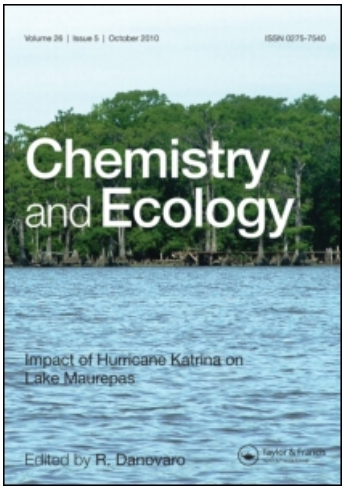

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MICROBIAL HYDROLYSIS OF POLYSACCHARIDES AND ORGANIC PHOSPHATES IN THE NORTHERN ADRIATIC SEA

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β-glucosidase and alkaline phosphatase, two ectoenzymes involved in the microbial decomposition of polysaccharides and organic phosphates, were evaluated in water samples collected from two areas of the Northern Adriatic Sea during the multidisciplinary Prisma II research project. The distribution of β-glucosidase and alkaline phosphatase is reported together with that of leucine aminopeptidase, an enzyme involved in the degradation of protein compounds. The data obtained showed the prevalence of β-glucosidase in summer months in the northern area, located in front of the Po delta, while in winter it is higher in the southern area (in front of Ancona). Phosphatase activity during February, 1998 had a maximum of 107 nm l^{-1} h^{-1} at a coastal station in the northern area; this enzyme appeared to be mainly associated with phytoplankton because the increase in alkaline phosphatase was associated with the increase of phytoplankton biomass, measured by chlorophyll a content. The relations between the microbial activities and environmental parameters are discussed.

Keywords: Enzymatic activities; polysaccharides; organic phosphate; Adriatic Sea

1. INTRODUCTION

In all marine environments, organic carbon represents the main trophic source for planktonic consumers. In the Adriatic Sea, particulated and dissolved carbon mainly derived from autochthonous (*i.e.* primary and secondary production) and allochthonous sources (Po inputs). The carbon substrates for bacterial development may result from algal exudation, cell lysis and zooplankton grazing and from bacterial colonization and hydrolysis of senescent algal cells and aggregates (Billen, 1990). The increase in bacterial biomass in relation to the increase in phytoplankton biomass may be delayed, *i.e.* 7–14 days in coastal areas, depending on the bacterial affinity for polymers (Karner and Herndl, 1992; Middelboe *et al.*, 1995). Polysaccharides and proteins represent the most abundant polymers of phytoplankton derived organic matter. Heterotrophic bacteria rapidly produce hydrolytic enzymes that transform dissolved polymeric substrates into readily utilisable monomers for their growth.

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The sum of the utilisation rates of monomers (monosaccharides, amino acids and carboxylic acids) may be used as an estimation of the total rate of carbon utilisation by heterotrophic bacteria (Lancelot and Billen, 1984).

The uncoupling between hydrolysis and uptake might be an important factor involved in mucillage formation, frequently observed in the Adriatic Sea (Herndl *et al.*, 1992), since the accumulation of organic matter could result from the inefficiency or from the occurrence of the two microbial processes at different times (Unanue *et al.*, 1998).

This paper focuses on the microbial hydrolysis processes acting on carbohydrates and organic phosphates present in the water column of the Northern Adriatic Sea. Bacterial extracellular hydrolysis was measured as the potential activity of two enzymes: β-glucosidase (GLU) and alkaline phosphatase (AP). β-glucosidase is a specific enzyme for hydrolysis of cellobiose present in polymers such as cellulose and mucopolysaccharides. It is generally known that β-glucosidase and peptidase activities are mostly associated with the heterotrophic bacteria (Hoppe, 1983; Chrost 1991). Some zooplanktonic species have been reported to possess these activities (Karner *et al.*, 1994; Bochdansky *et al.*, 1995).

Alkaline phosphatase, which hydrolyses organic phosphates, is not specific for bacteria and has been demonstrated also in association with phytoplankton and protozoans (Chrost, 1991). Most viable heterotrophic bacteria possess this activity, namely from 70 to 100% of the strains isolated from the Adriatic Sea (Zaccone *et al.*, 1998).

