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Vol. 43: 267–276, 2006 | AQUATIC MICROBIAL ECOLOGY
Aquat Microb Ecol | Published July 19

Pressure effects on surface Mediterranean prokaryotes and biogenic silica dissolution during a diatom sinking experiment

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ABSTRACT: This study examined the effect of increasing pressure on biogenic silica dissolution and on prokaryotic assemblages associated with diatom detritus. Experiments were carried out in hyperbaric bottles subjected to a gradual increase in pressure and compared to incubations at atmospheric pressure. To examine only pressure effects and to simulate detritus degradation in the Mediterranean Sea, the incubation temperature was kept constant (13°C), while pressure was increased by 1.5 MPa d^{-1}, simulating a fall of particles at a sinking rate of 150 m d^{-1} over 8 d. Aminopeptidase activity was significantly lower (nearly 5-fold) under increasing pressure than under atmospheric pressure conditions. Lower aminopeptidase activity under increasing pressure affected biogenic silica dissolution, at least at the beginning of the incubation, corresponding to a simulated depth of the first 800 m of the water column. Silicic acid regeneration rates were very low (0.07 ± 0.02 µmol l^{-1} h^{-1}) under increasing pressure conditions during the first 4 d (i.e. between 200 and 800 m), while rates were much higher under atmospheric pressure (0.32 ± 0.05 µmol l^{-1} h^{-1}). However, orthosilicic acid concentrations in the incubations under increasing pressure approached those of the atmospheric pressure incubations by the end of the experiment. In contrast, the taxonomic composition of prokaryotic communities was not affected by increasing pressure, but the input of fresh diatom detritus led to an increase in the relative abundance of the *Cytophago–Flavobacter* cluster and γ-*Proteobacteria*. These results suggest that hydrostatic pressure affects the function rather than the broad taxonomic structure of prokaryotic communities associated with sinking detrital particles.

KEY WORDS: Hydrostatic pressure · Particle sinking · Decomposition processes · Ectoenzymatic activity · Silica dissolution · Prokaryotic diversity · Mesopelagic waters · Bathypelagic waters

INTRODUCTION

Attached bacteria play an important biogeochemical role in the oceanic carbon flux and are implicated in the remineralization and enzymatic dissolution of particulate organic matter (POM) during its descent through the water column (Cho & Azam 1988, Turley & Mackie 1994, 1995). Remarkably little is known about the composition of POM as it sinks through mesopelagic and bathypelagic waters (Wakeham et al. 1997, Hedges et al. 2000). It is known that a large component of the sinking flux consists of diatoms, which dominate phytoplankton communities at specific times of the year in many oceanic systems (Honjo et al. 1995, Smith et al. 1996, Tréguer & Pondaven 2000).

The fate of sinking biogenic mineral particles depends on the remineralization of the organic matrix, as well as on the dissolution of the mineral matrix. Both processes could be mediated by microbial activities. Milliman et al. (1999), for instance, suggested the exis-

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tence of a biologically mediated dissolution of calcium carbonate. Other studies demonstrated that bacteria accelerate diatom opal dissolution by colonizing and enzymatically degrading the organic matrix of diatom frustules (Bidle & Azam 1999, 2001). Bidle et al. (2002) found that the control by temperature of marine bacteria degrading diatom detritus strongly influences the coupling of biogenic silica and organic carbon preservation.

Recently, based on data from the equatorial Pacific, Armstrong et al. (2002) and Francois et al. (2002) argued that fluxes of ballast minerals (biogenic silica or diatom opal, carbonate biominerals, and dust) determine deep-water particulate organic carbon (POC) fluxes, so that a mechanism-based model of POC export must simultaneously model both POC and the fluxes of ballast minerals. According to these models, the deep-water fluxes of organic carbon are directly proportional to the total fluxes of ballast minerals at these depths. Therefore, the ability to quantify correctly the remineralization processes at each depth in the water column is critical. This is especially necessary for using models of POC remineralization reliably. In fact, models of POC mineralization, such as that of Martin et al. (1987), assume that there is a tight quantitative relationship between export production and the amount of carbon reaching the sediments. If so, the organic carbon flux can be predicted at any depth solely from the carbon flux at some near-surface depth.

In spite of numerous studies showing that decompression of deep samples may lead to underestimates of prokaryotic activity rates (ZoBell 1970, Tabor et al. 1981, Jannasch & Wirsen 1982, Bianchi & Garcin 1993, Deming 2001, Tamburini et al. 2002, 2003), degradation rates of sinking particles are usually measured under atmospheric pressure (Bidle & Azam 1999, 2001, Sempéré et al. 2000, Bidle et al. 2002, Panagiotopoulos et al. 2002). As a consequence, current biogeochemical models of the silica cycle do not take into account the possible effects of hydrostatic pressure on decomposition during sinking. These models are based on the assumption that biogenic silica dissolution is controlled by temperature, zooplankton grazing, and diatom aggregation rate (Tréguer et al. 1989, Nelson et al. 1995, Dugdale & Wilkerson 1998), and implicitly assume that pressure has no effect.

