance.

TABLE 2. Results of the PCA performed with data from Riera Major, La Solana, river Ter and Breitenbach. Loadings for the factors 1 and 2 (PC I and PC II) and the eigenvalues and percentage of total variance explained by each factor are also shown.

Variable	PC I	PC II
β-glucosidase	0.90	-0.16
β-xylosidase	0.81	-0.44
Phosphatase	0.47	0.22
Chlorophyll-a	0.64	0.49
Bacterial density	0.10	0.84
Eigenvalue	2.11	1.22
% variance	42.3	24.4

The different streams were represented in the PCA by their scores (Fig. 1). On the left side of the first component were placed the Riera Major (sand and tiles), and Breitenbach, indicating a lower ectoenzymatic activity in these streams than in the river Ter and La Solana (placed on the right side of the first component). The scores for the PC I for the river Ter were similar to those for La Solana, indicating that ectoenzymatic activities were in the same range of values. The higher ectoenzymatic activity for the biofilms growing on calcareous watersheds (the river Ter at Montesquiu and La Solana, Table 3) could be in part a result of the higher concentration of the calcium and magnesium ions in these study sites (Sabater 1988, Martí and Sabater 1996, Table 3) than in Riera Major and the Breitenbach (Martí and Sabater 1996, Marxsen et al. 1997). A positive response of β-glucosidase to added calcium, and especially magnesium, has been observed in sediments (King 1986). Furthermore, these ions, especially magnesium, could act as activating cations for the enzyme reaction (Chróst 1990). Greater ectoenzymatic activity on calcareous watersheds was also observed for several streams in N England (Chapell and Goulder 1994a). These two sites (La

Solana and river Ter) showed higher respiratory activity (ETS, not included in the PCA) (Table 1), indicating more relevant heterotrophic activities than in the silicic sites.

However the river Ter and La Solana differ in their distribution throughout the second component. At the upper extreme are located the spring samples from the river Ter (Ter3 and Ter5) and the winter sample for the *Rivularia* community (R1). These biofilms are characterized by a low β-xylosidase activity and high bacterial and chlorophyll-a density. In constrast in the lower extreme of the second component, the samples from the mixed community in La Solana in autumn and winter (M8, M9, M11, M12, M1) were found. These are characterized by a high β-xylosidase activity and low bacterial and chlorophyll-a density. The

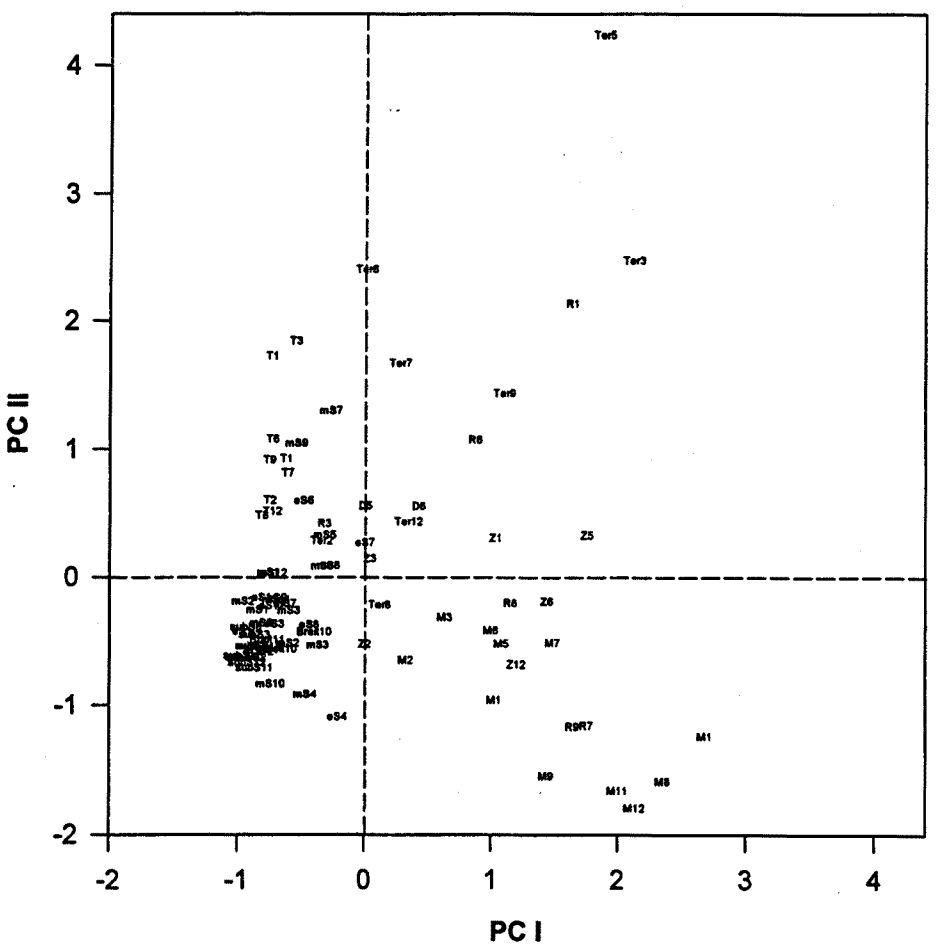


Fig. 1. Plot of the scores of the PCA performed with Riera Major mid-channel sand (mS), stream-edge sand (eS), subsurface sand (subS) and epilithic biofilm (T), the algal patches of La Solana cyanobacterial crust: Mixed community (M), *Rivularia* community (R), *Zygnema-Spirogyra* community (Z) and Diatom bloom (D), the river Ter epilithic biofilm (Ter), the Breitenbach epilithic biofilm (Breit). The numbers indicate the sampling month.

TABLE 3 . Physiographic features of Riera Major, La Solana, river Ter (4th order stretch) and Breitenbach watersheds.

	Riera Major	La Solana	Ter	Breitenbach
Location	41°56'N 2°25'E	42°70'N 2°13'E	41°N	51°N
Order	2	2	4	1
Watershed area (Km^2)	15.9	16.1	3010	0.0032
Altitude range (m above sea level)	960-460	980-500	680-460	350-220
Stream length (Km)	6	8.2	208	4.2
Lithology	granodiorite	calcite and dolomite	calcareous conglomerates	Bunter sandstone
Riparian vegetation	*Alnus glutinosa*	*Salix elaeagnus, Salix purpurea, Corylus avellana*	*Fraxinus excelsior, Betula pendula*	*Alnus glutinosa, Salix, Corylus avellana*
Discharge ($L\ s^{-1}$)	57.8	15.7	9600	26
Temperature (°C)	12.4	9.2	11.6	7.3
Conductivity ($\mu S\ cm^{-1}$)	206	413	310	165
DOC ($mg\ L^{-1}$)	2.7	3.8	6.3	1.6
Ca^{2+} and Mg^{2+} content ($mg\ L^{-1}$)	22.8 (Ca^{2+}) 4.1 (Mg^{2+})	59.2 (Ca^{2+}) 20.7 (Mg^{2+})	46.1 (Ca^{2+}) 5.8 (Mg^{2+})	17 (Ca^{2+}) 4.5 (Mg^{2+})
pH	7.9	8.2	8.2	7.1
Main benthic algae and cyanobacteria	*Phormidium autumnale*, diatoms, *Hildenbrandia rivularis, Cladophora glomerata*	*Rivularia biasolettiana, Schizothrix penicillata*, diatoms, *Mougeotia* spp, *Zygnema* spp.	Diatoms, *Cladophora glomerata*	*Chamaesiphon incrustans*, diatoms

detailed distribution throughout PC II can be interpreted in terms of efficiency of organic matter utilization. In the upper extreme are located the more eutrophic systems, less efficient in organic matter use and based on both autochthonous and allochthonous sources. In the bottom extreme the systems are more oligotrophic, more efficient in organic matter use and mainly based on allochthonous sources. Following this reasoning, the mixed community of La Solana cyanobacterial crust is the most efficient in the utilization of organic matter of all the biofilms studied (as has already been described in chapter 4.1). Moreover, the epilithic biofilms in Riera Major might be less efficient in organic matter use than the sandy biofilms (Fig. 1) coinciding with the higher capacity to degrade organic matter suggested for the sandy substrate than for the rocky substrate (chapter 3.1).

The Breitenbach had similar scores to those of Riera Major. Both streams have similar environmental characteristics (Table 3) which make these streams similar in their biofilm metabolisms (Table 1). Both streams have a catchment geology which results in a low ion concentration in the stream water (Bunter sandstone for the Breitenbach, Marxsen et al. 1997, and granodiorite for Riera Major, Martí and Sabater 1996). Riera Major was covered by a riparian forest (*Alnus glutinosa*) and the Breitenbach drains a highly forested area (*Fagus sylvatica* and *Pinus sylvestris*) with riparian vegetation (*Alnus glutinosa*) in some stretches. However, there were large differences in bacterial cell density (Table 1), which were very much lower in the Breitenbach.

Regularities in temporal variations in the studied streams

The seasonal distribution of the different biofilms in the PCA is presented separately for each stream (Fig. 2, 3 and 4). In Riera Major, variations are mainly attributable to the PC II, and thus due to bacterial and chlorophyll-a density variations (Fig. 2). This is especially clear for the tiles, which showed no variation on the first axis (PC I), indicating the lack of seasonality in the ectoenzymatic activities. For the sand, the higher values for the PCI were observed in the spring and summer months. As was described in the seasonal study of the Riera Major (chapter 3.1) there was no clear seasonality for the epilithic biofilm metabolism while the activities in the sandy biofilm followed seasonal variations. The few temporal fluctuations for the subsurface zone (chapter 3.2) are expressed by the very close distribution of all the samples from this habitat.

In La Solana, time variations were due to PC I and PC II which also distribute the different algal patches (Fig 3). The highest ectoenzymatic activities in the mixed community (M) (Table 1 and chapter 4.1) are expressed by the distribution of this algal patch in the right hand side of the PC I, while the lower ectoenzymatic values and score values of the PC I were characteristic of the diatom bloom (D). In between the M and D community, the *Zygnema-Spirogyra* (Z) and *Rivularia* (R) communities were found with much higher scores of the PC II for the R community, expressing the higher bacterial density in this algal patch. A seasonal pattern was observed for the *Rivularia* and the mixed community, which decrease throughout the second component in summer (R7, M8). This could indicate the decrease in bacterial and

chlorophyll-a density during the drought period but the rapid recovery of the ectoenzymatic activities (chapter 4.2) meaning a high level of efficiency of organic matter use especially in the R and M algal patches (lower values in PCII, as discussed above).

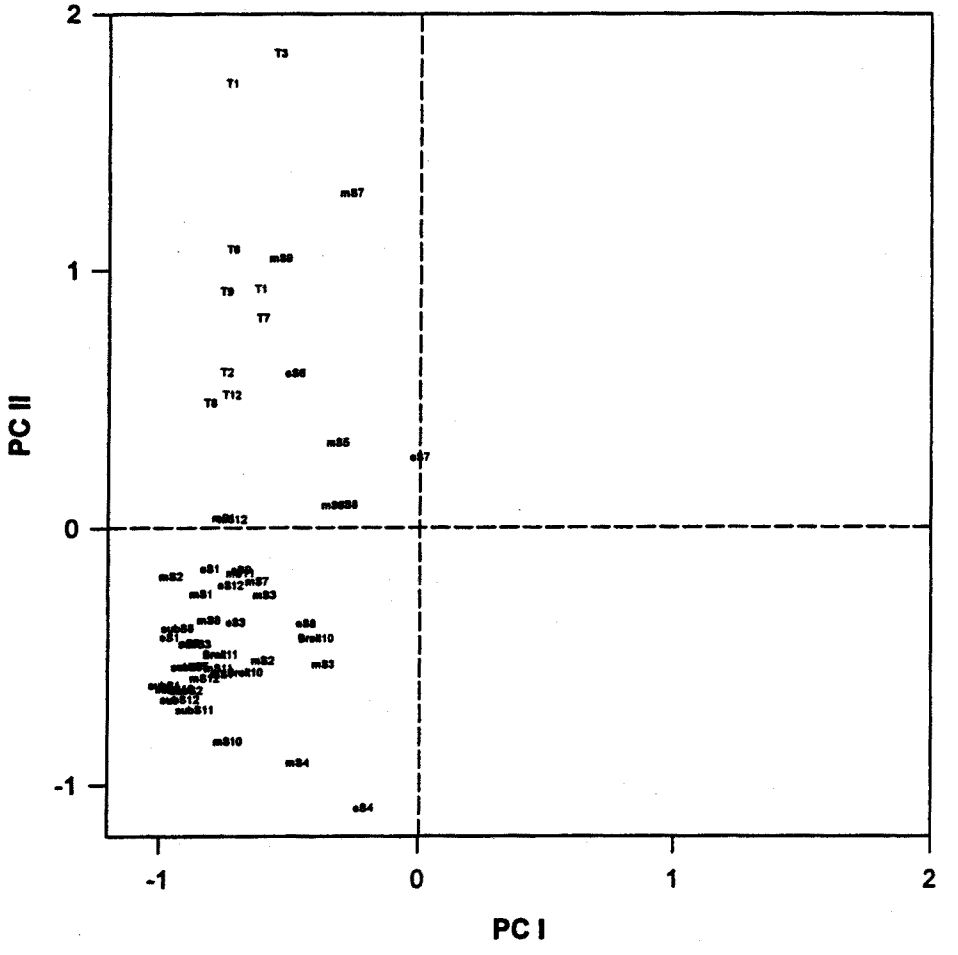


Fig. 2. Partial plot of the PCA (as in Fig. 1) for the Riera Major mid-channel sand (mS), stream-edge sand (eS), subsurface sand (subS), and tiles (T) and the Breitenbach (Breit).

The drastic seasonal changes in ectoenzymatic activities in the river Ter (chapter 6) are also expressed by the PCA analysis (Fig. 4). When drawing a line through the monthly samples, there seems to be a clear seasonality in epilithic biofilm metabolism for this Mediterranean river. The year of study (1994-95) was especially dry, determining the low summer activities and the high autumn activities when the flow was recovered (chapter 6).

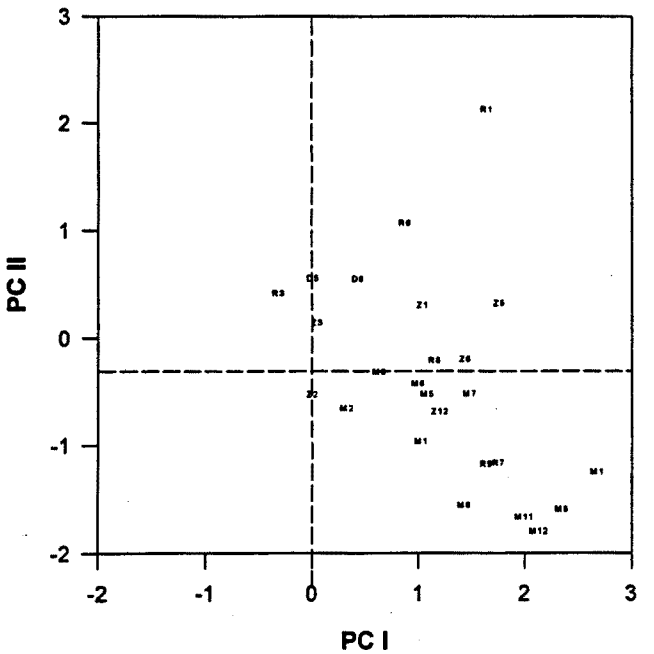


Fig. 3. Partial plot of the PCA (as in Fig. 1) for the algal patches of La Solana cyanobacterial crust: Mixed community (M), *Rivularia* community (R), *Zygnema-Spirogyra* community (Z), and diatom bloom (D).

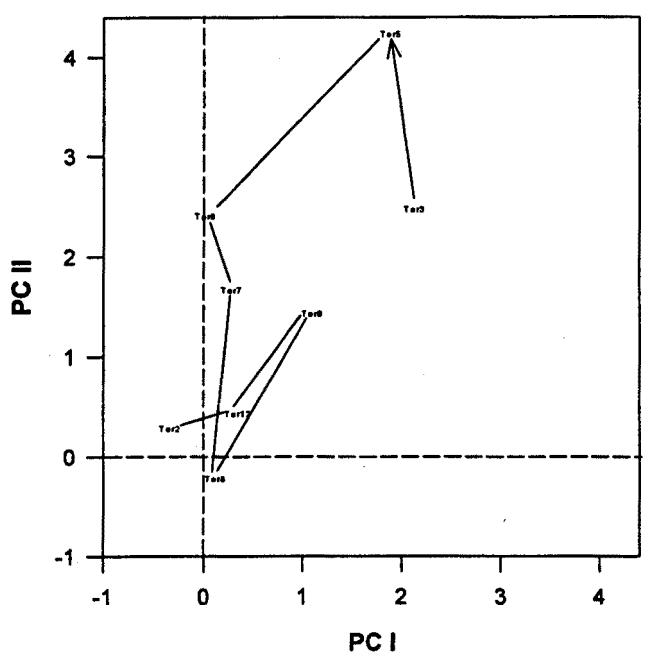


Fig. 4. Partial plot of the PCA (as in Fig. 1) for the river Ter. A line was drawn between the points to outline the seasonal sequence.