Enzymatic activities on polysaccharides and organic phosphates have been analysed together with leucine aminopeptidase, an ectoenzyme involved in the decomposition of natural peptide polymers. The mobilisation of carbon by this enzyme and by respiratory activity, which had been studied in the same CNR-MURST Prisma II project, has revealed a higher mineralization rate during the summer (La Ferla *et al.*, in press; Zaccone *et al.*, 1999).

2. MATERIALS AND METHODS

Sea water samples (total number 117) were collected during June, 1997 and February, 1998 in two areas of the Northern Adriatic Sea: the northern area, located in front of the Po delta, and the southern one, near Ancona (Fig. 1). Phosphatase activity was evaluated only in February, 1998.

Enzymatic assays were performed on intact water samples according to Hoppe (1983). Suitable amounts of fluorogenic methylumbelliferyl-derived substrates (4-MUF-β-D-glucopyranoside and 4-MUF phosphate (Sigma) for β-glucosidase and phosphatase determination, were added to 10 ml of sample in order to obtain a final concentration of 200 μM (saturating concentration, previously determined); the assay was performed in triplicate, using sterile sea water as a blank. The increase in fluorescence after 3 h of incubation at "*in situ*" temperature was measured with a spectrofluorometer Hitachi at 365 nm (excitation) and 445 nm (emission) wavelengths and converted into nM l^{-1} h^{-1} of MUF released through a calibration curve of methylumbelliferone standard. The same method was applied to measure the leucine aminopeptidase (LAP) activity, reading samples at 380 nm (excitation) and 440 nm

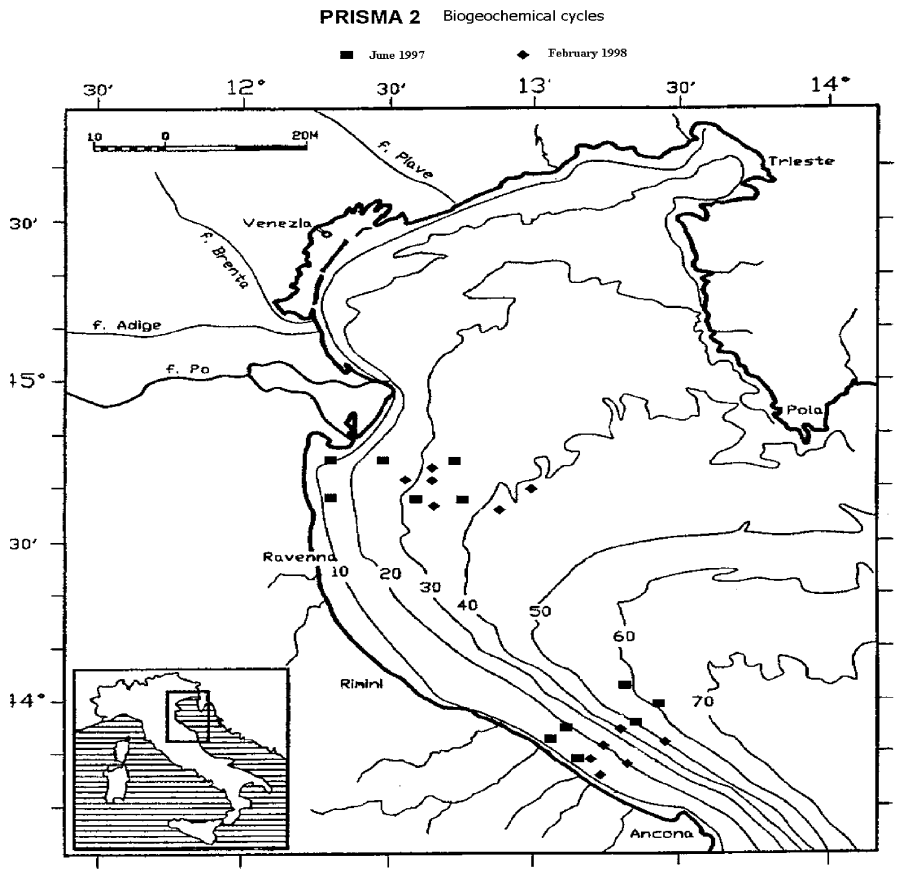

FIGURE 1 Sampling area.