The purpose of this study was to examine the effect of a gradual increase in pressure on surface prokaryotic communities enriched with axenic diatom detritus. Parallel incubations were set up under controlled pressure conditions, and prokaryotic ectoenzymatic activity and dissolution of biogenic silica rates, as well as the structure of the prokaryotic communities, were monitored over time. The experiments were based on the experimental design of Bidle & Azam (1999, 2001) and Bidle et al. (2002, 2003), but were modified to study pressure effects.

MATERIALS AND METHODS

Synthesis of diatom detritus from *Thalassiosira weissflogii*. An axenic culture of *T. weissflogii* (CCMP 1336) was obtained from the Provasoli Guillard Center for Culture of Marine Phytoplankton (West Boothbay Harbor, Maine, USA) and grown in *f*/2 medium (Guillard 1975). This culture was verified by microscopy to be axenic. Fresh diatom detritus was obtained from cell suspensions by rapidly freeze-thawing (dry ice/ethanol bath followed by a 55°C water bath) diatom cells for 7 cycles. The detritus was stored at –20°C until further use. A fresh diatom detritus sample was diluted with 0.8 µm filtered seawater to obtain a final concentration of 400 µg POC l^{-1} in order to mimic a decaying diatom bloom. This concentration corresponded to a cell density of 10^3 to 10^4 cells ml^{-1}.

Incubation under increasing pressure. Two experiments were carried out in January 2002 and February 2004. Mediterranean seawater (13°C) was collected off Marseille, France, above the Cassidaigne Trench at 200 m depth (February 2002) and at the surface (January 2004). Natural prokaryotic assemblages were obtained by gentle filtration through 0.8 µm polycarbonate filters. Incubations under increasing pressure were carried out in hyperbaric bottles, which can reproduce the increase in hydrostatic pressure observed in the ocean with increasing depth. These high-pressure bottles were the same as the ones used in previous studies to obtain undecompressed deep-sea water samples (Bianchi et al. 1999, Tamburini et al. 2003). The 500 ml high-pressure bottles were first autoclaved, filled with seawater containing the natural bacterial assemblage, and subsequently amended with the fresh diatom detritus mixture. Diatom detritus was kept in suspension by rotation (half-revolution each minute) using a specialized apparatus designed for high-pressure bottles. The incubation was at 13°C, corresponding to the average temperature of the water column below 200 m in the Mediterranean Sea. One bottle was kept at atmospheric pressure, while a second bottle was subjected to a pressure increase of 1.5 MPa d^{-1}, to simulate a sinking rate of 150 m d^{-1}. For the first experiment (January 2002), pressure was increased by 1.5 MPa d^{-1}. For the second one (February 2004), pressure was increased continuously by a programmable computer-driven system. The experimental system will be described in detail elsewhere (C. Tamburini et al. unpubl. data). According to laboratory and field studies (Alldredge & Gotschalk 1988), the sinking speed used in these experiments is applicable to mid-density particles such

as those from diatoms or clay aggregates. Settling velocities of marine snow range from 1 to 370 m d^{-1}, depending on the composition, size, and abundance of the particles, as well as on the hydrodynamic features of the environment. Two types of controls were performed: (1) one with sterilized seawater, i.e. without any prokaryotes, but with diatom detritus, and (2) another with the natural prokaryote assemblage, but without any added diatom detritus.

Every 2 d, community structure and silicic acid concentrations were sampled in the incubations, while maintaining the pressure conditions of the incubation. To measure total community aminopeptidase activity, 50 ml was transferred to 2 new sterilized high-pressure bottles (one for the atmospheric incubation, the other for the pressurized incubation); for the bottle maintained under atmospheric pressure, transfer and incubation were performed under atmospheric pressure. For the bottle under increasing pressurization, transfer and incubation were performed without any change in pressure. For example, on Day 4, the pressure of the culture under increasing pressure was 8 MPa, and transfer and incubation for aminopeptidase activity measurements were done at 8 MPa. All incubations were done at *in situ* temperature (13°C).

Aminopeptidase activity and silicic acid measurement. The fluorogenic substrate L-leucine-7-amino-4-methylcoumarin (Leu-MCA, Sigma Chemical; Chrost 1991) was added at a final concentration of 100 µM (Bidle & Azam 1999, 2001). Ectoenzymatic assays were done over 4 h, and the increase in fluorescence was measured using a Kontron SFM 25 spectrofluorometer (emission and excitation wavelengths at 455/365 nm). Orthosilicic acid [$Si(OH)_4$] concentrations were measured using a colorimetric method (Strickland & Parsons 1972) on a CECIL (CE 1011) spectrophotometer, with a detection limit of 50 nM.