General trends in the Mediterranean stream biofilms

Correlation analysis of the environmental and biological parameters for the Mediterranean streams was performed using product-moment Pearson coefficient. In addition a Canonical Correlation Analysis (CCA) was performed between the heterotrophic activities (β-glucosidase, β-xylosidase, phosphatase and ETS) and the physico-chemical and biological variables (bacterial density, chlorophyll-a, bacteria/algae biomass ratio, DOC, DIC, SRP, nitrate, ammonia, pH, temperature, conductivity and light). CCA was designed in order to elucidate which variables of this second variable group (physico-chemical and biological) had the greatest weight in determining the variation of the heterotrophic activities in the Mediterranean streams.

The correlation analysis performed with all data from Riera Major, La Solana and the river Ter, showed several highly significant relationships (Tables 4 and 5). The three enzymes, β-glucosidase, β-xylosidase and phosphatase were significantly correlated with respiratory activity (Table 4), which indicates that enzymatic activities are an expression of heterotrophic activity. A significant correlation between ectoenzymes and bacterial activity (thymidine incorporation) was observed especially for β-glucosidase (Somville 1984, Chróst and Overbeck 1990, Chróst 1991b, Chróst 1994) and for proteolytic activity (Admiraal and Tubbing 1991, Gajewski and Chróst 1995). It has been concluded that ectoenzymes may be a useful indicator of the bacterial activity in aquatic environments (Gajewski and Chróst 1995). However, although a strong relationship between bacterial production (thymidine incorporation) and enzymatic activity was also found in the Adriatic Sea, the two activity measurements followed a different trend during a diatom bloom leaving unclear whether extracellular enzymatic activity and bacterial production are mediated by the same part of the active proportion of the bacterial community (Karner et al. 1992). For the Mediterranean streams, no significant correlations were found between ectoenzymatic activities and bacterial cell density (Table 4). This contradictory finding is possibly due to the presence of non-active bacteria in certain biofilms (i.e. in sand, Bott and Kaplan 1985), which have been included in the data set.

Relationships with physico-chemical variables have also been explored. The three ectoenzymes were in general significantly correlated to conductivity, DIC and nitrate (Table 5). More scattered correlations were also found with oxygen, DOC, SRP and ammonia, indicating that the nutrient content in stream water must be an important parameter for the regulation of the ectoenzymatic activities. These correlations suggest that there is a negative relationship between the enzymatic activities and discharge as will be discussed after the CCA analysis.

TABLE 4. Significant Pearson correlation coefficients between enzymatic activities, respiratory activity, bacteria and chlorophyll-a for the three Mediterranean study sites (Riera Major, La Solana, Ter). The level of significance is expressed by the star: *p<0.05, **p<0.01, ***p<0.001, n=80.

	β-xyl	Phosp.	ETS	Bact	Chl-a	Bac. biovol.	Bac/alg ratio	xyl/glu ratio
β-glucosidase	0.77***		0.38**		0.45***			
β-xylosidase	--	0.22*	0.30**					0.57***
Phosphatase		--	0.61***		0.27*	0.75***		
ETS			--		0.61***		-0.23*	
Bacteria				--	0.24*			
Chlorophyll-a					--			
Bact. biovolume						--		
Bac./alg. ratio							--	

TABLE 5. Significant Pearson correlation coefficients between enzymatic activities, respiratory activity, bacteria and chlorophyll, and the physical and chemical parameters for the three Mediterranean study sites (Riera Major, La Solana, Ter). The level of significance is expressed by the star: *p<0.05, **p<0.01, ***p<0.001, n=80.

	Cond	Oxygen	DOC	DIC	NH_4	NO_3	SRP	pH	Light	Temp.
β-glucosidase	0.70***	-0.39***	0.40***	0.54***	0.27*	-0.33**				0.30**
β-xylosidase	0.62***			0.55***		-0.29**				
Phosphatase	0.50***			0.57***		-0.23*				
ETS	0.51***	-0.36**		0.60***		-0.37**			0.57***	0.22*
Bacteria							0.29**			
Chlorophyll-a	0.52***	-0.28*		0.48***	0.25*		0.55***	0.31**	0.62***	0.22*
Bac. biovol.	0.43**			0.54***		-0.27*				-0.27*
Bac./alg.	-0.31**			-0.31**		0.23*				

However, to distinguish which variables were the best predictors for the variability of the heterotrophic biofilm metabolism, a CCA was performed with the data. It is obvious that the variation in the heterotrophic activities could be determined not only by a unique environmental or biological variable but by a combination of variables which will also be elucidated by the CCA.

The CCA determines linear combinations within the variables of each set when obtaining the canonical variables. The canonical variables obtained for each set are equal to the number of variables in the set with fewer variables, which will be named U_1, U_2, ... U_n for the left set and V_1, V_2, ... V_m for the right set. These linear combinations are removed from each set in order to obtain the maximum correlation (canonical R) between them (U_1 with V_1, U_2 with V_2,...). The canonical roots are pairs of variables (U_1V_1, U_2V_2,...). The correlation

between U_1 and V_1 is maximum, as is that between U_2 and V_2 under the condition that they are not correlated with U_1 and V_1, and successively.

The two variable sets chosen for this analysis and the initial results are summarized in Table 6. The analysis showed that the four canonical roots removed were significant ($p<0.5$, Manly 1995) (Table 7).

TABLE 6. Results of the CCA and the variables considered for the left set and right set.

Canonical R: 0.90053

Chi^2 (d.f. 48) = 185.78 p=0.0000

	Left set		Right set	
No. of variables	4		12	
Variance extracted	100.000%		45.6287%	
Total redundancy	56.2016%		26.7921%	
Variables:	Respiratory activity	ETS	Bacterial density	BAC
	β-glucosidase activity	GLU	Chlorophyll-a density	CHLA
	β-xylosidase activity	XYL	Bacteria/algae ratio	BAC/ALG
	Phosphatase activity	P	Dissolved organic carbon	DOC
			Dissolved inorganic carbon	DIC
			Soluble reactive phosphorus	SRP
			Nitrate	NO3
			Ammonia	NH4
			pH	PH
			Temperature	TEMP
			Conductivity	COND
			Incident light	LIGHT

TABLE 7. Chi-Square tests with successive roots removed by the CCA. The canonical correlation (R), squared canonical correlation (R^2), Chi-square, degrees of freedom (df) and probability (p) are shown for each root. All roots removed are significant ($p<0.5$).

	R canonical	R^2 can.	Chi^2	df	p
0	0,901	0,811	185,779	48	4,9E-18
1	0,744	0,554	80,006	33	9,03E-06
2	0,506	0,256	28,740	20	0,0931
3	0,380	0,145	9,917	9	0,357

To interpret each canonical variable we used the correlation between the variables and the canonical roots in each set (Table 8). The most indicative variables for each canonical variable are those which have the highest correlation (> + 0.5, < -0.5).

TABLE 8. Correlation of each variable to the canonical roots removed.

Left set	Root 1	Root 2	Root 3	Root 4
	U_1	U_2	U_3	U_4
ETS	-0,838	0,106	-0,455	0,282
GLU	-0,760	-0,292	0,207	-0,543
XYL	-0,486	-0,720	-0,186	-0,458
P	-0,693	-0,354	0,195	0,597
Right set	Root 1	Root 2	Root 3	Root 4
	V_1	V_2	V_3	V_4
BAC	-0,220	0,436	0,117	-0,104
CHLA	-0,765	0,357	-0,001	-0,016
BAC/ALG	0,287	0,071	0,064	0,010
DOC	-0,308	-0,298	0,733	-0,257
DIC	-0,774	-0,432	-0,092	0,277
SRP	-0,247	0,280	0,164	-0,366
NO3	0,467	0,138	0,126	0,022
NH4	-0,141	-0,234	0,581	-0,303
PH	-0,251	0,015	-0,070	0,118
TEMP	-0,332	0,098	0,167	-0,254
COND	-0,838	-0,457	0,095	-0,114
LLUM	-0,492	0,259	-0,199	0,335

Each canonical variable of the left and right side could be interpreted as follows:

Left set

U_1: low respiratory and ectoenzymatic activities

U_2: low β-xylosidase activity

U_3: low respiratory activity

U_4: high phosphatase activity, low β-glucosidase and β-xylosidase activities

Right set

V_1: late autumn-winter conditions (low conductivity and DIC, low chlorophyll-a density, low light and temperature, high nitrate)

V_2: spring conditions (low conductivity and DIC), importance of benthic biomass (high bacterial and chlorophyll-a density).

V_3: high DOC and ammonia concentration

V_4: low nutrient content (low phosphorus and ammonia), high incident light

The most significant correlation (that between U_1 and V_1, Table 7), indicates that low values of ectoenzymatic and respiratory activities are related to low values of conductivity, DIC, chlorophyll-a density, light and temperature, and high nitrate content. These conditions in Mediterranean streams are usually found in late autumn and winter when there is a high discharge (floods usually occur in autumn), low nutrient and mineral content resulting in low conductivity in the stream water and low benthic biomass. The reverse situation (high conductivity, DIC and chlorophyll-a density) will be attained in late spring and summer with the highest values of ectoenzymatic and respiratory activity. The positive relationship to the DIC content express the higher ectoenzymatic activities measured in calcareous streams (as discussed above, in the section *comparison of the studied streams*). A similar positive relationship between epilithic ectoenzymatic activities and conductivity as well as variables related to water quality was found by Chapell and Goulder (1994a) for several English streams. In the Mediterranean streams a direct relationship has been observed between conductivity and discharge (especially for the river Ter and Riera Major) (Sabater F 1988, A. Butturini, pers. comm.). This general conclusion is in disagreement with the increase in the ectoenzymatic activities and discharge observed in the river Ter (chapter 6). Such a difference could be related to the higher DOC content in stream water in higher-order rivers during high discharge periods, which could provide organic substrates for the enzymatic hydrolysis. However, the lack of values from the high flood events for the river Ter as well as the representativeness of the seasonal study in an especially dry year (low heterotrophic activities in summer) could influence in this positive relationship between enzymatic activity and discharge in the river Ter, therefore contrasting to the negative relationship suggested in the lower-order streams (Riera Major and La Solana).

Algal biomass (chlorophyll-a density) appears as the most important biological factor for the variations in the ectoenzymatic and respiratory activities. Algae are an important source of high quality organic matter for heterotrophs (chapter 3, 4, 5 and 7) and a suitable site for bacteria attachment (chapter 8). Photosynthetic activity and biomass are related with ectoenzymatic activity (chapter 8). Concluding, algal biomass has a stimulating effect on heterotrophic activities in Mediterranean stream biofilms.

The second canonical root show that low values of β-xylosidase activity were related to high chlorophyll-a and bacterial density in the biofilm. This combination of variables indicates the conditions when β-xylosidase activity is enhanced: high conductivity, DIC and DOC in streamwater, and low chlorophyll-a and bacterial density. This could be a further evidence of the greater dependence of the β-xylosidase activity on allochthonous than on autochthonous organic matter.

The third canonical root relates low values of respiratory activity (ETS) with high DOC and ammonia in the stream water, conditions which were attained in the studied Mediterranean streams during the dry season. Specially dry conditions were found during the annual study (1994-95), compared to other annual periods (Guasch 1995, Martí 1995). Particularly in La Solana, low values of respiratory activity were found during the drought

period (chapter 4.1), coinciding with a high DOC and ammonia content in the stream water. Depletion of nutrients (e.g. inorganic phosphorus) during these dry periods might limit the bacterial and algal metabolic activity, in spite of having enough substrates available for heterotrophs (high DOC).

The fourth canonical root relates the low nutrient concentrations (soluble reactive phosphorus and ammonia) in stream water to high values of phosphatase activity and low values of polysaccharidic enzymes. Phosphatase activity may be important in Mediterranean streams to obtain inorganic phosphorus when it is scarce in the stream water. This has been observed for La Solana stream (chapters 3 and 5) where this activity is enhanced when there is a low phosphorus concentration in stream water. However, the general use of phosphatase activity to indicate the phosphorus state of natural waters has been under discussion (Jansson et al. 1988).

It is generally accepted that temperature regulates the metabolic processes (e.g. Peters et al. 1987, Kaplan and Bott 1989) and thus ectoenzymatic activities (Chróst 1991b, Münster et al 1992, Wiebe et al. 1992). However this was not revealed to be an important parameter for the ectoenzymatic activities in the river systems studied. Significant correlations with temperature were found only with β-glucosidase and ETS activity (Table 5). Analogous studies which have analysed seasonal variations found that ectoenzymatic activity and temperature is not always correlated (Jones and Lock 1993, Hoch et al. 1996). Sediment bacteria do not show significant differences in bacterial growth when the temperature is increased by 5 degrees (Bott et al. 1984). Substrate concentration was more important than temperature (in the range 8-25°C) for bacterial growth in culture (Barillier and Garnier 1993). It is possible that in more extreme conditions there would be a clear response to temperature, as has been observed in a boreal lake (Tulonen et al. 1994). Substrate availability and nutrient content are probably the main ectoenzyme regulators in temperate streams (Sinsabaugh and Linkins 1988, Jones and Lock 1993). The apparent relationship to temperature could also be masked by the retarded response of extracellular enzyme activity to changing water temperature (Hoppe et al 1988).

Comparison with other lotic systems

Comparison with other systems is difficult since few studies which gather enzymatic, bacterial, algal, and environmental data are available. Those which better fullfil these requirements have been included in Table 9.

The ectoenzymatic activities measured in the Mediterranean stream biofilms were in general higher than those reported from European streams (e.g. Jones and Lock 1993, Chapell and Goulder 1994, Table 9) but similar to the values reported for the St. Regis River (New York) (Sinsabaugh et al. 1991a) and for the the Billabong periphyton in Australia (lentic environment) (Scoltz and Boon 1993). The different climatic characteristics (pluviosity, temperature, incident light) in each region could be related with such differences in heterotrophic activities. The European streams considered, which were in fact north-European

TABLE 9. Benthic β-glucosidase, β-xylosidase and phosphatase activities measured in several streams. Most of the data were measured spectrofluorometrically (by methylumbellyferyl or aminomethylcoumarinyl substrates), except data from Sinsabaugh et al. 1991a and Golladay and Sinsabaugh 1991, which were measured spectrophotometrically (by p-nitrophenyl substrates). Values (in range) are expressed in nmol $cm^{-2} h^{-1}$.

Location	latitude	order	open (O)/ forested (F)	DOC mg L^{-1}	β-Glucosidase activity	β-Xylosidase activity	Phosphatase activity	Chlorophyll μg cm^{-2}	Substrate	Source
Ryans 1 Billabong (Australia)	36° S	–	F	–	4.4-8.4	–	42-52	–	periphyton: photic z.	Scholtz & Boon 1993
					1.4-2.2		13-18		periphyton: aphotic z.	
Ryans 2 Billabong (Australia)	36° S	–	F	–	1.3-2.2	–	24-26	–	periphyton: photic z.	"
					1.1-1.8		11-13		periphyton: aphotic z.	
River Ter (Spain)	41°N	4	O	6.3	0.6-118.6	0.5-29.9	1.3-161.2	3.48-89.7	tiles	This study
Riera Major (Spain)	41°N	2	F	2.7	2.9-36.8	1.2-18.6	1.6-55.7	0.03-5.04	mid-channel sand	"
					3.8-60.7	0.8-26.8	0.6-34.1	0.1-7.6	stream-edge sand	
					4.1-8.6	1.7-4.1	2.3-25.6	0.04-0.7	subsurface sand	
					1.3-28.7	0.3-126.4	2.7-131.3	0.9-10.8	tiles	
La Solana (Spain)	42°N	2	O	3.8	26.8-124.1	13.2-152.9	23.4-393.7	2.0-24.6	Mixed comm.	"
					5.9-123.9	1.8-70.3	41.3-1602	2.8-27.2	Rivularia comm.	
					5.8-116.1	5.3-42.9	67.0-431.2	4.6-57.5	Zygn.-Spir. comm.	
					4.16-34.3	3.1-12.7	27.9-89.9	19.4-42.6	Diatom bloom	
St. Regis River (EUA)	44° N	4	F	5-20	20-40	4-20	30-50	0.75-3.25	glass slides	Sinsabaugh et al. 1991a
					38-47	10-50	400-1300	1.1-3.9	wood slides	"
					100-600	–	–	0-0.05	leaves	Golladay &Sinsabaugh1991
Rohrwiesenbach (Germany)	51° N	1	F	4.7	0.32-0.50	–	0.1-0.3	–	glass beads (in dark)	Freeman et al. 1993
					0.06-0.11		0.08-0.12		sediment	Marxsen & Witzel 1991
Jossa (Germany)	51° N	1	F	6.3	0.07-0.13	–	0.83-1.47	–	glass beads (in dark)	Freeman et al. 1993
					0.08-0.12		0.45-0.70		sediment	Marxsen & Witzel 1991
Breitenbach (Germany)	51° N	1	O/F	1.6	0.60-1.00	–	0.21-0.35	–	glass beads (in dark)	Freeman et al 1993
					0.05-0.086	–	0.031-0.065	–	sediment	Marxsen & Witzel 1990
					0.30-0.47	0.17-0.23	0.49-0.59	0.02-0.04	sediment	Romani & Marxsen (in
					3.20-3.86	3.08-4.23	0.04-7.38	0.20-3.30	natural stones	prep.)
					5.1-13.7	2.7-7.4	14.2-51.2	2.8-14.1	tiles	This study
Nant Waen (North Wales)	54° N	1	O	1.7	0.2-9.8	0.1-0.8	–	0.07-2.09	glass beads	Jones & Lock 1993
River Clywedog (North Wales)	54° N	4	F	2.9	0.5-2.7	0.1-2.4	–	0.01-0.55	glass beads	"
Driffield Beck (England)	55° N	1	O	–	0.67-4.94	0.02-1.78	0.43-4.07	0-19	atural stones	Chapell & Goulder 1994a
Birk Gill (England)	55° N	1	F	–	0.27-4.06	0.07-1.26	0.78-23.2	0-2.7	natural stones	"
Long Gill (England)	55°N	1	O	–	0.10-5.31	0.002-0.65	0.29-73.2	0-2	atural stones	"
Weighton Beck (England)	55° N	–	–	–	1.78-6.14	–	2.36-12.5	0.7-29.7	atural stones	Chapell & Goulder 1994b
Calcareous streams (England)	55° N	1	O/F	–	0.70-8.44	0.38-1.80	1.24-15.0	0.28-25.7	natural stones	Chapell & Goulder 1992
Millstone-grit streams (England	55° N	1	O	–	0.46-8.38	0.12-1.20	2.66-35.7	0.02-3.15	atural stones	"

streams (latitude >50° N, Table 9) have a lower light irradiance and temperature and a higher rainfall (especially in the north Wales streams) than the Mediterranean streams (Margalef 1989). However, the light irradiance, temperature and pluviosity in New York is similar than in the Mediterranean region (Margalef 1989). The high activities in the Billabong periphyton in Australia (36° S) could be due to the different habitat (lentic), with a developed photic and aphotic zone, which makes difficult its comparison to the other stream substrates considered. However, this site may indicate that the ectoenzymatic activities in perifiton are higher than in benthic substrates.