(emission) wavelengths and using a calibration curve of MCA (7 amido 4-methyl-coumarin). Cultivable heterotrophic bacteria (M.A.) was determined on Marine Agar plates incubated at 20°C for 7 days.

Data related to other environmental variables were kindly provided by the research groups collaborating in the same project.

3. RESULTS AND DISCUSSION

Mean, minimal and maximal values of β-glucosidase, phosphatase, cultivable bacteria chlorophyll-a and dissolved carbohydrates are reported in Table I. Values from the northern area are clearly higher than those of the southern one during the summer. Dissolved carbohydrates (DCHO) constitute 18.4 and 14.2% of dissolved organic carbon (DOC) in June, 1997 and in February, 1998, respectively (Pettine *et al.*, in press).

Figures 2 and 3 show vertical profiles of β-glucosidase and aminopeptidase activities in the two sampling periods. In the northern area, enzymatic activities generally show a decreasing trend from the surface to the bottom in June, 1997. In February, 1998 the enzymatic activities did not show a clearly, decreasing pattern with water depth.

TABLE I. Range, mean value and standard error (S.E.) calculated for ß-glucosidase, dissolved carbohydrates (DCHO), chlorophyll a (CHLa) and phosphatase. (Number of samples, N)

		N	*Range*	*Mean*	*S.E.*
			β-glucosidase (nM l^{-1} h^{-1})		
Jun '97	Northern area	30	0.1–38.7	5.2	1.7
	Southern area	30	0.0–3.1	0.9	0.2
Feb '98	Northern area	30	0.0–9.9	2.1	0.5
	Southern area	27	0.5–39.0	5.0	1.9
			MA (CFU M l^{-1})		
Jun '97	Northern area	30	40–2717	874	159.49
	Southern area	30	100–1045	461	84.12
Feb '98	Northern area	30	55–1190	1387	70.62
	Southern area	27	40–4950	1067	204.0
			DCHO (µM l^{-1})		
Jun '97	Northern area	24	10.0–50.0	20.0	1.6
	Southern area	22	10.8–24.2	16.7	0.8
Feb '98	Northern area	20	7.0–39.4	13.8	2.1
	Southern area	22	6.7–19.4	11.3	0.7
			CHLa (µg l^{-1})		
Jun '97	Northern area	30	0.5–6.6	1.5	0.2
	Southern area	30	0.1–2.9	0.9	0.1
Feb '98	Northern area	30	0.6–8.0	2.2	0.4
	Southern area	27	0.4–9.2	2.8	0.6
			Phosphatase (nM l^{-1} h^{-1})		
Feb '98	Northern area	30	0.0–107.5	13.6	5.0
	Southern area	27	0.1–1.2	2.4	0.5

DCHO data were kindly provided by Dr. Pettine, IRSA-CNR, Roma and CHLa data by Dr. Decembrini IST-CNR, Messina.

The leucine aminopeptidase shows higher values than β-glucosidase (Figs. 2 and 3); along the water column the leucine aminopeptidase/β-glucosidase ratio (ranging from 5 to 300) may vary in relation to a shift in the qualitative composition of the organic matter available in the water. In fact, variations in vertical activity profiles may be related to changes in both availability and nutritional quality of degradable organic matter (Fabiano and Danovaro, 1998). Peptidase is the most important enzyme for bacterial growth, and the greater amount of leucine aminopeptidase with respect to β-glucosidase may be explained by the capability of leucine to provide a source of both carbon and nitrogen for bacteria (Middelboe *et al.*, 1995).

During the survey of June, 1997, β-glucosidase activity in the northern area was significantly correlated with all the parameters (POC, DOC, CHLa, bacterial carbon production (BCP) and DCHO) (Tab. II). Similar correlations were observed in the southern area, although to a lesser degree of significantivity. The particulated and dissolved organic matter constitute the substrate for microbial enzymes. In this season, the microbial attack of the lysis products of the phytoplankton is at its peak. The higher correlation between β-glucosidase and POC ($r^2 = 0.94$) suggests that the variability in microbial activity depends as much as 94% on the variations in particulated carbon.