Fluorescence *in situ* hybridization (FISH). The relative abundance of the major prokaryotic groups in natural communities was analyzed by FISH using Cy3-labeled oligonucleotides (Table 1). Samples were prepared following the method of Glöckner et al. (1996), as modified by Cottrell & Kirchman (2000a). Sub-samples were fixed with paraformaldehyde (final concentration 2%) and stored overnight at 4°C. They were sonicated to homogenize them and filtered onto 0.2 µm pore size polycarbonate filters (Millipore), rinsed twice with 0.2 µm filtered Milli-Q water, and stored at –20°C. For each probe, a filter piece (cell-adherent side) was placed on a Parafilm (Pechiney Plastic Packaging)-covered glass slide and overlaid with a 30 µl drop of hybridization solution using a final probe concentration of 2.5 ng $µl^{-1}$. We used the same concentration for unlabeled competitor probes, i.e. unlabeled Gam42a with labeled Bet42a and unlabeled Bet42a with labeled Gam42a (Manz et al. 1992). The glass slides were placed in a closed 50 ml tube and incubated overnight at 42°C. The hybridization solution contained 0.9 M NaCl, 20 mM Tris-HCl (pH 7.4), 5 mM edatic acid (EDTA), 0.01% sodium dodecyl sulfate (SDS), and the concentration of formamide was determined to achieve the best specificity for the bacterial groups targeted by the different probes (Zarda et al. 1997, Eilers et al. 2000). After hybridization, each piece of filter was transferred into 1 ml of a pre-warmed wash solution containing 20 mM Tris-HCl (pH 7.4), 10 mM EDTA, 0.01% SDS, and a concentration of NaCl appropriate for the probe (Zarda et al. 1997, Eilers et al. 2000). The concentrations of formamide and NaCl used are listed in Table 1. Filters were mounted using a mixture (4:1) of Citifluor (Ted Pella) and Vectashield (Vector Labs) containing 2 µg ml^{-1} of 4′,6-diamidino-2-phenylindole (DAPI). Cells were counted using semi-automated image analysis on an Olympus Provis AX70 microscope fitted with a Cy3 filter 41007a (Chroma) and a DAPI filter 31000 (Chroma) with a SPOT-RT (Diagnostic Instruments) and with ImagePro Plus (Media Cybernetics) software (Cottrell & Kirchman 2003).

Table 1. The 16S rRNA-targeted oligonucleotide probes used in this study. FA: formamide

Probe	Sequence (5′ to 3′) of probe	Target organisms	% FA	[NaCl] (mM)	Source
Eub338	GCT GCC TCC CGT AGG AGT	Domain of *Bacteria*	30	102	Amann et al. (1990)
NegControl	TAG TGA CGC GCT CGA	For non-specific probe binding	30	102	Karner & Fuhrman (1997)
Alf968	GGT AAG GTT CTG CGC GTT	Most of α-subclass of *Proteobacteria*	30	102	Glöckner et al. (1999)
Gam42a	GCC TTC CCA CAT CGT TT	γ-subclass of *Proteobacteria*	30	102	Manz et al. (1992)
Bet42a	GCC TTC CCA CTT CGT TT	β-subclass of *Proteobacteria*	30	102	Manz et al. (1992)
CF319a	TGG TCC GTG TCT CAG TAC	*Cytophaga–Flavobacter* cluster	35	80	Manz et al. (1996)
Arch915	GTG CTC CCC CGC CAA TTC CT	*Archaea*	20	308	Stahl & Amann (1991)
Cren537	TGA CCA CTT GAG GTG CTG	*Crenarchaea*	20	308	Teira et al. (2004)
Eury806	CAC AGC GTT TAC ACC TAG	*Euryarchaea*	20	308	Teira et al. (2004)