The ratio of β-xylosidase:β-glucosidase activity gives values rather similar for all the streams and substrates, being around 0.5 (Table 10), even though ectoenzymatic activities differ between geographical regions and stream benthic substrates. In all streams there is a major utilization of cellobiosic to xylobiosic molecules. The greater activity of β-glucosidase than β-xylosidase in all the stream biofilms considered could not be only a response to the composition of organic matter input (more cellulose than hemicellulose) but also a preference of bacteria to produce those ectoenzymes catalyzing more efficient reactions (Gazewski and Chróst 1995). The enzyme β-glucosidase splits β-linked polysaccharides found in a great variety of molecules, while β-xylosidase is involved in xylobiose degradation usually found in more complicated molecules.

It is suggested that a higher production of the β-xylosidase occurs only when it is strictly necessary to degrade organic matter with a large amount of hemicellulosic molecules, as will happen when allochthonous material is the main organic matter source. In this way, higher values of the β-xylosidase:β-glucosidase ratio (0.7-1) were observed in the mixed community of La Solana cyanobacterial crust, in the natural stones of the Breitenbach, in the wood substrates of the St. Regis River (Table 5), and in the dark-grown epilithic biofilms in Riera Major (chapter 8.2). These four substrates coincide in their low autochthonous input, allochthonous materials being the main organic matter source: low algal density in the mixed community of La Solana cyanobacterial crust; low chlorophyll, low incident light and accumulation of leaves in the Breitenbach natural stones in autumn; and low chlorophyll density and great accumulation of leaf material (from the riparian vegetation and the neighbouring high forested watershed) on the wood substrate of the St. Regis river). Therefore a high β-xylosidase:β-glucosidase ratio (ca. >0.6) in a given stream biofilm might indicate that allochthonous material is the major source of organic matter for the heterotrophs. In addition, the different substrate (xylobiosic or cellobiosic) that is being used by the heterotrophs might not be characteristic of a given stream but of a given streambed substrate, since in the same stream different values of this ratio have been calculated for the different substrates (Table 10). The microenvironment of each biofilm therefore plays a key role in the organic matter source to be used by the heterotrophs.

TABLE 10. β-xylosidase:β-glucosidase ratio in the different stream biofilms for the Mediterranean sites (left side) and for several European and American streams (right side) (from Table 9 when both β-glucosidase and β-xylosidase activities were available) (see bibliographic sources in Table 9). Values are means from monthly values and standard deviations (n as in Table 1) for the streams studied in this project and mean values for the results found in the bibliography.

β-xylosidase/β-glucosidase ratio

Mediterranean streams		European and American streams	
Site and substrate	Mean	Site and substrate	Mean
Riera Major		**Breitenbach**	
Current sand	0.48 (0.25)	Artificial substrates (tiles)	0.45 (0.22)
Littoral sand	0.44 (0.37)	Natural stones	1.03
Hyporheic sand	0.39 (0.08)	Sediment	0.52
Artificial substrates (tiles)	0.44 (0.24)	**St. Regis River**	
La Solana		Glass slides	0.40
Mixed community	0.71 (0.33)	Wood slides	0.71
Rivularia community	0.42 (0.08)	**Nant Waen** (glass beads)	0.25
Zygnema-Spirogyra community	0.49 (0.24)	**River Clywedog** (glass beads)	0.30
Diatom bloom	0.63 (0.3-0.9)	**Driffield Beck** (stones)	0.40
Ter		**Birk Gill** (stones)	0.38
Artificial substrates (tiles)	0.39 (0.29)	**Long Gill** (stones)	0.16
		Calcareous streams (stones)	0.33
		Millstone-grit streams (stones)	0.35

Relationships between ectoenzymatic activities and the bacterial/algal biomass ratio

The bacteria/algae ratio in terms of biomass (μg C cm^2 of biofilm) was calculated for the studied streams (Riera Major, La Solana, river Ter and Breitenbach) at the different substrate types, and for those streams from Table 9 where both bacterial density and chlorophyll was determined (from Jones and Lock 1993, Chapell and Goulder 1994a, Chapell and Goulder 1994b, Chapell and Goulder 1992) being all them North-european. Algal biomass was transformed from chlorophyll-a density using the conversion factor C:Chl of 60, which was in the middle of the range 20-100 suggested by Margalef for algae of the river benthos (1983) and applied in a mountain stream benthic community (Geesey et al. 1978). Although higher C:Chl ratios have been measured in cyanobacteria dominated communities (900-2500 for a *Nostoc* sp. dominated community, 227-1400 for *Phormidium* sp

dominated community, Vincent and Howard-Williams 1986; but 45.8 for *Phormidium* sp, Hawes 1993), measurements for diatoms, which were the most abundant in the biofilms studied, have always been lower (29.7-41.8 for *Thalassiosira* sp, Montagnes et al. 1994, 25-100 for *Thalassiosira pseudonana*, Geider and MacIntyre 1996). Similar conversion factors have been reported for natural phytoplankton (Banse 1977).

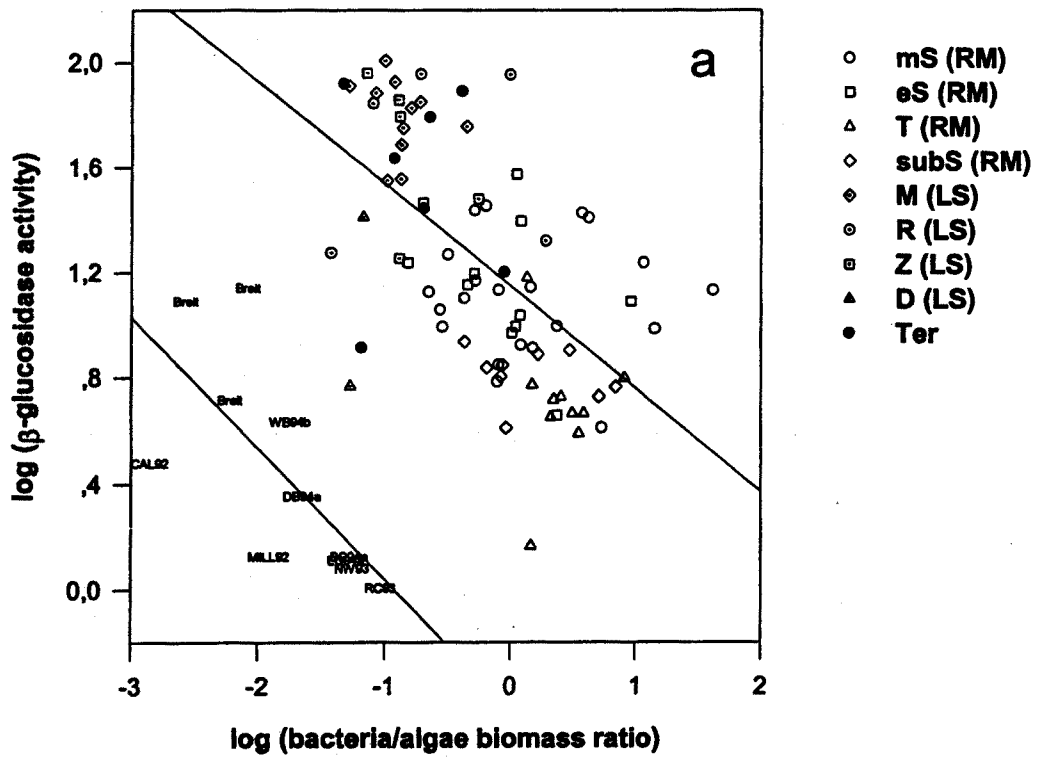
Bacterial biomass was calculated from bacterial cell biovolume using the conversion factor of 2.2 10^{-13} gC μm^{-3} (Bratbak and Dundas 1984, Kemp 1990) (chapter 2). For the results found in the bibliography bacterial biomass was calculated from bacterial density results and assuming a mean bacterial cell volume of 0.1 μm^3.

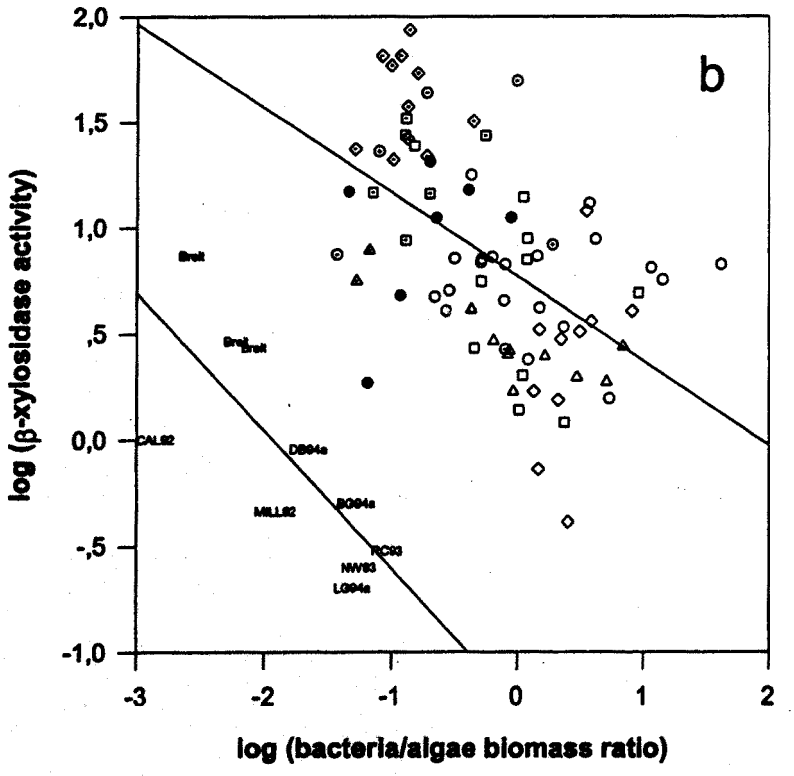
TABLE 11. Bacteria/algae biomass ratio in the different stream biofilms for the Mediterranean study sites (left side) and for several European streams (right site) (see bibliographic source in Table 9). Values are means from monthly values and standard deviations (n as in Table 1) for the streams studied in this project and mean values for the results found in the bibliography. The symbols for the European streams are those used in Fig. 5.

Bacteria/algae biomass ratio

Mediterranean streams		European streams		
Site and substrate	Mean	Site and substrate		Mean
Riera Major		**Breitenbach**		
Current sand	4.60 (9.52)	Artificial substrates (tiles)	Breit	0.006 (0.003)
Littoral sand	1.81 (2.63)	**Nant Waen** (glass beads)	NW93	0.055
Hyporheic sand	2.26 (2.31)	**River Clywedog** (glass beads)	RC93	0.092
Artificial substrates (tiles)	2.98 (2.01)	**Driffield Beck** (stones)	DB94a	0.022
La Solana		**Birk Gill** (stones)	BG94a	0.052
Mixed community	0.15 (0.10)	**Long Gill** (stones)	LG94a	0.049
Rivularia community	0.66 (0.72)	**Weighton Beck** (stones)	WB94b	0.018
Zygnema-Spirogyra community	0.20 (0.17)	**Calcareous streams** (stones)	CAL92	0.002
Diatom bloom	0.06 (0.009)	**Millstone-grit streams** (stones)	MILL92	0.012
Ter				
Artificial substrates (tiles)	0.25 (0.28)			

The different stream biofilms showed a different bacteria/algae biomass ratio (Table 11). The more heterotrophic biofilms (more abundant in bacteria) were found in Riera Major, especially in the sandy substrate, while La Solana and river Ter were more autotrophic (more abundant in algae). The Breitenbach epilithic biofilm had a drastically higher algal biomass than bacterial. The other European streams have also lower bacteria/algae biomass ratios than the Mediterranean streams (Table 11). The bacteria/algae biomass ratio can be used as an indicative value of the relative amount of algal and bacterial biomass on each biofilm. However, empirical values have to be managed with care, since the utilization of a single


a
log (β-glucosidase activity)
2,0
1,6
1,2
,8
,4
0,0
-3
-2
-1
0
1
2
log (bacteria/algae biomass ratio)
Brait
WB94b
CAL92
DB94a
MILL92
RC93
mS (RM)
eS (RM)
T (RM)
subS (RM)
M (LS)
R (LS)
Z (LS)
D (LS)
Ter


b
log (β-xylosidase activity)
2,0
1,5
1,0
,5
0,0
-,5
-1,0
-3
-2
-1
0
1
2
log (bacteria/algae biomass ratio)
Brait
CAL92
DB94a
MILL92
RC93
NW93
LG94a

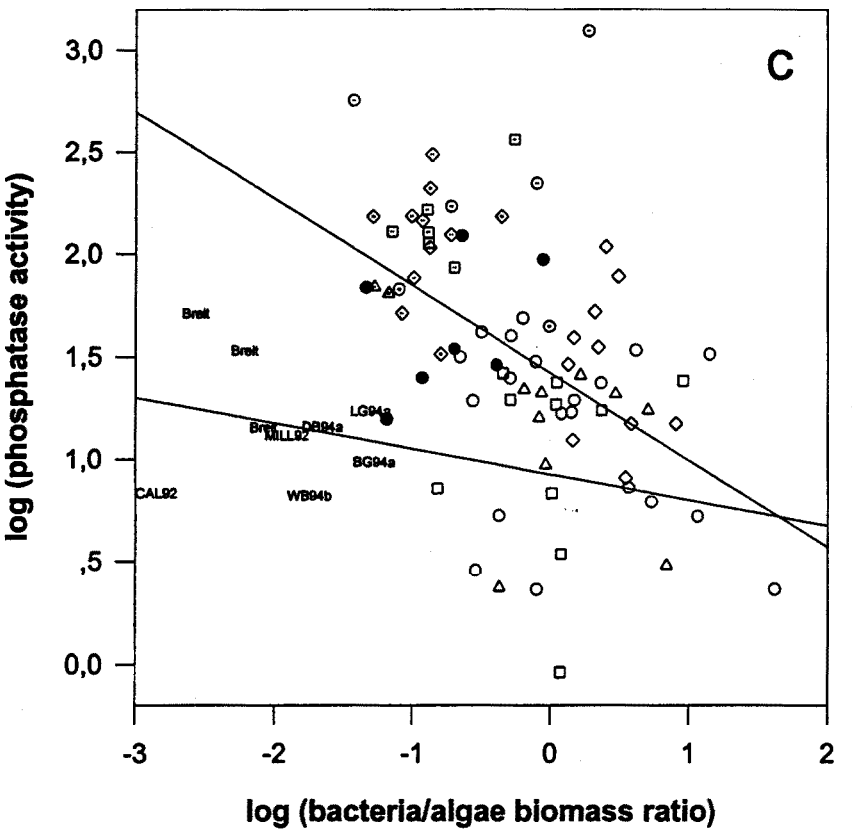


Fig. 5. Relationship between the bacteria/algae biomass ratio (in logarithms) and the ectoenzymatic activities (in logarithms) in the different stream biofilms: the Mediterranean streams: Riera Major (mid-channel sand, mS, stream-edge sand, eS, subsurface sand, subS, and tiles, T), La Solana (mixed community, M, Rivularia community, R, Zygnema-Spirogyra community, Z, diatom bloom, D), and river Ter; and several North European streams: the Breitenbach (Breit), Nant Waen (NW93), River Clywedog (RC93), Driffield Beck (DF94a), Birk Gill (BG94a), Long Gill (LG94a), Weighton Beck (WB94b), 7 English Calcareous streams (CAL92), 8 English Millstone-grit streams (MILL92). a) β-glucosidase, b) β-xylosidase and c) phosphatase. The significant regression line for each enzyme and for the Mediterranean and European streams is also shown following the equations indicated in Table 12.

conversion factor for the algal and bacterial biomass could imply deviations from the real values in diverse communities. Especially in La Solana, where the cyanobacteria are an important component of the biofilm species, the algal biomass is possibly underestimated, and thus this ratio would be lower than the calculated one.

In this section, regularities in the relationships between ectoenzymatic activities and the bacteria/algae biomass ratio are explored in the studied streams and compared to the North-european streams considered (Table 11). These relationships were analyzed after drawing the scatter plot and by performing non-linear regression analyses. Since a potential

relationship was found for the three enzymes, data were transformed to logarithms and a linear regression was performed. The relationship between the bacteria/algae biomass ratio and the respiratory activity was also investigated for the Mediterranean streams.