The significant correlation between β-glucosidase and autotrophic biomass, measured by chlorophyll-a (CHLa) content, indicates that most of this enzymatic activity may be associated with autotrophic cells. Also Chrost (1989) found a relationship between β-glucosidase activity and the release of a great quantity of polymeric matter

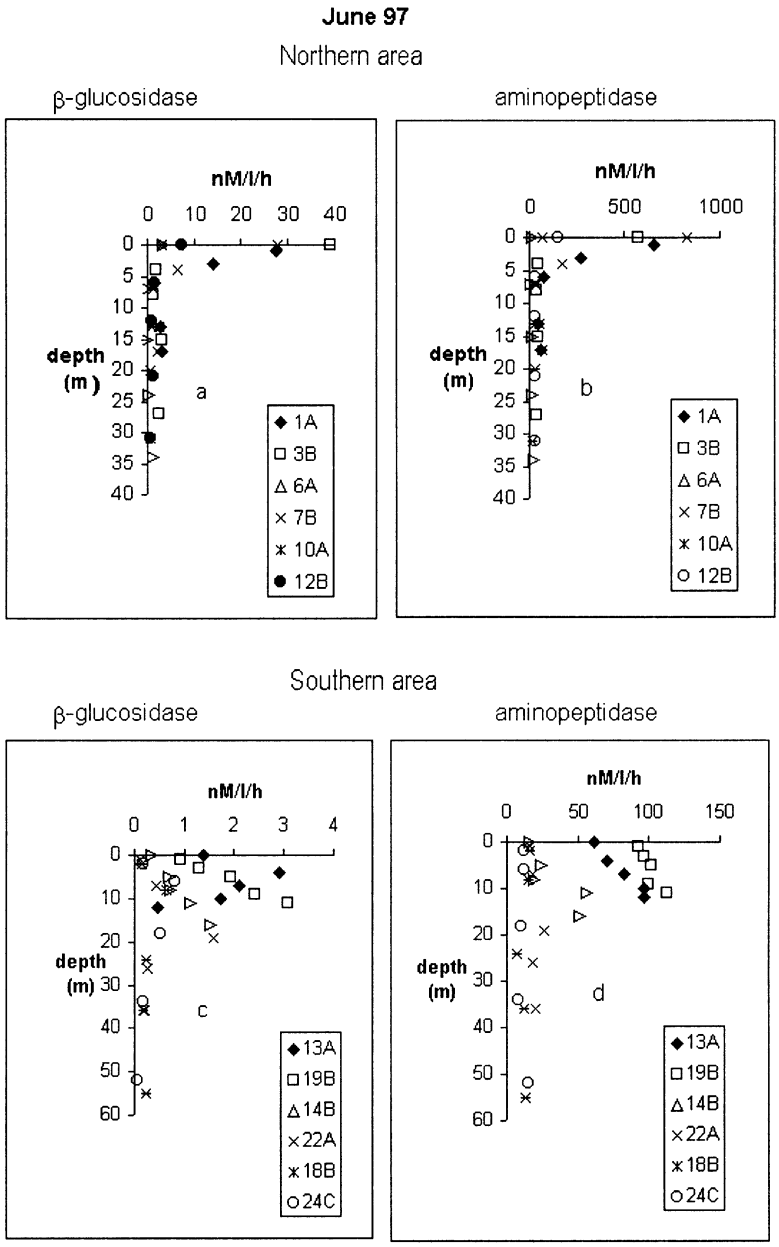

FIGURE 2 Vertical profiles of β-glucosidase (a) and aminopeptidase (b) in northern area; β-glucosidase (c) and aminopeptidase (d) in southern area in June, 1997.

(in our study, 20 μM l^{-1} polysaccharides in the northern area in the summer) that might induce the synthesis of β-glucosidase.

In February, 1998, however, the β-glucosidase was not correlated with any of the analysed parameters (Tab. II). The lack of correlations could be related to the availability of monosaccharides during the active growth of phytoplankton, when the excretion of photosynthetic products can inhibit activity and repress the synthesis of β-glucosidase (Chrost, 1989). The environmental conditions recorded during February in