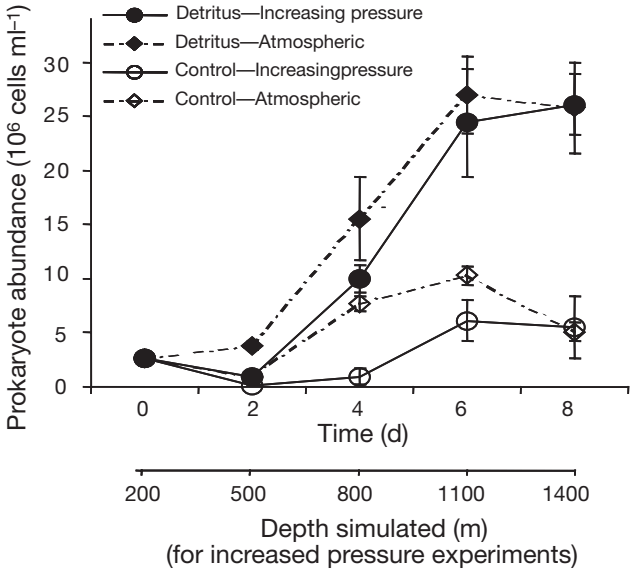

Fig. 1. Prokaryotic abundance over time. Detritus—Increasing pressure: incubation of natural prokaryotic community with fresh diatom detritus under increasing hydrostatic pressure conditions (1.5 MPa d^{-1} in order to simulate pressure experienced during a sinking fall of 150 m d^{-1}); Detritus—Atmospheric: incubation with detritus at atmospheric pressure; Control—Increasing pressure: incubation without detritus under increasing hydrostatic pressure conditions (1.5 MPa d^{-1}); Control—Atmospheric: incubation without detritus at atmospheric pressure. Sampling date: February 2004

RESULTS

Prokaryotic abundance over time

Prokaryotic abundances increased more quickly and reached higher levels in incubations with diatom detritus than in the control incubation without diatom detritus. Increasing pressure appears to cause a lag in bacterial growth compared to the sample incubated at atmospheric pressure, although growth rates and cell yields in both incubations reached similar levels (Fig. 1). For all incubations, after a lag of 2 d, prokaryotic abundance increased greatly until Day 6 and remained constant until Day 8. The abundance of prokaryotes increased from $2.8 \pm 0.4 \times 10^6$ cells ml^{-1} at the beginning of the incubations to $2.6 \pm 0.3 \times 10^7$ cells ml^{-1} at the end of the incubations, with diatom detritus both at atmospheric pressure and under increasing pressure conditions. Without diatom detritus, prokaryotic abundance reached only $5.2 \pm 0.4 \times 10^6$ cells ml^{-1} by the end of the incubations.

Aminopeptidase rates and $Si(OH)_4$ concentration

Total aminopeptidase activity was always lower when the pressure was increased over time than at

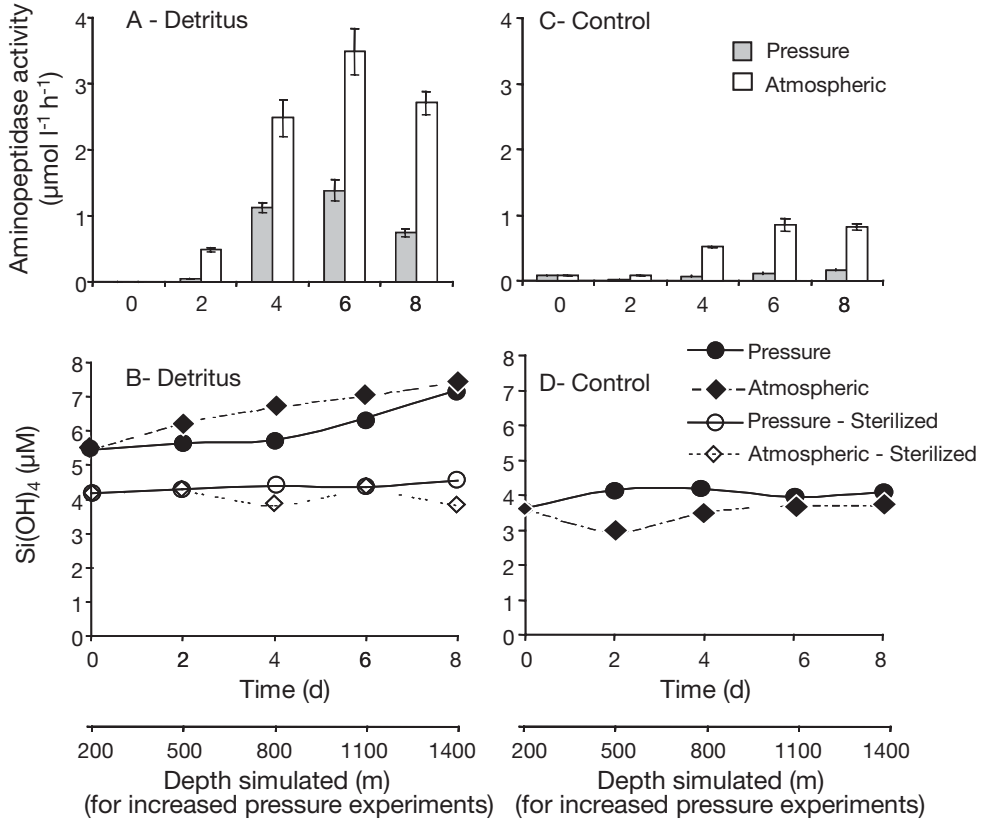

Fig. 2. (A,C) Aminopeptidase rates and (B,D) orthosilicic acid [$Si(OH)_4$] concentrations over time. Natural prokaryotic assemblages were incubated with fresh diatom detritus (A,B: Detritus) and without detritus (C,D: Control). Aminopeptidase activity was measured under increasing hydrostatic pressure conditions to simulate pressure experienced at a sinking rate of 150 m d^{-1} (1.5 MPa d^{-1}) and under atmospheric pressure. $Si(OH)_4$ concentrations were measured under increasing hydrostatic pressure conditions and under atmospheric pressure. Under sterilized conditions, $Si(OH)_4$ concentrations were measured under increasing hydrostatic pressure conditions and under atmospheric pressure. Sampling date: February 2004