Significant negative relationships were found between the bacteria/algae biomass ratio and the three ectoenzymatic activities for the Mediterranean streams studied (Fig. 5, Table 12). This relationship indicates that in highly heterotrophic biofilms (high values for the bacteria/algae biomass ratio) ectoenzymatic activities are lower than in more autotrophic biofilms (low values for the bacteria/algae biomass ratio) suggesting that there is a limitation to hydrolytic activities in the more heterotrophic biofilms. When the bacterial biomass of the biofilm is higher than the algal biomass, the ectoenzymatic activities do not increase but decrease. The highest heterotrophic biofilms were those found in Riera Major. Although allochthonous organic matter input may be an important carbon source for the heterotrophs in the Riera Major (chapter 3), ectoenzymatic activities could be substrate limited when the algal biomass is low. In contrast, when the bacteria/algae ratio is low (i.e. La Solana and river Ter, Table 11) ectoenzymatic activities were higher (Fig. 5). Therefore, higher algal biomass with respect to bacteria enhances the activity of the ectoenzymes. An extreme example of this tendency is the low ectoenzymatic activities measured in the dark incubated biofilms of Riera Major (chapter 8.2), where algae were very scarce (bacteria/algae biomass ratio =8.8, on average). It seems that given a bacteria/algae biomass ratio of a stream biofilm, the potential ectoenzymatic activities have a thereshold which is not exceeded. A part from stream differences there seems to be an upper limit for the enzymatic activities.

Related with this pattern, it is observed a positive correlation between β-glucosidase and phosphatase to chlorophyll-a (Table 4). This indicates the importance of algae as an organic matter source for the heterotrophs, which is cleaved faster than allochthonous materials (chapter 5).

Significant negative relationships between the bacteria/algae biomass ratio and β-glucosidase and β-xylosidase activities were also found when considering the Breitenbach and other 8 European stream sites described in Table 9 (Fig. 5, Table 12). However, any significant relationship could be stablished for the phosphatase activity. The slopes of the regression lines were similar but the constants were lower than those found in the Mediterranean streams (Table 12), as a result of the lower ectoenzymatic characteristic of the European streams. Similar slopes in Mediterranean and European streams indicates that, at least for the studied systems, there is a general decrease in ectoenzymatic activities when the bacteria/algae biomass ratio increases. It is worth to be noted that calcareous streams are placed on the left side of the graph indicating a lower bacteria/algae biomass ratio and a higher ectoenzymatic activity in such habitats (La Solana and Ter for the Mediterranean streams and the seven headwater English calcareous streams, CAL92, for the European streams, Fig. 5).

TABLE 12. Equations for the linear regressions obtained between the (log) ectoenzymatic activities and the (log) bacteria/algae biomass ratio (bac/alg) with Mediterranean and North-european streams. Coefficients of determination (R^2), degrees of freedom (df) and significance of the F-Fischer (F and signif. F) are also shown.

Equation	R^2	df	F	signif F
Mediterranean streams				
log β-glucosidase = 1.15-0.39 (log bac/alg)	0.355	79	43.55	0.000
log β-xylosidase = 0.77-0.40 (log bac/alg)	0.286	78	31.18	0.000
log Phosphatase = 1.42-0.42 (log bac/alg)	0.231	79	23.72	0.000
log ETS = -0.02-0.56 (log bac/alg)	0.437	72	55.99	0.000
North-european streams				
log β-glucosidase = -0.46-0.49 (log bac/alg)	0.500	9	9.01	0.015
log β-xylosidase = -1.26-0.65 (log bac/alg)	0.587	8	11.37	0.010
log Phosphatase= 0.92-0.12 (log bac/alg)	0.051	7	0.38	0.558

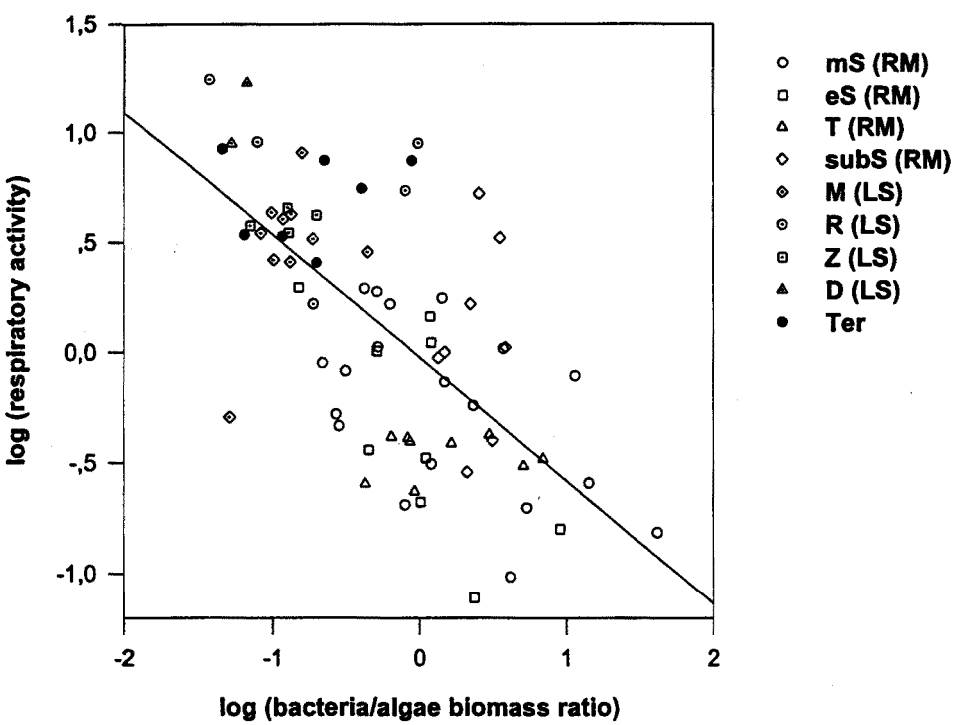


Fig. 6. Relationship between the bacteria/algae biomass ratio (in logarithms) and the respiratory activity in Riera Major, La Solana and river Ter biofilms (symbols as in Fig. 5). The significant regression line is also shown and the equation is indicated in Table 12.

Respiratory activity also showed a significant negative relationship with the algae/bacteria biomass ratio (Fig. 6). The higher slope than those found for ectoenzymatic activities might indicate that there is a considerable contribution of algae to the respiratory activity. Furthermore, it also stresses that algae are relevant for the heterotrophic metabolism of the biofilm.

Conclusions and perspectives

Autochthonous organic matter, also known as high quality material for its lability, plays an important role for the hydrolysis capacity of the heterotrophs in biofilms with low chlorophyll density. The algal content of a highly heterotrophic biofilm in an oligotrophic stream is a valuable organic matter source for the heterotrophs. In more autotrophic biofilms, the role of algae decreases as a regulator parameter for the heterotrophic activity while the geochemical features and environmental conditions of the stream become important for the microbial activity.

In this study, special attention has been given to the role of the autotrophs in the biofilm metabolism, and it is concluded that they play an important role. Less importance is generally given to the autochthonous input for the microbial loop in flowing waters than to the allochthonous input (e.g. Meyer 1994), possibly as a result of more study sites described in the literature being devoted to forested streams.

Organic matter use in stream biofilms could experience variations in the short-time scale. An analysis of the hourly and daily changes in ectoenzymatic activity probably would give more light into the detailed dynamics of organic matter processing, especially on what concerns to the role of heterotrophs and their link with the autotrophs. In the planktonic environment it has been observed an hourly variation of ectoenzymatic activities following the variations in the primary production (Karner and Rassoulzadegan 1995).

Furthermore, to widely analyse the factors which control the hydrolytic capacity of the stream biofilms, we should also study allochthonous input, such as the specific analysis of DOC composition. In a large number of studies of organic matter cycling in streams, the influence of DOC composition for microbial uptake and growth, rather than DOC concentration, has been suggested and noted (e.g. Bärlocher and Murdoch 1989, Hedin 1990, Middelboe and Sondergaard 1993, Koetsier III et al. 1997), strongly encouraging this analysis to a major knowledge of the microbial loop in stream ecosystems (Meyer 1994). The DOC composition of the stream water, and therefore the lability or recalcitrancy of the compounds being transported will determine the quantity of ectoenzymes synthesized and their activities.

On the other hand, a wider approach to the bacterial heterotrophic activity (i.e measuring incorporation of organic substrates and bacterial production) should be planned as

a complement to the ectoenzymatic activities since they are direct measures of heterotrophic activity.

Multidisplinarity is probalby necessary since the complication of all different appoaches to the study of organic matter use in stream environments. However, by knowing the natural substrate concentration of a given organic compound, the kinetic parameters of the specific ectoenzyme and the incorporation of this compound by the heterotrophs, we will be able to calculate the real hydrolytic activity and thus the total organic matter which is being used by the biofilm community and therefore the self depuration capacity of a stream stretch.

References

Abdelahad, N. and Bazzichelli, G. (1989) Structure and Composition of Living Stromatolitic Mats from the Terrestrial Environment (Locality: Grotta dell'Inferniglio, Latium, Italy). Cryptogamic Botany 1: 219-225.

Admiraal, G. and Tubbing, G. M. J. (1991) Extracellular enzyme activity associated with suspended matter in the River Rhine. Freshwater Biology 26: 507-517.

Amon, R. M. W. and Benner, R. (1996) Bacterial utilization of different size classes of dissolved organic matter. Limnology and Oceanography 41 (1): 41-51.

APHA (1989) Standard Methods for the examination of water and wastewater, 17 th Edition. American Public Health Association, Washington, 1134 pp.

Armengol, J., Sabater, S., Vidal, A., Sabater, F. (1991). Using the rescaled range analysis for the study of hydrological records: the river Ter as an example. Oecologia aquatica 10: 21-33.

Armengol, J., Guasch, H., Martí, E., Sabater, F., Sabater, S. and Romaní, A. M. (1993) Variacions en la càrrega de nutrients a la conca del riu Ter i el seu efecte sobre les comunitats d'indicadors biològics. Memòria final. Departament d'Ecologia de la Universitat de Barcelona i Junta de Sanejament del Departament de Medi Ambient, 60 pp.

Atlas, R. M. and R. Bartha (1987) Biogeochemical Cycling: Carbon, Hydrogen, and Oxygen. In: R. M. Atlas and R. Bartha (eds.). Microbial Ecology, Fundamentals and Applications. Second Edition. The Benjamin/Cummings Publishing Company, Inc. , p. 307-332.

Azam, F. and Hodson, R. E. (1981) Multiphasic kinetics for D-glucose uptake by assemblages of natural marine bacteria. Marine Ecology Progress Series 6: 213-222.

Azam, F., Fenchel, T., Field, J. G., Gray, J. S., Meyer-Reil, L. A. and Thingstad, F. (1983) The Ecological Role of Water-Column Microbes in the Sea. Marine Ecology Progress Series 10: 257-263.

Azam, F. and Cho, B. C. (1987) Bacterial utilization of organic matter in the sea. In: M. Fletcher, T. R. J. Gray and J. G. Jones (eds.) Ecology of Microbial Communities. Cambridge University Press, Cambridge, p. 261-281.

Banse, K. (1977) Determining the Carbon-to-Chlorophyll Ratio of Natural Phytoplankton. Marine Biology 41: 199-212.

Barillier, A. and Garnier, J. (1993) Influence of Temperature and Substrate Concentration on Bacterial Growth Yield in Seine River Water Batch Cultures. Applied and Environmental Microbiology 59 (5):1678-1682.

Bärlocher, F. and Murdoch, J. H. (1989) Hyporheic biofilms - a potential food source for interstitial animals. Hydrobiologia 184: 61-67.

Bateston, M. M. and Ward, D. M. (1988) Photoexcretion and fate of glycolate in a hot spring cyanobacterial mat. Applied and Environmental Microbiology 54: 1738-1743.

Benner, R., Lay, J., K'nees, E. and Hodson, R. E. (1988) Carbon conversion efficiency for bacterial growth on lignocellulose: Implications for detritus-based food webs. Limnology Oceanography 33 (6, part 2): 1514-1526.

Biggs, B. J. and Close, M. E. (1989) Periphyton biomass dynamics in gravel bed rivers: the relative effects of flows and nutrients. Freshwater Biology 22: 109-231.

Biggs, B. J. F. and Thomsen, H. A. (1995) Disturbance of stream periphyton by perturbations in shear stress: time to structural failure and differences in community resistance. Journal of Phycology 31: 233-241.

Bilby, R. E. and Bisson, P. A. (1992) Allochthonous versus autochthonous organic matter contributions to the trophic support of fish populations in clear-cut and old-growth forested streams. Canadian Journal of Fisheries and Aquatic Sciences 49: 540-551.

Bird, D. F. and Kalff, J. (1984) Empirical Relationships between Bacterial Abundance and Chlorophyll Concentration in Fresh and Marine Waters. Canadian Journal of Fisheries and Aquatic Sciences 41: 1015-1023.

Bjoernsen, P. K. (1988) Phytoplankton exudation of organic matter: Why do healthy cells do it? Limnology and Oceanography 33(1): 151-154.

Blenkinsopp, S. A. and Lock, M. A. (1990) The measurement of Electron Transport System activity in river biofilms. Water Research 24 (4):441-445.

Blenkinsopp, S. A. and Costerton, J. W. (1991) Understanding bacterial biofilms. Tibtech 9: 138-143.

Blenkinsopp, S. A., Gabbott, P. A., Freeman, C. and Lock, M. A. (1991) Seasonal trends in river biofilm storage products and electron transport system activity. Freshwater Biology 26: 21-34.

Blenkinsopp, S. A. and Lock, M. A. (1992) Impact of storm-flow on electron transport system activity in river biofilms. Freshwater Biology 27: 397-404.

Boavida, M. J. (1991) Search for phosphomonoesters in Lake Maggiore (N. Italy) during summer. Memorie dell'Istituto Italiano di Idrobiologia "Dott. Marco De Marchi" 49: 19-27.

Bögershausen, A. and Marxsen, J (1995) Die Enzymspezifität von Methylumbelliferyl-Verbindungen. In: Bögershausen, A., Hendel, B., Marxsen, J. and Preuss, G. Messung extrazellulärer Enzymaktivitäten im Grundwasser. Deutscher Verband für Wasserwirtschaft und Kulturbau e. V. (DVWK)- Fachausschuss Grundwasserbiologie, Bonn, p. 1-77.

Boon, P. I. (1989) Organic matter degradation and nutrient regeneration in Australian freshwaters: I. Methods for exoenzyme assays in turbid aquatic environments. Archiv für Hidrobiologie 115: 339-359.

Boon, P. I. (1991) Enzyme activities in Billabongs of Southeastern Australia. In: R. J. Chróst (ed.) Microbial enzymes in aquatic environments. Springer-Verlag, New York, p. 286-297.

Boon, P. I. (1993) Organic matter degradation and nutrient regeneration in Australian fresh waters: III. Size fractionation of phosphatase activity. Archiv für Hydrobiologie 126(3): 339-360.

Boschker, H. T. S., Bertilsson, S. A., Dekkers, E. M. J. and Cappenberg, T. E. (1995) An inhibitor-based method to measure initial decomposition of naturally occurring polysaccharides in sediments. Applied and Environmental Microbiology 61(6): 2186-2192.

Boston, H. L. and Hill, W. R. (1991) Photosynthesis-light relations of stream periphyton communities. Limnology and Oceanography 36: 644-656.

Bott, T. L., Kaplan, L. A. and Kuserk, F. T. (1984) Benthic bacterial biomass supported by streamwater dissolved organic matter. Microbial Ecology 10: 335-344.

Bott, T. L., Brock, J. T., Dunn, C. S., Naiman, R. J., Ovink, R. W. and Petersen, R. C. (1985) Benthic community metabolism in four temperate stream systems: An inter-biome comparison and evaluation of the river continuum concept. Hydrobiologia 123: 3-45.

Bott, T. L. and Kaplan, L. A. (1985) Bacterial Biomass, Metabolic State, and Activity in Stream Sediments: Relation to Environmental Variables and Multiple Assay Comparisons. Applied and Environmental Microbiology 50 (2): 508-522.

Boulton, A. J. and Boon, P. I. (1991) A review of methodology used to measure leaf litter decomposition in lotic environments: time to turn over and old leaf? Australian Journal of Marine and Freshwater Research 42: 1-43.

Bratbak, G. and Dundas, I. (1984) Bacterial dry matter content and biomass estimations. Applied and Environmental Microbiology 48: 755-757.

Bretschko, G. (1995) Running water ecosystems - a bare field for modelling? Ecological Modelling 78(1-2): 77-81.

Bretschko, G. and Leightfried, M. (1988) Distribution of organic matter and fauna in a second order, alpine gravel stream (Ritordat-Lunz study area, Austria). Verhandlungen der Internationalen Vereinigung für Theoretische und Andgewandte Limnologie 23: 1333-1339.

Brock, T. D. and Clyne, J. (1984) Significance of Algal Excretory Products for Growth of Epilimnetic Bacteria. Applied and Environmental Microbiology 47(4): 731-734.

Burkholder, J. M., Wetzel, R. G. and Klomparens, K. L. (1990) Direct comparison of phosphate uptake by adnate and loosely attached microalgae within an intact biofilm matrix. Applied and Environmental Microbiology 56: 2882-2890.

Burns, R. G. (1983) Extracellular enzyme-substrate interactions in soil. In: J. H. Slater, R. Whittenbury and J. W. T. Wimpenny (eds.) Microbes in their Natural Environments. Symposium 34. The Society for General Microbiology. Cambridge University Press, Cambridge, p. 249-298.