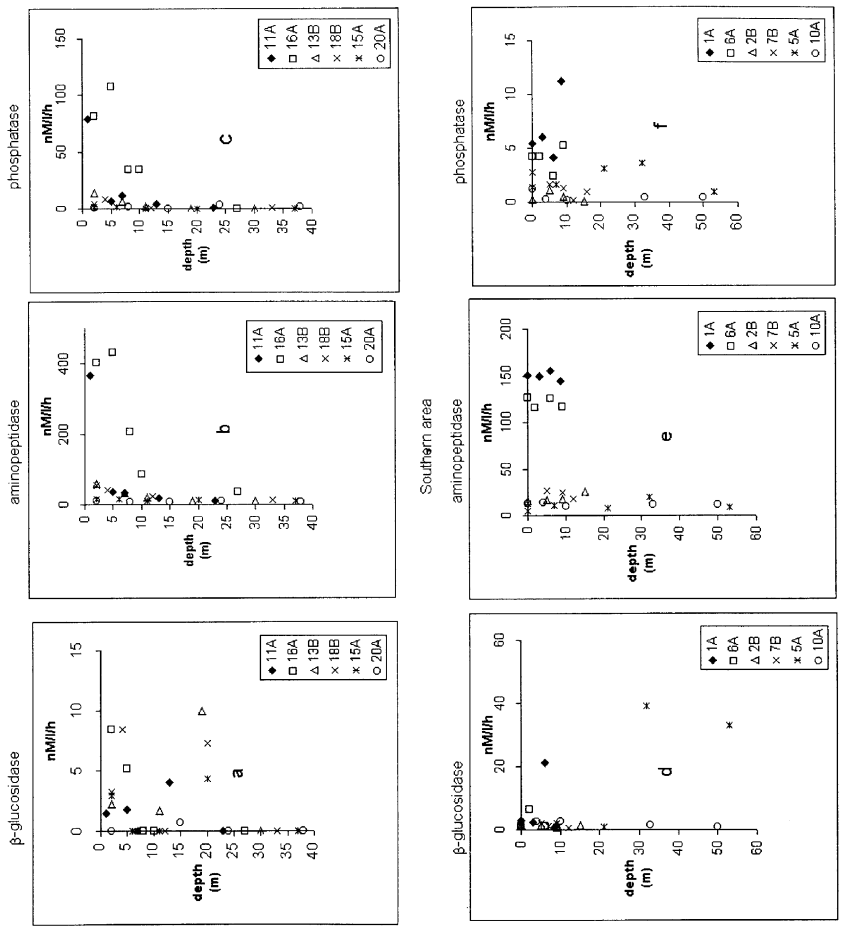

FIGURE 3 Vertical profiles of β-glucosidase (a) aminopeptidase (b) and phosphatase (c) in northern area; β-glucosidase (d) aminopeptidase (e) and phosphatase (f) in southern area in February, 1998.

TABLE II Correlation between enzyme activities and other environmental parameters

	DOC	*BCP*	*GLU*	*LAP*	*POC*	*MA*	*CHL*	*DCHO*	*DAPI*	
June 97	Northern area									
GLU	0.63**	0.91**	1	0.94**	0.97**	0.69**	0.92**	0.71**	0.16	
LAP	0.67**	0.99**	0.94**	1	0.94**	0.63**	0.83**		0.16	
	$n=30$									
	$n=24$ with DCHO									
	Southern area									
GLU	0.17	0.01	1	0.77**	0.70**	0.24	0.69**	0.50**	0.59**	
LAP	0.56**	−0.08	0.77**	1	0.91**	0.28	0.84**		0.76**	
	$n=30$									
	$n=23$ with DOC									
	$n=15$ with POC									
	DOC	*BCP*	*GLU*	*LAP*	*AP*	*POC*	*MA*	*CHL*	*DCHO*	*DAPI*
February 98	Northern area									
GLU	0.12	0.26	1	0.30	0.28	n.d.	0.25	0.24	0.23	0.15
LAP	0.73**	0.98**	0.30	1	0.98**	n.d.	0.49**	0.87**		0.07
AP	0.72**	0.95**	0.28	0.98**	1	n.d.	0.50**	0.92**	0.85**	0.05
	$n=30$									
	$n=20$ with DCHO									
	Southern area									
GLU	0.07	−0.02	1	−0.01	0.06	n.d.	0.23	0.01	0.06	0.10
LAP	0.87**	0.98**	−0.01	1	0.79**	n.d.	0.71**	0.99**		0.70**
AP	0.69**	0.79**	0.06	0.79**	1	n.d.	0.65**	0.76**	0.81**	0.34
	$n=27$									
	$n=22$ with DCHO									

DOC, dissolved organic carbon; BCP, bacterial carbon production; GLU, β-glucosidase; LAP, leucine-aminopeptidase; POC, particulate organic carbon; MA, viable plate counts on marine agar; CHL, chlorophyll a; DCHO, dissolved carbohydrates; DAPI, total bacterial counts; AP, alkaline-phosphatase.

the northern area seem to be typical of a phytoplanktonic growth phase as confirmed by chlorophyll and phytoplankton counts (Cabrini M., personal communication).