atmospheric pressure (Fig. 2A). During the incubation with diatom detritus, aminopeptidase rates increased from 0.15 µmol MCA-Leu hydrolyzed l^{-1} h^{-1} at Time 0 to 1.39 µmol MCA-Leu hydrolyzed l^{-1} h^{-1} at Day 6, when the pressure was 11 MPa (1100 m simulated depth), 2.5-fold lower than the atmospheric control.

The second experiment confirmed that aminopeptidase activity under increasing pressure was lower than at atmospheric pressure (Fig. 3A). The differences in aminopeptidase activity were due to differences in cell-specific activity (amol $cell^{-1}$ h^{-1}) rather than in the abundance of prokaryotes. Aminopeptidase rates were 6.3 to 311.3 amol $cell^{-1}$ h^{-1} as the pressure increased over time, around half the rates at atmospheric pressure (10.1 to 508.7 amol $cell^{-1}$ h^{-1}).

During the first 4 d of incubation, $Si(OH)_4$ concentrations were higher in the atmospheric pressure incubation than in the incubation with increasing pressure, in both experiments (Figs. 2B & 3B). The initial silicic acid regeneration rates were very low (0.07 ± 0.02 µmol l^{-1} h^{-1}) during the first 4 d of incubation under increasing pressure (simulating a fall of particles between 200 and 800 m), whereas rates under atmospheric pressure conditions were easily measured (0.32 ± 0.05 µmol l^{-1} h^{-1}). However, $Si(OH)_4$ concentrations in the increasing pressure incubations approached those of the atmospheric pressure incubations by the end of the experiment. Overall, rates were 0.21 ± 0.05 and 0.24 ± 0.03 µmol l^{-1} h^{-1} under increasing pressure and atmospheric pressure conditions, respectively, at the end of the first experiment (Fig. 2B) and 0.70 ± 0.08 and 0.68 ± 0.29 µmol l^{-1} h^{-1}, respectively, at the end of the second experiment (Fig. 3B).

Two controls were performed: one with sterilized seawater and diatom detritus and the other in the same seawater without fresh diatom detritus. $Si(OH)_4$ concentrations did not change under atmospheric pressure or under increasing pressure over time in these controls.

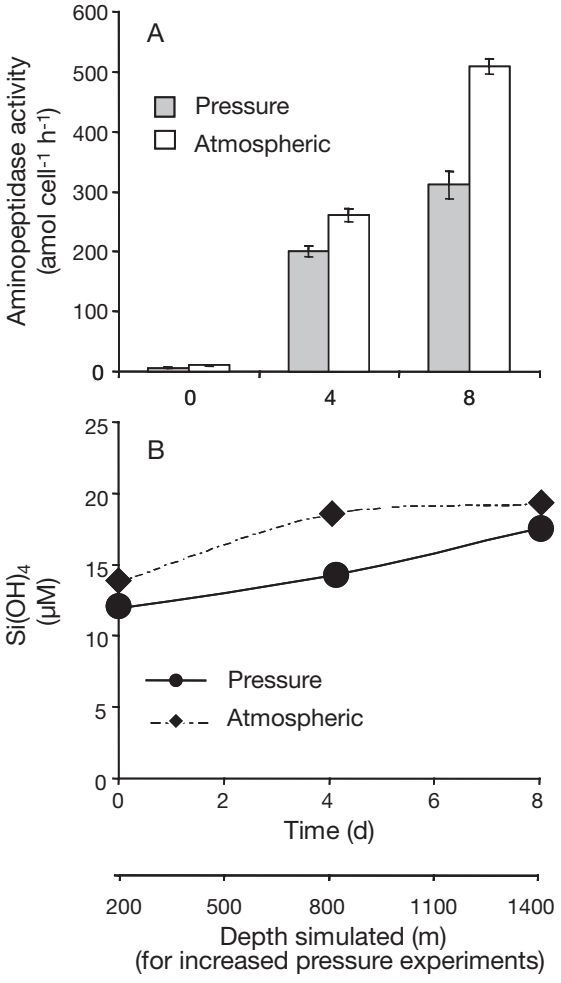

Fig. 3. Cell-specific (A) aminopeptidase rates and (B) $Si(OH)_4$ concentrations over time. Natural prokaryotic assemblages were incubated with detritus under increasing hydrostatic pressure conditions simulating a particle fall of 150 m d^{-1} (1.5 MPa d^{-1}) and under atmospheric pressure. Sampling date: January 2002