Button, D. K. (1991) Biochemical Basis for Whole-Cell Uptake Kinetics: Specific Affinity, Oligotrophic Capacity, and the Meaning of the Michaelis Constant. Applied and Environmental Microbiology 57(7): 2033-2038.

Button, D. K. (1994) The physical base of marine bacterial ecology. Microbial Ecology 28: 273-285.

Cammen, L. M. (1982) Effect of Particle Size on Organic Content and Microbial Abundance Within Four Marine Sediments. Marine Ecology Progress Series 9: 273-280.

Canfield, D. E. and Des Marais, D. J. (1993) Biogeochemical cycles of carbon, sulfur, and free oxygen in a microbial mat. Geochimica et Cosmochimica Acta 57: 3971-3984.

Carlson, C. A. and Ducklow, H. W. (1996) Growth of bacterioplankton and consumption of dissolved organic carbon in the Sargasso Sea. Aquatic Microbial Ecology 10: 69-85.

Chafetz, H. S. and Buczynski, C. (1992) Bacterially Induced Lithification of Microbial Mats. Palaios 7: 277-293.

Chapell, K. R. and Goulder, R. (1992) Epilithic extracellular enzyme activity in acid and calcareous headstreams. Archiv für Hydrobiologie 125 (2): 129-148.

Chapell, K. R. and Goulder, R. (1994a) Seasonal variation of extracellular enzyme activity in three diverse headstreams. Archiv für Hydrobiologie 130(2): 195-214.

Chapell, K. R. and Goulder, R. (1994b) Enzymes as river pollutants and the response of native epilithic extracellular-enzyme activity. Environmental Pollution 86: 161-169.

Chapell, K. R. and Goulder, R. (1995) A Between-River Comparison of Extracellular-Enzyme Activity. Microbial Ecology 29: 1-17.

Characklis, W. G., McFeters, G. A. and Marshall, K. C. (1990) Physiological ecology in biofilm systems. In: W. G. Characklis and K. C. Marshall (eds.) Biofilms. John Wiley & Sons, Inc., New York, p 341-394.

Cho, B. C., Choi, J.-K., Chung, C.-S. and Hong, G. H. (1994) Uncoupling of bacteria and phytoplankton during a spring diatom bloom in the mouth of the Yellow Sea. Marine Ecology Progress Series 115: 181-190.

Christensen, B. E. and Characklis, W. G. (1990) Physical and chemical properties of biofilms. In: W. G. Characklis and K. C. Marshall (eds.) Biofilms. John Wiley & Sons, Inc., New York, p. 93-130.

Christensen, H., Olsen, R. A. and Bakken, L. R. (1995) Flow cytometric measurements of cell volumes and DNA contents during culture of indigenous soil bacteria. Microbial Ecology 29: 49-62.

Chróst, R. J. (1981) The composition and bacterial utilization of DOC released by phytoplankton. Kieler Meeresforsch., Sonderh. 5: 325-332.

Chróst, R. J. (1989) Characterization and significance of β-glucosidase activity in lake water. Limnology and Oceanography 34(4): 660-672.

Chróst, R. J. (1990) Microbial Ectoenzymes in Aquatic Environments. In: J. Overbeck and R. J. Chróst (eds.) Aquatic Microbial Ecology: Biochemical and Molecular Approaches. Springer-Verlag, New York, p. 47-78.

Chróst, R. J. (1991a) Ectoenzymes in aquatic environments: Microbial strategy for substrate supply. Verhandlungen der Internationalen Vereinigung für Theoretische und Andgewandte Limnologie 24: 2597-2600.

Chróst, R. J. (1991b) Environmental Control of the Synthesis and Activity of Aquatic Microbial Ectoenzymes. In: J. Chróst (ed) Microbial Enzymes in Aquatic Environments. Brock/ Springer Verlag, p. 29-59.

Chróst, R. J. (1994) Microbial Enzymatic Degradation and Utilitzation of Organic Matter. In: R. C. Jürgen Overbeck (ed) Microbial Ecology of Lake Pluβsee. Springer-Verlag, p. 118-174.

Chróst, R. J. and Krambeck, H. J. (1986) Fluorescence correction for measurements of enzyme activity in natural waters using methylumbelliferyl-substrates. Archiv für Hydrobiologie 106(1): 79-90 .

Chróst, R. J., Münster, U., Rai, H., Albrecht, D., Witzel, K. and Overbeck, J. (1989) Photosynthetic production and exoenzymatic degradation of organic matter in the euphotic zone of a eutrophic lake. Journal of Plankton Research 11(2): 223-242.

Chróst, R. and Overbeck, J. (1990) Substrate-ectoenzyme interaction: significance of β-glucosidase activity for glucose metabolism by aquatic bacteria. Archiv für Hydrobiologie Ergebnisse der Limnologie 34: 93-98.

Chróst, R. J. and Rai, H. (1993) Ectoenzyme Activity and Bacterial Secondary Production in Nutrient-Impoverished and Nutrient-Enriched Freshwater Mesocosms. Microbial Ecology 25: 131-150.

Chróst, R. J. and Riemann, B. (1994) Storm-stimulated enzymatic decomposition of organic matter in benthic/pelagic coastal mesocosms. Marine Ecology Progress Series 108: 185-192.

Chrzanowski, T. H., Crotty, R. D. and Hubbard, G. J. (1988) Seasonal Variation in Cell Volume of Epilimnetic Bacteria. Microbial Ecology 16: 155-163.

Cohen, Y. and Rosenberg, E. (1989) Microbial mats: Physiological ecology of benthic microbial communities. American Society of Microbiology, Washington.

Cole, J. J. (1982) Interactions between bacteria and algae in aquatic ecosystems. Annual Review of Ecology and Systematics 13: 291-314.

Cole, J. J., Findlay, S. and Pace, M. L. (1988) Bacterial production in fresh and saltwater ecosystems: a cross-system overview. Marine Ecology Progress Series 43: 1-10.

Conners, M. E. and Naiman, R. J. (1984) Particulate allochthonous inputs: relationships with stream size in an undisturbed watershed. Canadian Journal of Fisheries and Aquatic Sciences 41: 1473-1484.

Cooksey, K. E. and Wigglesworth-Cooksey, B. (1995) Adhesion of bacteria and diatoms to surfaces in the sea: a review. Aquatic Microbial Ecology 9: 87-96.

Cotner, J. B. and Wetzel, R. G. (1991) Bacterial phosphatases from different habitats in a small, hardwater lake. In: R. J. Chróst (ed.) Microbial Enzymes in Aquatic Environments. Springer-Verlag, New York, p. 187-205.

Cotner, J. B. and Wetzel, R. G. (1992) Uptake of dissolved inorganic and organic phosphorus compounds by phytoplankton and bacterioplankton. Limnology and Oceanography 37(2): 232-243.

Cox, E. J. (1990a) Studies on the algae of a small softwater stream. I. Occurence and distribution with particular reference to the diatoms. Archiv für Hydrobiologie, Supplement 83(4): 525-552.

Cox, E. J. (1990b) Studies on the algae of a small softwater stream II. Algal standing crop (measured by chlorophyll-a) on soft and hard substrata. Archiv für Hydrobiologie, Supplement 83(4): 553-566.

Coxson, D. S. and Kershaw, A. K. (1983) Rehydration response of nitrogenase activity and carbon fixation in terrestrial *Nostoc commune* from *Stipa-Bouteloa* grassland. Canadian Journal of Botany 61: 2658-2668.

Cummins, K. W., Sedell, J. R., Swanson, F. J., Minshall, G. W., Fisher, S. G., Cushing, C. E., Petersen, R. C. and Vannote (1983) Organic matter budgets for stream ecosystems: problems in their evaluation. In: J. R. Barnes and G. W. Minshall (eds.) Stream ecology: application and testing of general ecological theory. Plenum Press, New York, p. 299-353.

Cummins, K. W., Wilbach, M. A., Gates, D. M., Perry, J. B. and Taliaferro, W. B. (1989) Shredders and riparian vegetation. BioScience 39: 24-30.

Dahm, C. N. (1981) Pathways and Mechanisms for Removal of Dissolved Organic Carbon from Leaf Leachates in Streams. Canadián Journal of Fisheries and Aquatic Sciences 38: 68-76.

Décamps, H. and Naiman, R. J. (1989) La ecología de los ríos. Mundo Científico 91: 470-479.

Decho, A. M. (1990) Microbial exopolymer secretions in ocean environments: their role(s) in food webs and marine processes. Oceanography and Marine Biology Annual Review 28: 73-153.

Decho, A. W. and Herndl, J. (1995) Microbial activities and the transformation of organic matter within mucilagenous material. The Science of the Total Environment 165: 33-42.

Deshpande, V. and Eriksson, K.-E. (1988) 1,4-β-Glucosidases of *Sporotrichum pulverulentum*. In: W. A. Wood and S. T. Kellog (eds.) Methods in Enzymology Volume 160 ParA Cellulose and Hemicellulose. Academic Press, Inc., p. 415-424.

Dubois, J. D. and Kapustka, L. A. (1983) Freeze-Recovery physiology of nitrogenase activity in terrestrial *Nostoc* sp. colonies. Applied and Environmental Microbiology 46(4): 773-778.

Ducklow, H. W. (1994) Modeling the Microbial Food Web. Microbial Ecology 28: 303-319.

Ellis, B. K. and Stanford, J. A. (1988) Phosphorus bioavailability of fluvial sediments determined by algal assays. Hydrobiologia 160: 9-18.

Elwood, J. W., Mulholland, P. J. and Newbold, J. D. (1988) Microbial activity and phosphorus uptake on decomposing leaf detritus in a heterotrophic stream. Verhandlungen der Internationalen Vereinigung für Teoretische und Andgewandte Limnologie 23: 1209-1218.

Epstein, S. S. and Shiaris, M. P. (1992) Rates of Microbenthic and Meiobenthic Bacterivory in a Temperate Muddy Tidal Flat Community. Applied and Environmental Microbiology 58(8): 2426-2431.

Epstein, S. S. and Rossel, J. (1995) Enumeration of sandy sediment bacteria: search for optimal protocol. Marine Ecology Progress Series 117: 289-298.

Esteve, I., Martínez-Alonso, M., Mir, J. and Guerrero, R. (1992) Distribution, typology and structure of microbial mat communities in Spain: a preliminary study. Limnetica 8: 185-195.

Feminella, J. W., Power, M. E. and Resh, V. H. (1989) Periphyton response to invertebrate grazing and riparian canopy in three northern California coastal streams. Freshwater Biology 22: 445-457.

Fenchel, T. (1969) The ecology of marine microbenthos IV. Structure and function of the benthic ecosystem, its chemical and physical factors and the microfauna communities with special reference to the ciliated Protozoa. Ophelia 6: 1-182.

Ferguson, R. L. and Rublee, P. (1976) Contribution of bacteria to standing crop of coastal plankton. Limnologie and Oceanography 21(1): 141-145.

Findlay, S. (1995) Importance of surface-subsurface exchange in stream ecosystems: The hyporheic zone. Limnology and Oceanography 40 (1): 159-164.

Findlay, S., Meyer, J. and Risley, R. (1986) Benthic bacterial biomass and production in two blackwater rivers. Canadian Journal of Fisheries and Aquatic Sciences 43(6): 1271-1276.

Findlay, S., Pace, M. L., Lints, D., Cole, J. J., Caraco, N. F. and Peierls, B. (1991) Weak coupling of bacterial and algal production in a heterotrophic ecosystem: The Hudson River estuary. Limnology and Oceanography 36(2): 268-278.

Findlay, S. and Howe, K. (1993) Bacterial-algal relationships in streams of the Hubbard Brook Experimental Forest. Ecology 74(8): 2326-2336.

Findlay, S., Strayer, D., Goumbala, C. and Gould, K. (1993) Metabolism of streamwater dissolved organic carbon in the shallow hyporheic zone. Limnology and Oceanography 38(7): 1493-1499.

Fisher, S. G. (1995) Stream ecosystems of the Western United States. In: C. E. Cushing, K. W. Cummins and G. W. Minshall (eds.) River and stream ecosystems. Elsevier, Amsterdam, p. 61-88.

Fisher, S. G. and Likens, G. E. (1973) Energy flow in Bear Brook, New Hampshire: an integrative approach to stream ecosystem metabolism. Ecology 43: 421-439 .

Fogg, G. E. (1966) The extracellular products of algae. Oceanography and Marine Biology Annual Review 4: 195-212.

Fogg, G. E. (1977) Excretion of organic matter by phytoplankton. Limnology and Oceanography 22(3): 576-577.

Fogg, G. E., Stewart, W. D. P., Fay, P. and Walsby, A. E. (1973): The blue-green algae. Academic Press Inc. London Ltd. and New York. p. 459.

Fontigny, A., Billen, G. and Vives-Rego, J. (1987) Some Kinetic Characteristics of Exoproteolytic Activity in Coastal Seawater. Estuarine, Coastal and Shelf Science 25(1): 127-133.

Ford, T. E. and Lock, M. A. (1985) A temporal study of colloidal and dissolved organic carbon in rivers: apparent molecular weight spectra and their relationship to bacterial activity. Oikos 45: 71-78.

Francko, D. A. (1991) Filtration and buoyant density characterization of algal alkaline phosphatase. In: R. J. Chróst (ed) Microbial enzymes in aquatic environments. Springer-Verlag, New York, p. 227-238.

Freeman, C., Lock, M. A., Marxsen, J. and Jones, S. E. (1990) Inhibitory effects of high molecular weight dissolved organic matter on metabolic processes in contrasted rivers and streams. Freshwater Biology 24: 159-166.

Freeman, C. and Lock, M. (1992) Recalcitrant High-Molecular-Weight Material, an Inhibitor of Microbial Metabolism in River Biofilms. Applied and Environmental Microbiology 58(6): 2030-2033.

Freeman, C., Lock, M. A. and Marxsen, J. (1993) Poly-beta-hydroxy alkanoate and the support of river biofilm metabolism following radical changes in environmental conditions. Hydrobiologia 271: 159-164.

Freeman, C., Chapman, P., Gilman, K., Lock, M., Reynolds, B. and Wheater, H. (1995) Ion exchange mechanisms and the entrapment of nutrients by river biofilms. Hydrobiologia 297: 61-65.

Freeman, C., Liska, G., Ostle, N. J., Jones, S. E. and Lock, M. A. (1995) The use of fluorogenic substrates for measuring enzyme activity in peatlands. Plant and Soil 175: 147-152.

Freeman, C. and Lock, M. A. (1995) The biofilm polysaccharide matrix: A buffer against changing organic substrate supply? Limnology and Oceanography 40(2): 273-278.

Frűnd, C. and Cohen, Y. (1992) Diurnal cycles of sulfate reduction under oxic conditions in cyanobacterial mats. Applied and Environmental Microbiology 58: 70-77.

Gajewski, A. J. and Chróst, R. J. (1995) Microbial enzyme activities and phytoplankton and bacterial production in the pelagial of the Great Mazurian Lakes (North-eastern Poland) during summer stratification. Ekologia Polska 43(3-4): 245-265.

Gasol, J. M. (1993) Benthic Flagellates and Ciliates in Fine Freshwater Sediments: Calibration of a Live Counting Procedure and Estimation of Their Abundances. Microbial Ecology 25: 247-262.

Geesey, G. G., Mutch, R., Costerton, J. W. and Green, R. B. (1978) Sessile bacteria: An important component of the microbial population in small mountain streams. Limnology and Oceanography 23(6): 1214-1223.

Geider, R. J. and MacIntyre, H. L. (1996) A dynamic model of photoadaptation in phytoplankton. Limnology and Oceanography 41(1): 1-15.

Gessner, M. O. and Schwoerbel, J. (1991) Fungal biomass associated with decaying leaf litter in a stream. Oecologia 87: 602-603.

Gessner, M. O. and Chauvet, E. (1994) Importance of stream microfungi in cotrolling breakdown rates of leaf litter. Ecology 75(6): 1807-1817.

Gocke, K. (1977) Comparison of Methods for Determining the Turnover Times of Dissolved Organic Compounds. Marine Biology 42: 131-141.

Golladay, S. W. and Sinsabaugh, R. L. (1991) Biofilm development on leaf and wood surfaces in a boreal river. Freshwater Biology 25: 437-450.

González, E. and Pozo, J. (1997) Longitudinal and temporal patterns of benthic coarse particulate organic matter in the Agüera stream (northern Spain). Aquatic Sciences 58(4): 355-366.

Gonzalez, J. M., Sherr, E. B. and Sherr, B. F. (1990) Size-Selective Grazing on Bacteria by Natural Assemblages of Estuarine Flagellates and Ciliates. Applied and Environmental Microbiology 56(3): 583-589.

Gordon, N. D., McMahon, T. and Finlayson, B. L. (1993) Stream Hydrology, An Introduction for Ecologists. Chichester, 526 pp.

Gotschalk, C. C. and Alldredge, A. L. (1989) Enhanced primary productivity and nutrient regeneration within aggregated marine diatoms. Marine Biology 103: 119-129.

Graham, J. M., Auer, M. T., Canale, R. P. and Hoffman, J. P. (1982) Ecological studies and mathematical modelling of *Cladophora* in Lake Huron. 4. Photosynthesis and respiration as functions of light and temperature. Journal of Great Lakes Research 8(1): 100-111.

Grasshoff, K., Ehrhardt, M. and Kremling, K. (1983) Methods of Seawater Analysis. Second, Revised and Extended Edition. Verlag Chemie, Weinheim, 419 pp.