The normalisation of the β-glucosidase to the cell count (defined as specific activity) shows higher values in June and at the coastal stations. Also in a Caribbean reef lagoon, Rath *et al.* (1993) found that the activity per cell decreases from eutrophic to oligotrophic stations. However, this finding still remains controversial, since enzymatic activity may also be associated with other non-bacterial cells and moreover, not all bacterial cells are active or produce the same amount of enzyme (Fabiano and Danovaro, 1998).

Previous studies showed that bacterial strains that possess β-glucosidase constitute only a minor fraction (ranging from 0 to 30%) of heterotrophic bacteria able to grown in media, during a seasonal study (Zaccone *et al.*, 1998). β-glucosidase activity values obtained in this study are similar to those found by Chrost (1989) during the spring phytoplankton bloom (18.25–47.75 $\mathrm{nmol\,l^{-1}\,h^{-1}}$) in lake PluBsee, but one order of magnitude higher than those observed in the Baltic Coast by Chrost and Velimirov (1991).

The amounts of β-glucosidase compared to the total organic carbon content (DOC + POC) and to the dissolved carbohydrate concentrations allowed us to estimate that this enzyme potentially mobilizes along the water column a percentage of 0.1–0.6% and 9.7–77.0% per day.

Alkaline phosphatase represents an important enzyme involved in organic phosphorus remineralization; it is particularly efficient in phosphate limited conditions.

Alkaline phosphatase generally decreases with increasing water depth in the northern area, where higher values were reported at coastal stations. In the southern area a clear trend was not observed (Fig. 3).

Alkaline phosphatase values reported for the northern area were on average comparable with those observed by Chrost *et al.* (1989) during phytoplankton growth, however in a wider range with a maximum of 107 $\mathrm{nM\,l^{-1}\,h^{-1}}$ in the coastal station 16A. The same authors found low levels of alkaline phosphatase during algal bloom and increased levels in post bloom condition. During bloom phosphatase activity was associated both with phytoplankton and bacterioplankton cells. By contrast, in post bloom phase most of the activity was related to bacterial cells.

In the Northern Adriatic Sea, in February, 1998 (in conditions of a presumed phytoplankton growth) phosphatase seemed to be associated with the bacterial component (with bacteria able to grow on Marine Agar medium, $r = 0.50$ and 0.65 in the two areas) but above all with the phytoplankton biomass measured as CHLa ($r = 0.92$ and 0.76 in the two areas).

The positive correlation between alkaline phosphatase and chlorophyll indicates, according to Middelboe *et al.* (1995), a more dependent enzymatic activity on phytoplankton than on bacteria. Alkaline phosphatase values decreasing along a trophic gradient were also found by Rath *et al.* (1993).

Phosphatase was significantly correlated with most of the analysed parameters (excepting bacteria direct counts). The significant correlation between alkaline phosphatase and bacterial secondary production suggests that this enzyme may supply key elements for bacterial metabolism phosphorus (*i.e.* inorganic phosphorus). In fact,

bacterial production may increase by addition of inorganic phosphorus (Pettine *et al*., in press).

Nutrient conditions may also affect distribution of alkaline phosphatase as indicated by Nausch and Nausch (1999) in the Baltic Sea. In The North Adriatic Sea the limited phosphorus condition showed by DOP budget (Cozzi *et al*., in press) probably induces the enzyme production.

4. CONCLUSIONS

This study indicates that the quantity and quality of enzymes produced by bacteria may vary in relation to trophic conditions of the environment. During summer the notable contribution of lysis products of phytoplankton stimulates the production of β-glucosidase other than protease.

The production of phosphatase constitutes an interesting index since it supplies inorganic phosphorus both for bacterial as well as for phytoplanktonic growth. Further studies will however be necessary to evaluate the contribution to the activity of bacteria and phytoplankton in different seasonal conditions.

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