Response of prokaryotic community structure to pressure and diatom detritus

At the beginning of the incubation, the prokaryotic community consisted of 17% of the *Cytophaga–Flavobacter* cluster, 23% of α-*Proteobacteria* and 33% of γ-*Proteobacteria*, and 25% of *Crenarchaea* and 13% of *Euryarchaea* (Fig. 4). From Day 2, *Bacteria* were dominant (close to 90%) among prokaryotes, increasing 2-fold from Day 0. In contrast, *Archaea* (*Crenarchaea* and *Euryarchaea*) drastically decreased to negligible levels by the end of the incubation. *Crenarchaea* represented <2% of total prokaryotes and *Euryarchaea* <0.5% of total prokaryotes.

Among the *Bacteria*, the *Cytophaga–Flavobacter* cluster appeared to dominate in incubations with diatom detritus; this cluster was always more numerous and constituted a higher percentage of the total prokaryotes than the γ-*Proteobacteria* group, which was the second most abundant group. Without detritus, the γ-*Proteobacteria* group dominated the first half of the incubation (Days 0 to 4), but drastically decreased after Day 4, when the *Cytophaga–Flavobacter* cluster became dominant until the end of the incubation. Community structure changed under increasing pressure conditions in a fashion similar to how it changed in the atmospheric pressure condition treatment. Whatever the conditions, the α-*Proteobacteria* group appeared negligible from Day 2, constituting <3% of the total prokaryotes.