Gregory, S. V., Swanson, F. J., McKee, W. A. and Cummins, K. W. (1991) An ecosystem perspective of riparian zones. BioScience 41(8): 540-551.

Grimm, N. B. and Fischer, S. G. (1984) Exchange between interstitial and surface water: implications for stream metabollism and nutrient cycling. Hydrobiologia 111: 219-228.

Guasch, H. (1995) Primary production of epilithic biofilms in Mediterranean streams. PhD thesis, Departament d'Ecologia, Universitat de Barcelona.

Guasch, H. and Sabater, S. (1994) Primary production of epilithic communities in undisturbed Mediterranean streams. Verhandlungen der Internationalen Vereinigung für Theoretische und Andgewandte Limnologie 25: 1761-1764.

Guasch, H., Martí, E. and Sabater, S. (1995) Nutrient enrichment effects on biofilm metabolism in a Mediterranean stream. Freshwater Biology 33: 373-383.

Guasch, H. and Sabater, S. (1995) Seasonal variations in photosynthesis-irradiance responses by biofilms in Mediterranean streams. Journal of Phycology 31: 727-735.

Guerrero, R. and Pedrós-Alió, C. (1992) The microbial component in Spanish aquatic ecosystems. Limnetica 8: 175-184.

Haack, S. K., Burton, T. and Ulrich, K. (1988) Effects of Whole-Tree Harvest on Epilithic Bacterial Populations in Headwater Streams. Microbial Ecology 16: 165-181.

Haack, T. K. and McFeters, G. A. (1982a) Microbial Dynamics of an Epilithic Mat Community in a High Alpine Stram. Applied and Environmental Microbiology 43(3): 702-707.

Haack, T. K. and McFeters, G. A. (1982b) Nutritional Relationships Among Microorganisms in an Epilithic Biofilm Community. Microbial Ecology 8: 115-126.

Hakenkamp, C. C., Valett, H. M. and Boulton, A. J. (1993) Perspectives on the hyporheic zone: integrating hydrology and biology. Concluding remarks. Journal of the North American Benthological Society 12(1): 94-99.

Halemejko, G. Z. and Chróst, R. J. (1986) Enzymatic hydrolysis of proteinaceous particulate and dissolved material in an eutrophic lake. Archiv für Hydrobiologie 107(1): 1-21.

Hall, S. L. and Fisher, F. M. (1983) Organic compounds released from a natural population of epibenthic bluegreen algae. Journal of Phycology 19: 365-368.

Hamilton, W. A. (1987) Biofilms: microbial interactions and metabolic activities. In: M. Fletcher, J. R. G. Gray, J. G. Jones (eds.) Ecology of Microbial Communities. Symposium 41. Cambridge University Press. The Society for General Microbiology, p. 361-385.

Hansmann, E. W. and Phinney, H. K. (1973) Effects of logging on periphyton in coastal streams of Oregon. Ecology 54: 194-199.

Hansson, L.-A. (1989) The influence of a periphytic biolayer on phosphorus exchange between substrate and water. Archiv für Hydrobiologie 115(1): 21-26.

Hart, D. D. (1992) Community organization in streams: the importance of species interactions, physiscal factors, and chance. Oecologia 91: 220-228.

Hawes, I. (1993) Photosynthesis in thick cyanobacterial films: a comparison of annual and perennial antarctic mat communities. Hydrobiologia 252: 203-209.

Hawkins, C. P. and Sedell, J. R. (1981) Longitudinal and seasonal changes in functional organization of macroinvertebrate communities in four Oregon streams. Ecology 62: 387-397.

Hawkins, C. P., Murphy, M. L. and Anderson, N. H. (1982) Effects of canopy, substrate composition, and gradient on the structure of macroinvertebrate communities in Cascade Range streams of Oregon. Ecology 63(6): 1840-1856.

Hedin, L. O. (1990) Factors controlling sediment community respiration in woodland stream ecosystems. Oikos 57: 94-105.

Hendricks, S. P. (1993) Microbial ecology of the hyporheic zone: a perspective integrating hydrology and biology. Journal of the North American Benthological Society 12(1): 70-78.

Herbert, R. A. (1990) Methods for enumerating microorganisms and determining biomass in natural environments. Methods in Microbiology 22: 1-39.

Herndl, G. J. (1992) Marine snow in the Northern Adriatic Sea: possible causes and consequences for a shallow ecosystem. Marine Microbial Food Webs 6(2): 149-172.

Hill, B. H., Gardner, T. J., Ekisola, O. F. and Henebry, G. M. (1992) Microbial use of leaf litter in prairie streams. Journal of the North American Benthological Society 11(1): 11-19.

Hill, W. (1996) Effects of Light. In: R. J. Stevenson, M. L. Bothwell and R. L. Lowe (eds.) Algal Ecology. Freshwater Benthic Ecosystems. Academic Press, p. 121-148.

Hill, W. R. and Knight, A. W. (1988) Nutrient and light limitation of algae in two northern California streams. Journal of Phycology 24: 125-132.

Hill, W. R. and Boston, H. L. (1991) Effects of community development on photosynthesis-irradiance relations in stream periphyton. Lymnology and Oceanography 36: 1375-1389.

Hoagland, K. D., Rosowski, J. R., Gretz, M. R. and Roemer, S. C. (1993) Diatom extracellular polymeric substances: function, fine structure, chemistry, and physiology. Journal of Phycology 29: 537-566.

Hobbie, J. E., Daley, R. J. and Jasper, S. (1977) Use of Nucleopore Filters for Counting Bacteria by Fluorescence Microscopy. Applied and Environmental Microbiologie 33(5): 1225-1228.

Hobbie, J. E. and Likens, G. E. (1973) Output of phosphorus, dissolved organic carbon, and fine particulate carbon from Hubbard Brook watersheds. Limnology and Oceanography 18(5): 734-742.

Hoch, B., Berger, B., Kavka, G. and Herndl, G. J. (1996) Influence of wastewater treatment on the microbial ecology of a large, temperate river system - the Danube River. Hydrobiologia 321: 205-218.

Hollibaugh, J. T. and Azam, F. (1983) Microbial degradation of dissolved proteins in seawater. Limnology and Oceanography 28(6): 1104-1116.

Hoppe, H.-G. (1983) Significance of exoenzymatic activities in the ecology of brackish water: measurement by means of methylumbelliferyl-substrates. Marine Ecology Progress Series 11: 299-308.

Hoppe, H.-G., Kim, S.-J. and Gocke, K. (1988) Microbial Decomposition in Aquatic Environments: Combined Process of Extracellular Enzyme Activity and Substrate Uptake. Applied and Environmental Microbiology 54(3): 784-790.

Hoppe, H.-G., Ducklow, H. and Karrasch, B. (1993) Evidence for dependency of bacterial growth on enzymatic hydrolysis of particulate organic matter in the mesopelagic ocean. Marine Ecology Progress Series 93: 277-283.

Horner, R. R. and Welch, E. B. (1981) Stream periphyton development in relation to current velocity and nutrients. Canadian Journal of Fisheries and Aquatic Sciences 38: 449-457.

Hudson, J. J., Roff, J. C. and Burnison, B. K. (1992) Bacterial Productivity in Forested and Open Streams in Southern Ontario. Canadian Journal of Fisheries and Aquatic Sciences 49: 2412-2422.

Humphrey, K. P. and Stevenson, R. J. (1992) Responses of benthic algae to pulses in current and nutrients during simulations of subscouring spates. Journal of the North American Benthological Society 11(1): 37-48.

Hunding, C. and Hargrave, B. T. (1973) A comparison of benthic microalgal production measured by ^{14}C and oxygen methods. Journal of Fisheries Research Board of Canada 30: 309-312.

Jackson, C. R., Foreman, C. M. and Sinsabaugh, R. L. (1995) Microbial enzyme activities as indicators of organic matter processing rates in a Lake Erie coastal wetland. Freshwater Biology 34: 329-342.

Jacobsen, T. R. and Rai, H. (1991) Aminopeptidase activity in lakes of different eutrophication. In: R. J. Chróst (ed.) Microbial Enzymes in Aquatic Environments. Springer-Verlag, New York, p. 155-164.

Jansson, M., Olsson, H. and Pettersson, K. (1988) Phosphatases; origin, characteristics and function in lakes. Hydrobiologia 170: 157-175.

Jeffrey, S. W. and Humphrey, G. F. (1975) New spectrophotometric equations for determining chlorophylls a, b, and c in higher plants, algae and natural phytoplankton. Biochemie Physiologie Pflanzen 167: 191-194.

Johnson, S. L. and Covich, A. P. (1997) Scales of observation of riparian forests and distributions of suspended detritus in a prairie river. Freshwater Biology 37: 163-175.

Jones, J. G. (1971) Studies on freshwater bacteria: factors which influence the population and its activity. Journal of Ecology 59: 593-613.

Jones, S. E. and Lock, M. A. (1989) Hydrolytic extracellular enzyme activity in heterotrophic biofilms from two contrasting streams. Freshwater Biology 22: 289-296.

Jones, S. E. and Lock, M. A. (1991) Peptidase activity in river biofilms by product analysis. In: R. J. Chróst (ed) Microbial Enzymes in Aquatic Environments. Springer-Verlag, p. 144-154.

Jones, S. E. and Lock, M. A. (1993) Seasonal determinations of extracellular hydrolytic activities in heterotrophic and mixed heterotrophic/autotrophic biofilms from two contrasting rivers. Hydrobiologia 257: 1-16.

Jorgensen, B. B., Revsbech, N. P. and Cohen, Y. (1983) Photosynthesis and structure of benthic microbial mats: Microelectrode and SEM studies of four cyanobacterial communities. Limnology and Oceanography 28(6): 1075-1093.

Jorgensen, N. O. G. (1987) Free aminoacids in lakes: concentrations and assimilation rates in relation to phytoplankton and bacterial production. Limnology and Oceanography 32: 97-111.

Kaplan, L. A. and Bott, T. L. (1982) Diel fluctuations of DOC generated by algae in a piedmont stream. Limnology and Ocenaography 27(6): 1091-1100.

Kaplan, L. A. and Bott, T. L. (1983) Microbial heterotrophic utilization of dissolved organic matter in a piedmont stream. Freshwater Biology 13: 363-377.

Kaplan, L. A. and Bott, T. L. (1985) Acclimation of stream-bed heterotrophic microflora: metabolic responses to dissolved organic matter. Freshwater Biology 15: 479-492.

Kaplan, L. A. and Bott, T. L. (1989) Diel fluctuations in bacterial activity on streambed substrata during vernal algal blooms: Effects of temperature, water chemistry, and habitat. Limnology and Oceanography 34(4): 718-733.

Karl, D. M. (1994) Accurate Estimation of Microbial Loop Processes and Rates. Microbial Ecology 28: 147-150.

Karner, M., Fuks, D. and Herndl, G. (1992) Bacterial Activity along a Trophic Gradient. Microbial Ecology 24: 243-257.

Karner, M. and Rassoulzadegan, F. (1995) Extracellular Enzyme Activity: Indications for a High Short-Term Varability in a Coastal Marine Ecosystem. Microbial Ecology 30: 143-156.

Kemp, P. F. (1990) The fate of benthic bacterial production. Aquatic Sciences 2(1): 109-124.

Kepner, R. L. and Pratt, J. R. (1994) Use of fluorochromes for direct enumeration of total bacteria in environmental samples: past and present. Microbiological Review 58: 603-615.

King, G. M. (1986) Characterization of β-glucosidase activity in intertidal marine sediments. Applied and Environmental Microbiology 51(2): 373-380.

Klotz, B. (1985) Factors controlling phosphorus limitation in stream sediments. Limnology and Oceanography 30(3): 543-553.

Klotz, R. L. (1992) Factors Influencing Alkaline Phosphatase Activity of Stream Epilithon. Journal of Freshwater Ecology 7(2): 233-242.

Koetsier III, P., McArtur, J. V. and Leff, L. G. (1997) Spatial and temporal response of stream bacteria to sources of dissolved organic carbon in a blackwater stream system. Freshwater Biology 37: 79-89.

Kühl, M. and Jorgensen, B. B. (1992) Microsensor measurements of sulfate reduction and sulfide oxidation in compact microbial communities of aerobic biofilms. Applied and Environmental Microbiology 58: 1164-1174.

Kuserk, F. T., Kaplan, L. A. and Bott, T. L. (1984) In Situ Measures of Dissolved Organic Carbon Flux in a Rural Stream. Canadian Journal of Fisheries and Aquatic Sciences 41: 964-973.

Lachke, A. H. (1988) 1,4-β-D-Xylan Xylohydrolase of *Sclerotium rolfsii*. In: W. A. Wood and S. T. Kellog (eds.) Methods in Enzymology. Volume 160. Par A Cellulose and Hemicellulose. Academic Press, Inc., p. 679-684.

Ladd, T. I., Ventullo, R. M., Wallis, P. M. and Costerton, J. W. (1982) Heterotrophic activity and biodegradation of labile and refractory compounds by groundwater and stream microbial populations. Applied and Environmental Microbiology 44(2): 321-329.

Lai, D. (1996) Comparison study of AR models on the Canadian lynx data: A close look at BDS statistic. Computational Statistics and Data Analysis 22: 409-423.

Lange, W. (1976) Speculations on a possible essential function of the gelatinous sheath of blue-green algae. Canadian Journal of Microbiology 22: 1181-1185.

Lau, Y. L. and Liu, D. (1993) Effect of flow rate on biofilm accumulation in open channels. Water Research 27(3): 355-360.

Le, J., Wehr, J. D. and Campbell, L. (1994) Uncoupling of Bacterioplankton and Phytoplankton Production in Fresh Waters Is Affected by Inorganic Nutrient Limitation. Applied and Environmental Microbiology 60(6): 2086-2093.

Leatherbarrow, R. J. (1987) Enzfitter. A non-linear Regression Data Analysis Program for the IBM PC/PS2. Elsevier-Biosoft, Cambridge, UK.

Leightfried, M. (1985) Organic matter in gravel streams (Project Ritordat-Lunz). Verhandlungen der Internationalen Vereingung für Theoretische und Andgewandte Limnologie 22:2058-2062.

Leland, H. V. (1995) Distribution of phytobenthos in the Yakima River basin, Washington, in relation to geology, land use, and other environmental factors. Canadian Journal of Fisheries and Aquatic Sciences 52(5): 1108-1129.

Lewis, D. L., Hodson, R. E. and Hwang, H.-M. (1988) Kinetics of Mixed Microbial Assemblages Enhance Removal of Highly Dilute Organic Substrates. Applied and Environmental Microbiology 54(3): 2054-2057.

Lindell, M. J., Graneli, W. and Tranvik, L. J. (1995) Enhanced bacterial growth in response to photochemical transformation of dissolved organic matter. Limnology and Oceanography 40: 195-199.

Livingstone, D. and Whitton, B. A. (1984) Water chemistry and phosphatase activity of the blue-green alga *Rivularia* in Upper Teesdale streams. Journal of Ecology 72: 405-421.

Lock, M. A. (1981) River epilithon- A light and organic energy transducer. In: M. A. Lock and D. D. Williams (eds.) Perspectives in Running Water Ecology. Plenum Press, p. 1-40.

Lock, M. A. (1990) The dynamics of dissolved and particulate organic material over the substratum of water bodies. In: R. J. Whitton (ed.) The Biology of Particles in Aquatic Systems. CRC, p. 117-144.

Lock, M. A. (1993) Attached microbial communities in rivers. In: T. E. Ford (ed.) Aquatic microbiology: An ecological approach. Blackwell Scientific Publications, p. 113-138.

Lock, M. A. and Hynes, H. B. (1976) The fate of dissolved organic carbon derived from autumn-shed maple leaves (*Acer saccharum*) in a temperate hard-water stream. Limnology and Oceanography 21: 436-443.

Lock, M. A. and John, P. H. (1979) The effects of flow patterns on uptake of phosphorus by river periphyton. Limnology and Oceanography 24(2): 376-383.

Lock, M. A., Wallace, R. R., Costerton, J. W., Ventullo, R. M. and Charlton, S. E. (1984) River epilithon: toward a structural-functional model. Oikos 42: 10-22.

Lock, M. A. and Ford, T. E. (1986) Colloidal and dissolved organic carbon in undisturbed boreal forest catchments: a seasonal study of apparent molecular weight spectra. Freshwater Biology 16: 187

Lohman, K. and Priscu, J. C. (1992) Physiological indicators of nutrient deficiency in *Cladophora* (Chlorophyta) in the Clark Fork of the Columbia River, Montana. Journal of Phycology 28: 443-448.

López, N. I. (1993) Actividades bacterianas del sedimento superficial marino y su importancia ecológica en la producción de los sistemas litorales. PhD thesis, Universitat Politècnica de Catalunya, Barcelona.

Lorenz, R. C. and Herdendorf, C. E. (1982) Growth dynamics of *Cladophora glomerata* in western Lake Erie in relation to some environmental factors. Journal of Great Lakes Research 8(1): 42-53.

Low, C. S. F. and White, D. C. (1989) Regulation of External Polymer Production in Benthic Microbial Communities. In: Y. Cohen and E. Rosenberg (eds.) Microbial Mats: physiological ecology of benthic microbial communities. American Society for Microbiology, p. 228-238.

Lowe, R. L., Golladay, S. W. and Webster, J. R. (1986) Periphyton response to nutrient manipulation in streams draining clearcut and forested watersheds. Journal of the North American Benthological Society 5(3): 221-229.

Lundgren, B. (1981) Fluorescein diacetate as a stain of metabolically active bacteria. Oikos 36: 17-22.

Lütge, U., Büdel, B., Ball, E., Strube, F. and Weber, P. (1995) Photosynthesis of terrestrial cyanobacteria under light and desiccation stress as expressed by chlorophyll fluorescence and gas exchange. Journal of Experimental Botany 46: 309-319.

MacLeod, F. A., Guiot, S. R. and Costerton, J. W. (1990) Layered structure of bacterial aggregates produced in an upflow anaerobic sludge bed and filter reactor. Applied and Environmental Microbiology 56(6): 1598-1607.

Manly, B. F. J. (1995) Multivariate statistical methods, A primer. Second Edition, Chapman & Hall, London pp.

Mann, K. H. (1988) Production and use of detritus in various freshwater, estuarine, and coastal marine ecosystems. Limnology and Oceanography 33(4, part 2): 910-930.

Margalef, R. (1960) Ideas for a synthetic approach to the ecology of running waters. Internationale Revue der gesamten Hydrobiologie 45(1): 133-153.

Margalef, R. (1983) Limnología. Ediciones Omega, S.A., Barcelona, 1010 p.

Margalef, R. (1989) Ecología. Ediciones Omega S.A., Barcelona, 951 p.

Margalef, R. (1990) Ecosistemas fluviales, transporte horizontal y dinámica sucesional evolutiva. Interciencia 15(6): 334-336.

Margalef, R. (1991) Teoría de los sistemas ecológicos. Publicacions de la Universitat de Barcelona, Estudi General 1, Ciències Experimentals i Matemàtiques, Barcelona, 290 pp.

Margalef, R. (1997) Our Biosphere. In: O. Kinne (de.) Excellence in Ecology, Ecology Institute, Germany, book 10, 176 pp.

Marquart, D. W. (1963) An algorithm for least-squares estimation of non-linear parameters. J. Soc. ind. appl. Math. 11: 431-441.

Marshall, K. C. (1988) Adhesion and growth of bacteria at surfaces in oligotrophic habitats. Canadian Journal of Microbiology 34: 503-506.

Martí, E. (1995) Nutrient dynamics in two Mediterranean streams differing in watershed physiographic features. PhD thesis, Departament d'Ecologia, Universitat de Barcelona.

Martí, E., Armengol, J. and Sabater, F. (1994) Day and night nutrient uptake differences in a calcareous stream. Verhandlungen der Internationalen Vereinigung für Theoretische und Andgewandte Limnologie 25: 1756-1760.

Martí, E. and Sabater, F. (1996) High variability in temporal and spatial nutrient retention in Mediterranean streams. Ecology 77(3): 854-869.

Marxsen, J. (1988) Evaluation of the importance of bacteria in the carbon flow of a small open grassland stream, the Breitenbach. Archiv für Hydrobiologie 111(3): 339-350.

Marxsen, J. (1996) Measurement of bacterial production in stream-bed sediments via leucine incorporation. FEMS Microbiology Ecology 21: 313-325.

Marxsen, J. and Witzel, K.-P. (1990) Measurement of exoenzymatic activity in streambed sediments using methylumbelliferyl-substrates. Archiv für Hydrobiologie Ergebnisse der Limnologie 34: 21-28.

Marxsen, J. and Witzel, K.-P. (1991) Significance of Extracellular Enzymes for Organic Matter Degradation and Nutrient Regeneration in Small Streams. In: R. J. Chróst (ed.) Microbial Enzymes in Aquatic Environments. Springer-Verlag, p. 270-285.

Marxsen, J. and Fiebig, D. M. (1993) Use of perfused cores for evaluating extracellular enzyme activity in stream-bed sediments. FEMS Microbiology Ecology 13: 1-12.

Marxsen, J. and Schmidt, H.-H. (1993) Extracellular phosphatase activity in sediments of the Breitenbach, a Central European mountain stream. Hydrobiologia 253: 207-216.

Marxsen, J., Schmidt, H.-H. and Fiebig, D. M. (1997) Organic matter dynamics in the Breitenbach, Germany. Journal of the North American Benthological Society 16(1): 28-32.

McDowell, W. H. and Fisher, S. G. (1976) Autumnal processing of dissolved organic matter in a small woodland stream ecosystem. Ecology 57: 561-569.

McKinley, V. L. and Vestal, J. R. (1992) Mineralization of Glucose and Lignocellulose by Four Arctic Freshwater Sediments in Response to Nutrient Enrichment. Applied and Environmental Microbiology 58(5): 1554-1563.

Metzler, G. M. and Smock, L. A. (1990) Storage and Dynamics of Subsurface Detritus in a Sand-Bottomed Stream. Canadian Journal of Fisheries and Aquatic Sciences 47: 588-594.

Meyer, J. L. (1979) The role of sediments and bryophytes in phosphorus dynamics in a headwater stream ecosystem. Limnology and Oceanography 24(2): 365-375.

Meyer, J. L. (1988) Benthic bacterial biomass and production in a blackwater river. Verhandlungen der Internationalen Vereinigung für Theoretische und Andgewandte Limnologie 23: 1832-1838.

Meyer, J. L. (1994) The Microbial Loop in Flowing Waters. Microbial Ecology 28: 195-199.

Meyer, J. L., Edwards, R. T. and Risley, R. (1987) Bacterial Growth on Dissolved Organic Carbon from a Blackwater River. Microbial Ecology 13: 13-29.

Meyer, J. L. and Edwards, R. T. (1990) Ecosystem metabolism and turnover of organic carbon along a blackwater river continuum. Ecology 71(2): 668-677.

Meyer-Reil, L.-A. (1986) Measurement of hydrolytic activity and incorporation of dissolved organic substrates by microorganisms in marine sediments. Marine Ecology Progress Series 31: 143-149.

Meyer-Reil, L.-A. (1987) Seasonal and Spatial Distribution of Extracellular Enzymatic Activities and Microbial Incorporation of Dissolved Organic Substrates in Marine Sediments. Applied and Environmental Microbiology 53(8): 1748-1755.

Meyer-Reil, L.-A. and Köster, M. (1992) Microbial life in pelagic sediments: the impact of environmental parameters on enzymatic degradation of organic material. Marine Ecology Progress Series 81: 65-72.

Meyer-Reil, L.-A. (1994) Microbial life in sedimentary biofilms - the challenge to microbial ecologists. Marine Ecology Progress Series 112: 303-311.

Middelboe, M. and Sondergaard, M. (1993) Bacterioplankton growth yield: seasonal variations and coupling to substrate lability and β-glucosidase activity. Applied and Environmental Microbiology 59(11): 3916-3921.

Middelboe, M., Sondergaard, M., Letarte, Y. and Borch, N. H. (1995) Attached and Free-Living Bacteria: Production and Polymer Hydrolysis During a Diatom Bloom. Microbial Ecology 29: 231-248.

Millie, D. F., Ingram, D. A. and Dionigi, C. P. (1990) Pigment and photosynthetic responses of *Oscillatoria agardhii* (Cyanophita) to photon flux density and spectral quality. Journal of Phycology 26: 660-666.

Minshall, G. W., Petersen, R. C., Cummins, K. W., Bott, T. L., Sedell, J. R., Cushing, C. C. and Vanotte, R. L. (1983) Interbiome comparison of stream ecosystem dynamics. Ecological Monographs 53: 1-25.

Minshall, G. W., Cummins, K. W., Petersen, R. C., Cushing, C. E., Bruns, D. A., Sedell, J. R. and Vannote, R. L. (1985) Development in Stream Ecosystem Theory. Canadian Journal of Fisheries and Aquatic Sciences 42: 1045-1055.

Mir, J., Martínez-Alonso, M., Esteve, I. and Guerrero, R. (1991) Vertical stratification and microbial assemblage of a microbial mat in the Ebro Delta (Spain). FEMS Microbiology Ecology 86: 59-68.

Montagnes, D. J. S., Berges, J. A., Harrison, P. J. and Taylor, F. J. R. (1994) Estimating carbon, nitrogen, protein, and chlorophyll-a from volume in marine phytoplankton. Limnology and Oceanography 39(5): 1044-1060.

Moran, M. A. and Hodson, E. (1992) Contributions of three subsystems of a freshwater marsh to total bacterial secondary productivity. Microbial Ecology 24: 161-170.

Mulholland, P. J., Steinman, A. D., Palumbo, A. W. and Elwood, J. W. (1991) Role of nutrient cycling and herbivory in regulating periphyton communities in laboratory streams. Ecology 72: 966-982.

Mulholland, P. J., Steinman, A. D., Marzolf, E. R., Hart, D. R. and DeAngelis, D. L. (1994) Effect of periphyton biomass on hydraulic characteristics and nutrient cycling in streams. Oecologia 98: 40-47.

Münster, U., Einió, P. and Nurminen, J. (1989) Evaluation of the measurements of extracellular enzyme activities in a polyhumic lake by means of studies with 4-methylumbelliferyl-substrates. Archiv für Hydrobiologie 115(3): 321-337.

Münster, U. and Chróst, R. J. (1990) Origin, Composition, and Microbial Utilization of Dissolved Organic Matter. In: J. Overbeck and R. J. Chróst (eds.) Aquatic Microbial Ecology: Biochemical and Molecular Approaches. Springer-Verlag, New York, p. 8-46.

Münster, U., Einiö, P., Nurminen, J. and Overbeck, J. (1992) Extracellular enzymes in a polyhumic lake: important regulators in detritus processing. Hydrobiologia 229: 225-238.

Murray, R. E., Cooksey, K. E. and Priscu, J. C. (1986) Stimulation of bacterial DNA synthesis by algal exudates in attached algal-bacterial consortia. Applied and Environmental Microbiology 52(5): 1177-1182.

Naiman, R. J. (1983) The annual pattern and spatial distribution of aquatic oxygen metabolism in boreal forest watersheds. Ecological Monographs 53(1): 73-94.

Naiman, R. J. and Sedell, J. R. (1981) Stream ecosystem research in a watershed perspective. Verhandlungen der Internationalen Vereinigung für Theoretische und Andgewandte Limnologie 21: 804-811.

Naiman, R. J., Melillo, J. M., Lock, M. A., Ford, T. E. and Reice, S. R. (1987) Longitudinal patterns of ecosystem processes and community structure in a subarctic river continuum. Ecology 68(5): 1139-1156.

Naiman, R. J., Décamps, H., Pastor, J. and Johnston, C. A. (1988) The potential importance of boundaries to fluvial ecosystems. Journal of the North American Benthological Society 21: 804-811.

Newbold, J. D., Elwood, J. W., O'Neill, R. V. and van Winkle, W. (1981) Measuring Nutrient Spiralling in Streams. Environmental Sciences Division 1755: 860-863.

Newbold, J. D., Mulholland, P. J., Elwood, J. W. and O'Neill, R. V. (1982) Organic carbon spiralling in stream ecosystems. Oikos 38: 266-272.

Nivens, D. E., Palmer, R. J. and White, D. C. (1995) Continuous nondestructive monitoring of microbial biofilms: a review of analytical techniques. Journal of Industrial Microbiology 15: 263-276.

Norrman, B., Zweifel, U. L., Hopkinson, C. S. and Fry, B. (1995) Production and utilization of dissolved organic carbon during an experimental diatom bloom. Limnology and Oceanography 40(5): 898-907.

Novitsky, J. A. (1983) Microbial Activity at the Sediment-Water Interface in Halifax Harbor, Canada. Applied and Environmental Microbiology 45(6): 1761-1766.

Oppenheim, D. R. and Paterson, D. M. (1990) The fine structure of an algal mat from a freshwater maritime antarctic lake. Canadian Journal of Botany 68: 174-183.

Opsahl, S. and Benner, R. (1993) Decomposition of senescent blades of the seagrass *Halodule wrightii* in a subtropical lagoon. Marine Ecology Progress Series 94: 191-201.

Paerl, H. W., Bebout, B. M., Joye, S. B. and Des Marais, D. J. (1993a) Microscale characterization of dissolved organic matter production and uptake in marine microbial mat communities. Limnology and Oceanography 38(6): 1150-1161.

Paerl, H. W., Joye, S. B. and Fitzpatrick, M. (1993b) Evaluation of nutrient limitation of CO_2 and N_2 fixation in marine microbial mats. Marine Ecology Progress Series 101: 297-306.

Palmer, M. A. (1993) Experimentation in the hyporheic zone: challenges and prospectus. Journal of the North American Benthological Society 12: 84-93.

Peters, G. T., Webster, J. R. and Benfield, E. F. (1987) Microbial activity associated with seston in headwater streams: effects of nitrogen, phosphorus and temperature. Freshwater Biology 18: 405-413.

Peters, R. H. (1983) The Ecological Implications of Body Size. Cambridge University Press.

Petersen, R. C., Cummins, K. W. and Ward, G. M. (1989) Microbial and animal processing of detritus in a woodland stream. Ecological Monographs 59(1): 21-39.

Peterson, B. J., Hobbie, J. E., Hershey, A. E., Lock, M. A., Ford, T. E., Vestal, J. R., McKinley, V. L., Hullar, M. A. J., Miller, M. C., Ventullo, R. M. and Volk, G. S. (1985) Transformation of a tundra river from heterotrophy to autotrophy by addition of phosphorus. Science 229: 1383-1386.

Peterson, B. J., Deegan, L., Helfrich, J., Hobbie, J. E., Hullar, M. and Moller, B. (1993) Biological responses of a tundra river to fertilization. Ecology 74(3): 653-672.

Peterson, C. G. and Stevenson, R. J. (1992) Resistance and resilience of lotic algal communities: importance of disturbance timing and current. Ecology 73(4): 1445-1461.

Pfeifer, R. F. and McDiffett (1975) Some factors affecting primary productivity of stream riffle communities. Archiv für Hydrobiologie 75: 306-317.

Pinay, G., Fabre, A., Vervier, P. and Gazelle, F. (1992) Control of C, N, P distribution in soils of riparian forest. Landscape Ecology 6(3): 121-132.

Pinckney, J., Paerl, H. W. and Fitzpatrick, M. (1995a) Impacts of seasonality and nutrients on microbial mat community structure and function. Marine Ecology Progress Series 123: 207-216.

Pinckney, J., Paerl, H. W., Reid, R. P. and Bebout, B. (1995b) Ecophysiology of Stromatolitic Microbial Mats, Stocking Island, Exuma Cays, Bahamas. Microbial Ecology 29: 19-37.

Poremba, K. (1995) Hydrolytic enzymatic activity in deep-sea sediments. FEMS Microbiology Ecology 16: 213-222.

Poremba, K. and Hoppe, H.-G. (1995) Spatial variation of benthic microbial production and hydrolytic enzymatic activity down the continental slope of the Celtic Sea. Marine Ecology Progress Series 118: 237-245.

Porter, K. G. and Feig, Y. S. (1980) The use of DAPI for identifying and counting aquatic microflora. Limnology and Oceanography 25(5): 943-948.

Prat, N. (1991) Present trends in river studies. In: J. D. Ros and N. Prat (eds.) Homage to Ramon Margalef; or Why there is such pleasure in studying nature. Publicacions de la Universitat de Barcelona, Oecologia aquatica 10: 1-12.

Prenant, A. 1960. Études écologiques sur les sables intercotidaux. I. Questions de méthode granulometrique. Applications à trois anses de la Baie de Quiberon. Cahiers de Biologie Marine 1: 295-340.

Psenner, R. (1990) From image analysis to chemical analysis of bacteria: A long-term study? Limnology and Oceanography 31(1): 234-237.

Pusch, M. and Schwoerbel, J. (1994) Community respiration in hyporheic sediments of a mountain stream (Steina, Black Forest). Archiv für Hydrobiologie 130(1): 35-52.

Quesada, A., Mouget, J. L. and al. (1995) Growth of Antarctic cyanobacteria under ultraviolet radiation: UVA counteracts UVB inhibition. Journal of Phycology 31(2): 242-248.

Ramsing, N. B., Kühl, M., Jorgensen, B. B. (1993) Distribution of sulfate-reducing bacteria, O_2, and H_2S in photosynthetic biofilms determined by oligonucleotide probes and microelectrodes. Applied and Environmental Microbiology 59: 3840-3849.

Reichardt, W., Overbeck, J. and Steubing, L. (1967) Free Dissolved Enzymes in Lake Waters. Nature 216: 1345-1347.

Roberson, E. B. and Firestone, M. K. (1992) Relationship between dessication and exopolysaccharide production in a soil *Pseudomonas* sp. Applied and Environmental Microbiology 58(4): 1284-1291.

Rodriguez, G. G., Phipps, D., Ishiguro, K. and Ridgway, H. F. (1992) Use of a Fluorescent Redox Probe for Direct Visualization of Actively Respiring Bacteria. Applied and Environmental Microbiology 58(6): 1801-1808.

Rogers, H. J. 1961. The dissimilation of high molecular weight organic substances. In I. C. Gunsalus and R. Y. Stanier (eds.), The bacteria. Academic Press: 261-318.

Romaní, A. M. and Sabater, S. (1997) Metabolism recovery of a stromatolitic biofilm after drought in a Mediterranean stream. Archiv für Hydrobiologie, in press.

Rosemond, A. D. (1993) Interactions among irradiance, nutrients, and herbivores constrain a stream algal community. Oecologia 94: 585-594.

Ross, H. H. (1963) Stream communities and terrestrial biomes. Archiv für Hydrobiologie 59(2): 235-242.

Rutherford, J. E. and Hynes, H. B. N. (1987) Dissolved organic carbon in streams and groundwater. Hydrobiologia 154: 33-48.

Sabater, F. (1987) Estudi integrat del riu Ter i de la seva conca. PhD thesis, Departament d'Ecologia, Universitat de Barcelona.