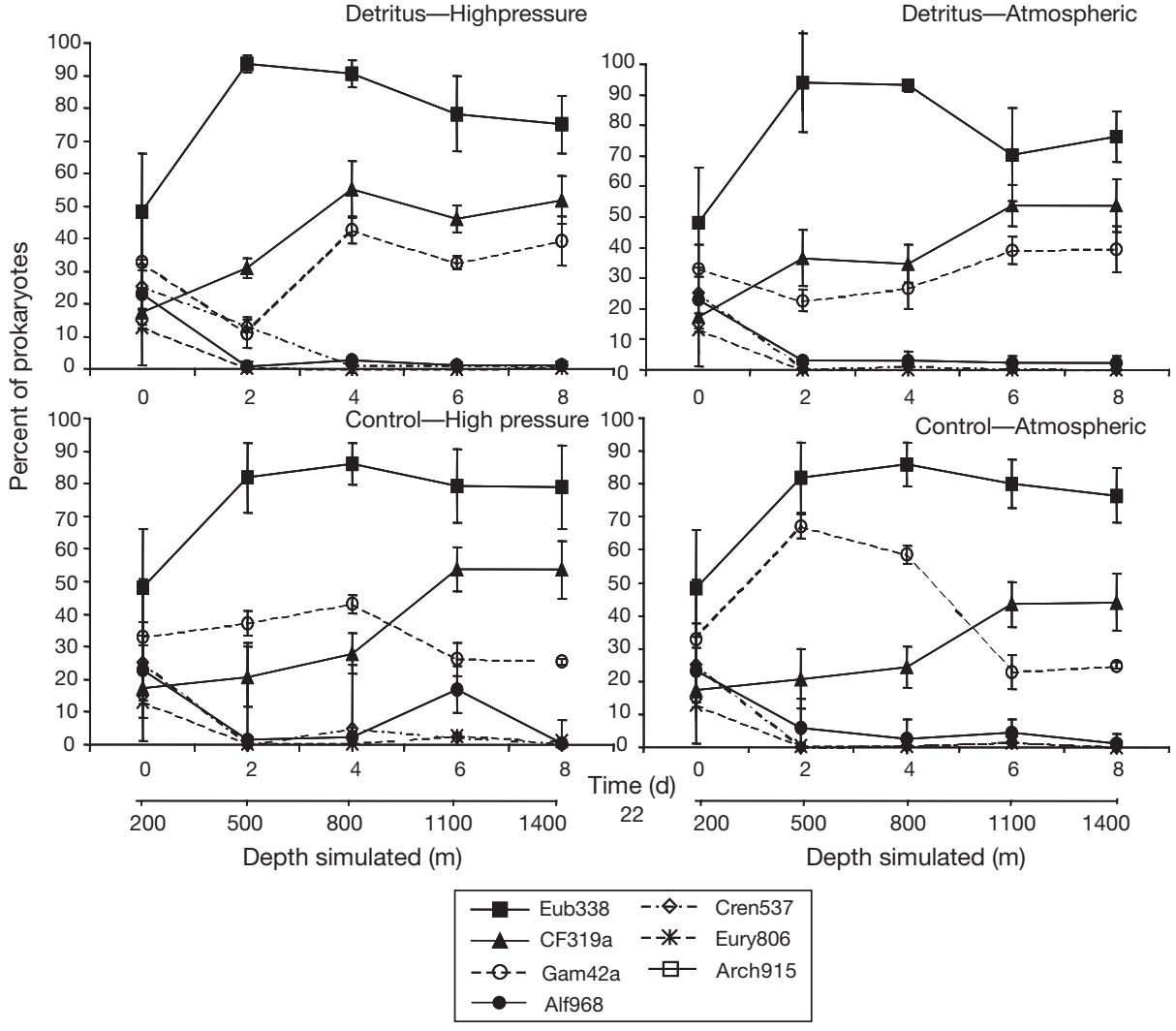

Fig. 4. Percent of total prokaryotes (DAPI-stained cells) detected by fluorescence *in situ* hybridization over time. Detritus—High pressure: incubation with fresh diatom detritus under increasing hydrostatic pressure conditions; Detritus—Atmospheric: incubation with detritus at atmospheric pressure; Control—High pressure: incubation without detritus under increasing hydrostatic pressure conditions; Control—Atmospheric: incubation without detritus at atmospheric pressure; Eub338: *Bacteria*; CF319a: *Cytophaga–Flavobacter* cluster of the *Cytophaga–Flavobacter–Bacteroides* division; Gam42a, Alf968: γ-subclass and α-subclass of *Proteobacteria*; Cren537: *Crenarchaea*; Eury806: *Euryarchaea*; Arch915: *Archaea.* Sampling date: February 2004

DISCUSSION

When diatoms die, they tend to aggregate and sink through the water column (Alldredge et al. 1995). Because it is difficult to follow *in situ* degradation of organic matter and regeneration of biogenic elements considered as mineral ballast (e.g. silica, calcium carbonates, and dust), we designed an experiment to simulate the sinking of diatom detritus. Our experimental design was based on that of Bidle & Azam (1999, 2001) and Bidle et al. (2002, 2003), changing only 1 parameter: the pressure. Temperature was maintained at 13°C, which corresponds to the temperature in the mesopelagic and bathypelagic zones of the Mediterranean. A gradual increase in pressure was applied to surface water samples during the incubation. This pressure increase corresponded to a sinking rate of 150 m d^{-1}. This sinking rate is well within the vast range of rates reported in the literature. For example, Smayda (1970) gave a settling range for the phytoplankton of 1 to 510 m d^{-1}. Shanks & Trent (1980) calculated a sinking rate for marine snow of between 43 and 95 m d^{-1} (with a mean of 68 m d^{-1}), while McCave (1975) estimated a sinking rate of 105 m d^{-1}. Other field and experimental studies report values ranging from 1 to 370 m d^{-1} (see references in Alldredge et al. 1995).

All measurements were done on the entire prokaryotic community, not just on the prokaryotes firmly attached to the diatom detritus, because it was impossible to separate free from attached bacteria in these experiments. In any case, there is a tight coupling between particle fluxes and free-living prokaryotes (Cho & Azam 1988, Nagata et al. 2000). Moreover, sinking particles are surrounded by a plume of nutrients, carbon, and microbes that can create hotspots of growth and carbon cycling by free-living bacteria (Kiørboe & Jackson 2001, Long & Azam 2001). Also, in the experiments of this study, it seems likely that pressure affected both free-living and attached bacteria. Turley (1993) demonstrated that both free-living bacteria and bacteria attached to sinking particles were affected by pressure increases.

The results indicate that increasing hydrostatic pressure significantly affected the aminopeptidase activity of prokaryotic populations during our incubation under increasing pressure conditions and certainly when particles were sinking. In fact, the activity rates were always lower under increasing pressure than under atmospheric pressure. This low aminopeptidase activity was associated with low initial silicic acid regeneration rates, consistent with the results of Bidle & Azam (1999, 2001). These previous studies hypothesized that regeneration of silicic acid from diatom detritus depends on hydrolysis of proteins in the silicic acid–protein matrix of the frustrales. However, eventually silicic acid concentrations under pressure approached those observed in the atmospheric pressure incubation, although aminopeptidase activity did not, suggesting that even low aminopeptidase activity is sufficient to eventually release silicic acid from diatom frustrales. These results suggest some degree of uncoupling between organic material degradation and silicic acid regeneration as particles sink through the water column. Experiments on diatom aggregates at atmospheric pressure have shown that dissolution rates of silica are lower for aggregated cells than for dispersed cells (Passow et al. 2003). Organic substances might chemically protect diatom frustules within aggregates and reduce dissolution by prokaryotic activity. Our data showing that pressure decreases ectoenzymatic activity suggest that dissolution rates are reduced when diatoms embedded within sinking aggregates fall through the water column.