Sabater, F. (1988) The geochemical features of the Ter River watershed (NE Spain). Oecologia aquatica 9: 77-87.

Sabater, F. and Armengol, J. (1986) Chemical characterization of the Ter River. Limnetica 2 :75-84.

Sabater, F., Guasch, H., Martí, E., Armengol, J. and Sabater, S. (1995) The Ter: a Mediterranean river case-study in Spain. In: C. E. Cushing, K. W. Cummins and G. W. Minshall (eds.) River and stream ecosystems. Elsevier, p. 419-438.

Sabater, S. (1987) Estudi de les poblacions d'algues del riu Ter. PhD thesis, Departament d'Ecologia, Universitat de Barcelona.

Sabater, S. (1988) Composición y ciclo de pigmentos clorofílicos en las poblaciones del fitobentos del río Ter durante un período de nueve meses. Oecologia aquatica 9: 61-75.

Sabater, S. (1989) Encrusting algal assemblages in a Mediterranean river basin. Archiv für Hydrobiologie 114: 55-57.

Sabater, S. and Sabater, F. (1988) Diatom assemblages in the River Ter. Archiv für Hydrobiologie 111(3): 397-408.

Sabater, S. and Sabater, F. (1992) Longitudinal changes of benthic algal biomass in a mediterranean river during two high production periods. Archiv für Hydrobiologie 124(4): 475-487.

Sabater, S., Guasch, H., Martí, E., Armengol, J. and Sabater, F. (1992) The Ter, a Mediterranean river system in Spain. Limnetica 8: 141-149.

Sabater, S. and Romaní, A. M. (1996) Metabolic changes associated with biofilm formation in an undisturbed Mediterranean stream. Hydrobiologia 335: 107-113.

Sabater, S., Butturini, A., Muñoz, I., Romaní, A., Wray, J., and Sabater, F. (1997) Effects of removal of riparian vegetation in algae and heterotrophs of a Mediterranean stream. In press.

Sala, M. M. 1995. Caracterización de las activitdades enzimáticas de las comunidades microbianas en el litoral del lago de Constanza. PhD Thesis, Universitat Autònoma de Barcelona.

Schallenberg, M., Kalff, J. and Rasmussen, J. B. (1989) Solutions to Problems in Enumerating Sediment Bacteria by Direct Counts. Applied and Environmental Microbiology 55(5): 1214-1219.

Schallenberg, M. and Kalff, J. (1993) The ecology of sediment bacteria in lakes and comparisons with other aquatic ecosystems. Ecology 74(3): 919-934.

Scherer, S., Ernst, A., Chen, T.-W. and Böger, P. (1984) Rewetting of drought-resistant blue-green algae: Time course of water uptake and reappearance of respiration, photosynthesis, and nitrogen fixation. Oecologia 62: 418-423.

Scherer, S., Chen, T. W. and Böger, P. (1988) A New UV-A/B Protecting Pigment in the Terrestrial Cyanobacterium *Nostoc commune*. Plant Physiology 88: 1055-1057.

Schönborn, W. (1992) The role of protozoan communities in freshwater and soil ecosystems. Acta Protozool. (Hung.) 31: 11

Scholz, O. and Boon, P. I. (1993) Alkaline phosphatase, aminopeptidase and β-D-glucosidase activities associated with billabong periphyton. Archiv für Hydrobiologie 126(4): 429-443.

Scholz, O. and Marxsen, J. (1996) Sediment phosphatases of the Breitenbach, a first-order Central European stream. Archiv für Hydrobiologie 135(4): 433-450.

Sell, A. F. (1994) Phytoplankton-Excreted Organic Carbon. In: J. Overbeck and R. J. Chróst (eds.) Microbial Ecology of Lake Plubsee, Ecological Studies 105. Springer-Verlag, p. 81-91.

Servais, P. and Billen, G. (1993) Dynamics of Heterotrophic Bacteria in Aquatic Systems: The HSB Model. In: R. Guerrero and C. Pedrós-Alió (eds.) Trends in Microbial Ecology, Proceedings of the Sixth International Symposium on Microbial Ecology (Barcelona 6-11 September 1992). Spanish Society for Microbiology, Barcelona, p

Sinsabaugh, R. L. and Linkins, A. E. (1988) Exoenzyme activity associated with lotic epilithon. Freshwater Biology 20: 249-261.

Sinsabaugh, R. L., Golladay, S. W. and Linkins, A. E. (1991a) Comparison of epilithic and epixylic biofilm development in a boreal river. Freshwater Biology 25: 179-187.

Sinsabaugh, R. L., Repert, D., Weiland, T., Golladay, S. W. and Linkins, A. E. (1991b) Exoenzyme accumulation in epilithic biofilms. Hydrobiologia 222: 29-37.

Sinsabaugh, R. L., Weiland, T. and Linkins, A. E. (1992) Enzymic and molecular analysis of microbial communities associated with lotic particulate organic matter. Freshwater Biology 28: 393-404.

Sinsabaugh, R. L., Antibus, R. K., Linkins, A. E., McClaugherty, C. A., Rayburn, L., Repert, D. and Weiland, T. (1993) Wood decomposition: Nitrogen and phosphorus dynamics in relation to extracellular enzyme activity. Ecology 74(5): 1585-1593.

Sinsabaugh, R. L., Moorhead, D. L. and Linkins, A. E. (1994a) The enzymic basis of plant litter decomposition: emergence of an ecological process. Applied Soil Ecology 1: 97-111.

Sinsabaugh, R. L., Osgood, M. P. and Findlay, S. (1994b) Enzymatic models for estimating decomposition rates of particulate detritus. Journal of the North American Benthological Society 13(2): 160-169.

Sinsabaugh, R. L. and Findlay, S. (1995) Microbial Production, Enzyme Activity, and Carbon Turnover in Surface Sediments of the Hudson River Estuary. Microbial Ecology 30 :127-141.

Siuda, W., Wcislo, R. and Chróst, R. J. (1991) Composition and bacterial utilization of photosynthetically produced organic matter in an eutrophic lake. Archiv für Hydrobiologie 121(4): 473-484.

Smith, D. C., Simon, M., Alldredge, A. L. and Azam, F. (1992) Intense hydrolytic enzyme activity on marine aggregates and implications for rapid particle dissolution. Nature 359: 139-142.

Smith, D. C., Steward, G. F., Long, R. A. and Azam, F. (1995) Bacterial mediation of carbon fluxes during a diatom bloom in a mesocosm. Deep Sea Research II 42(1): 75-97.

Snaddon, C. D., Stewart, B. A. and Davies, B. R. (1992) The effect of discharge on leaf retention in two headwater streams. Archiv für Hydrobiologie 125(1): 109-120.

Sobczak, W. V. (1996) Epilithic bacterial responses to variations in algal biomass and labile dissolved organic carbon during biofilm colonization. Journal of the North American Benthological Society 15(2): 143-154.

Sommaruga, R. and Psenner, R. (1995) Permanent Presence of Grazing-Resistant Bacteria in a Hypertrophic Lake. Applied and Environmental Microbiology 61(9): 3457-3459.

Somville, M. (1984) Measurement and Study of Substrate Specificiy of Exoglucosidase Activity in Eutrophic Water. Applied and Environmental Microbiology 48(6): 1181-1185.

Somville, M. and Billen, G. (1983) A method for determining exoproteolytic activity in natural waters. Limnology and Oceanography 28(1): 190-193.

Stal, L. J. (1995) Tansley Review No. 84 Physiological ecology of cyanobacteria in microbial mats and other communities. New Phytologist 131(1): 1-32.

Stal, L. J., Gemerden, H. V. and Krumbein, W. E. (1985) Structure and development of a benthic marine microbial mat. FEMS Microbiology Ecology 31: 111-125.

Starink, M., Bär-Gilissen, M.-J., Bak, R. P. M. and Cappenberg, T. E. (1996) Seasonal and spatial variations in heterotrophic nanoflagellate and bacteria abundances in sediments of a freshwater littoral zone. Limnology and Oceanography 41(2): 234-242.

Statzner, B. and Higler, B. (1985) Questions and comments on the River Continuum Concept. Canadian Journal of Ficheries and Aquatic Sciences 42: 1038-1044. ????

Steinman, A. D. and Parker, A. F. (1990) Influence of substrate conditioning on periphytic growth in a heterotrophic woodland stream. Journal of the North American Benthological Society 9(2): 170-179.

Stevenson, R. J. (1984) How currents on different sides of substrates in streams affect mechanisms of benthic algal accumulation. Internationale Revue der gesamten Hydrobiologie 69(2): 241-262.

Stevenson, R. J. (1996) An introduction to algal ecology in freshwater benthic habitats. In: R. J. Stevenson, M. L. Bothwell and R. L. Lowe (eds.) Algal Ecology, Freshwater Benthic Ecosystems. Academic Press, p. 3-30.

Stevenson, R. J. and Glover, R. (1993) Effects of algal density and current on ion transport through periphyton communities. Limnology and Oceanography 38: 1276-1281.

Stock, M. S. and Ward, A. K. (1989) Establishment of a Bedrock Epilithic Community in a Small Stream: Microbial (Algal and Bacterial) Metabolism and Physical Structure. Canadian Journal of Fisheries and Aquatic Sciences 46: 1874-1883.

Sumner, W. T. and Fischer, S. G. (1979) Periphyton production in Fort River, Massachussets. Freshwater Biology 9: 205-212.

Sundh, Y. and R. T. Bell. 1992. Extracellular dissolved organic carbon released from phytoplankton as a source of carbon for heterotrophic bacteria in lakes of different humic content. Hydrobiologia 229: 93-106.

Tait, C. K., Li, J. L., Lamberti, G. A., Pearsons, T. N. and Li, H. W. (1994) Relationships between riparian cover and the communitiy structure of high desert streams. Journal of the North American Benthological Society 13(1): 45-56.

Tett, P., Gallegos, C., Kelly, M. G., Hornberger, G. M. and Cosby, B. J. (1978) Relationships among substrate, flow, and benthic microalgal pigment density in the Mechums River, Virginia. Limnology and Oceanography 23(4): 785-797.

Thurmann, E. M. (1985) Organic Geochemistry of Natural Waters. Nijhoff/Junk, Dordrecht.

Triska, F. J., Sedell, J. R. and Gregory, S. V. (1982) Coniferous forest streams. In: R. L. Edmonds (ed.) Analysis of coniferous forest ecosystems in the Western United States. Hutchinson Ross Publ., Stroudsburg, p. 292-332.

Triska, F. J., Kennedy, V. C., Avanzino, R. J. and Reilly, B. N. (1983) Effect of simulated canopy cover on regulation of nitrate uptake and primary production by natural periphyton assemblages. In: T. D. Fontaine and S. M. Bartell (eds.) Dynamics of Lotic Ecosystems. Annual Arbor Science Publishers, Michigan, p. 129-159.

Triska, F. J., Kennedy, V. C., Avanzino, R. J., Zellweger, G. W. and Bencala, K. E. (1989) Retention and transport of nutrients in a third-order stream: channel processes. Ecology 70: 1877-1892.

Tulonen, T., Kankaala, P., Ojala, A. and Arvola, L. (1994) Factors controlling production of phytoplankton and bacteria under ice in a humic, boreal lake. Journal of Plankton Research 16(10): 1411-1432.

Turley, C. (1994) Controls of the Microbial Loop: Nutrient Limitation and Enzyme Production, Location and Control. Microbial Ecology 28: 287-289.

Utermöhl, H. (1958). Zur Vervollkommung der quantitativen Phytoplankton-Methodik. Mitt. internationalen Vereingung für Limnolologie 9: 1-39.

Van Gaans, P. F. M. (1989) WATEQX- A restructured, generalized and extended Frtran 77 computer code and database format for the WATEQ aqueous chemical model for element speciation and mineral saturation, for use on personal computers or mainframes. Computers and Geosciences 15: 843-887.

Vannote, R. L., Minshall, G. W., Cummins, K. W., Sedell, J. R. and Cushing, C. E. (1980) The River Continuum Concept. Canadian Journal of Fisheries and Aquatic Sciences 37: 130-137.

Verrecchia, E., Yair, A., Kidron, G. J. and Verrecchia, K. (1995) Physical properties of the psammophile cryptogamic crust and their consequences to the water regime of sandy soils, north-western Negev Desert, Israel. Journal of Arid Environments 29(4): 427-437.

Vincent, W. F. and Howard-Williams, C. (1986) Antarctic stream ecosystems: physiological ecology of a blue-green algal epilithon. Freshwater Biology 16: 219-233.

Vives-Rego, J., Billen, G., Fontigny, A. and Somville, M. (1985) Free and attached proteolytic activity in water environments. Marine Ecology Progress Series 21: 241-249.

Volk, C. J., Volk, C. B. and Kaplan, L. A. (1997) Chemical composition of biodegradable dissolved organic matter in streamwater. Limnology and Oceanography 42 (1):39-44.

Vrba, J. (1992) Seasonal extracellular enzyme activities in decomposition of polymeric organic matter in a reservoir. Archiv für Hydrobiologie, Beih. 37: 33-42.

Vrba, J,, Nedoma, J., Simek, K. and Seda, J. (1992) Microbial decomposition of polymer organic matter related to plankton development in a reservoir: activity of α-, β-glucosidase, and β-N-acetylglucosaminidase and uptake of N-acetylglucosamine. Archiv für Hydrobiologie 126(2): 193-211.

Vrba, J., Simek, K., Nedoma, J. and Hartman, P. (1993) 4-Methylumbelliferyl-β-N-Acetylglucosaminide Hydrolysis by a High-Affinity Enzyme, a Putative Marker of Protozoan Bacterivory. Applied and Environmental Microbiology 59(9): 3091-3101.

Ward, J. V. (1984) Ecological perspectives in the management of aquatic insects habitat. In: V. H. Resh and R. M. Rosenberg (eds.) The ecology of aquatic insects. Praeger, New York, p. 558-577.

Ward, J. V. and Stanford, J. A. (1991) Research directions in stream ecology. In: J. Menon (ed.) Advances in Ecology. Council Science Research, Trivandrum, India, p. 121-132.

Webster, J. R. and Waide, J. B. (1982) Effects of forest clearcutting on leaf breakdown in a southern Appalachian stream. Freshwater Biology 12: 331-344.

Wellnitz, T. A., Rader, R. B. and Ward, J. V. (1996) Light and a grazing mayfly shape periphyton in a Rocky Mountain stream. Journal of the North American Benthological Society 15(4): 496-507.

Wetzel, R. G. (1991) Extracellular enzymatic interactions: storage, redistribution, and interspecific communication. In: R. J. Chróst (ed.) Microbial Enzymaes in Aquatic Environments. Springer-Verlag, New York, p. 6-28.

Wetzel, R. G. (1993) Humic compounds from wetlands: complexation, inactivation, and reactivation of surface-bound and extracellular enzymes. Verhandlungen der Internationalen Vereinigung für Theoretische und Andgewandte Limnologie 25: 122-128.

White, D. S. (1993) Perspectives on defining and delineating hyporheic zones. Journal of the North American Benthological Society 12(1): 61-69.

Whitford, L. A. and Schumacher, G. J. (1961) Effect of current on mineral uptake and respiration by a freshwater alga. Limnology and Oceanography 6: 423-425.

Whitton, B. A. (1970) Biology of *Cladophora* in freshwaters. Water Research 4: 457-476.

Whitton, B. A. (1991) Use of Phosphatase Assays with Algae to Assess Phosphorus Status of Aquatic Environments. Bioindicators and Environmental Management, p. 295-310.

Wiebe, W. J., Sheldon, J. W. M. and Pomeroy, L. R. (1992) Bacterial growth in the cold: evidence for an enhanced substrate requirement. Applied and Environmental Microbiology 58: 359-364.

Williams, D. D. and Hynes, H. B. N. (1976) The ecology of temporary streams I. The faunas of two Canadian streams. Internationale Revue der gesamten Hydrobiologie 61: 761-787.

Williams, P. J. I. (1973) The validity of the application of simple kinetic analysis to heterogeneous microbial populations. Limnology and Oceanography 18: 159-165.

Winsborough, B. M. and Seeler, J.-S. (1986) The Relationship of Diatom Epiflora to the Growth of Limnic Stromatolites and Microbial Mats. In: M. Ricard (ed.) Proceedings of the Eighth International Diatom Symposium (Paris, 1984). Koeltz Scientific Books. Koenigstein., p. 395-407.

Winsborough, B. M. and Golubic, S. (1987) The role of diatoms in stromatolite growth: two examples from modern freshwater settings. Journal of Phycology 23: 195-201.

Winsborough, B. M., Seeler, J.-S., Golubic, S. and Folk, R. L. (1994) Recent fresh-water lacustrine stromatolites, stromatolitic mats and oncoids from northeastern Mexico. In: J. Bertrand-Sarfati and C. Monty (eds.) Phanerozoic Stromatolites II. Kluwer Academic Publishers.Netherlands., p. 71-100.

Wolfaardt, G. M., Lawrence, J. R., Robarts, R. D. and Caldwell, D. E. (1995) Bioaccumulation of the Herbicide Diclofop in Extracellular Polumers and Its Utilization by a Biofilm Community during Starvation. Applied and Environmental Microbiology 61(1): 152-158.

Wright, R. T. and Burnison, B. K. (1979) Heterotrophic activity measured with radiolabelled organic substrates. In: J. W. Costerton and R. R. Colwell (eds.) Native Aquatic Bacteria: Enumeration, Activity, and Ecology. ASTM Special Technical Publication 695. American Society for Testing and Materials, Philadelphia, p. 140-155.