It is well known that major environmental variables, such as temperature (Ward et al. 1998), nutrient status (Broughton & Gross 2000), pollutants (Muller et al. 2001, Castle & Kirchman 2004), and predation (Jürgens & Matz 2002) influence the composition of prokaryotic communities. To our knowledge, this is the first study to examine the effect of pressure on the structure of prokaryotic communities. Results from FISH and denaturing gradient gel electrophoresis (data not shown) indicated that increasing pressure did not affect prokaryotic community structure any differently than constant atmospheric pressure during the 8 d of incubation. However, *Archaea* decreased in all incubations as soon as the experiment started. These results are consistent with those from previous experiments carried out on surface samples, suggesting that *Archaea* do not play a significant role in promoting silicic acid regeneration (Bidle & Azam 2001). Since our data confirm that *Archaea* are not enriched on particles in surface waters (Simon et al. 2002), we suggest that the high densities of free-living *Archaea* found in the deep sea (Delong 1998, Delong et al. 1999, Karner et al. 2001, Church et al. 2003, Teira et al. 2004) are not related to vertical transport by sinking particles.

In parallel treatments with and without increasing pressure, the diatom detritus seemed to have an influence on prokaryotic diversity during the 8 d of incubation. In particular, the enrichment with axenic diatom detritus stimulated an increase in the relative abundance of the *Cytophaga–Flavobacter–Bacteroides* group and of the γ-*Proteobacteria*, the second most dominant group. These results agree with those of several studies carried out on the diversity of prokaryotic communities degrading organic carbon and nitrogen in diatom aggregates (Bidle & Azam 2001, Grossart & Ploug 2001). The *Cytophaga–Flavobacter*, α-*Proteobacteria*, and γ-*Proteobacteria* clusters seem to dominate communities associated with marine snow in oceanic systems (DeLong et al. 1993, Ploug & Grossart 1999, Simon et al. 2002) and in the Mediterranean Sea (Acinas et al. 1999, Moeseneder et al. 2001). Using FISH, Ploug & Grossart (1999) showed that members of the *Cytophaga–Flavobacter* cluster constituted around 30% of the DAPI-stained cells on marine snow in the Southern California Bight. Bidle & Azam (2001) also analyzed the bacterial community on aggregates of diatom detritus incubated with a natural bacterial assemblage in coastal waters off California by denaturing gradient gel electrophoresis and found a dominance of the γ-*Proteobacteria* and *Cytophaga–Flavobacter* clusters. These observations are consistent with other studies, suggesting that, although the *Cytophaga–Flavobacter* and γ-*Proteobacteria* clusters play a major role in the degradation of high molecular weight organic carbon (Cottrell & Kirchman 2000b, Kirchman 2002), α-*Proteobacteria* seems to dominate uptake of low molecular weight material (Cottrell & Kirchman 2000a, Malmstrom et al. 2005).

This study found that organic matrix hydrolysis and silica dissolution are influenced by pressure as diatoms and associated prokaryotes fall through the water column. This initial study focused on a single factor (pressure) and its effect on the remineralization of sinking

particles. The instrumentation used in this study can be modified to examine the other factors, such as temperature, that may affect detritus degradation and silica dissolution. However, this initial study should help us to understand the role of prokaryotes in the mineralization of organic matter and their contribution to regeneration of the mineral ballast (e.g. silica and carbonates). This information is essential for improving models to describe organic matter mineralization by prokaryotes and the transfer of carbon and energy to the dark ocean and for exploring the consequences of these processes in the global carbon cycle. Moreover, the strategy proposed in this study will provide us with useful information about links between the structure and function of the bacterial compartment involved in the transfer of organic carbon from the surface to the deep layers of the water column.

Acknowledgements. This work was funded by the Institut National des Sciences de l'Univers with the PROOF-SINPAS (see www.com.univ-mrs.fr/LMGEM/sinpas) program (awarded to C.T.) and the DOE BIOMP program (awarded to D.L.K.). We thank M. Cottrell for skillful assistance and his development of image analysis for FISH, L. Yu and the rest of the Kirchman laboratory. We also thank B. Quéguiner for his help with the silicic acid analysis. C.T. is especially grateful to Armand Bianchi, to whom this article is dedicated, for his continuous support and his valuable scientific insights. We also thank L. Giuliano and D. Lefèvre for helpful comments and discussion of the manuscript.

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Editorial responsibility: Fereidoun Rassoulzadegan, Villefranche-sur-Mer, France

Submitted: December 12, 2005; Accepted: March 20, 2006
Proofs received from author(s): June 22, 2006