Accepted Article

DR. BEGONA AYO (Orcid ID : 0000-0002-3073-9696)

Article type : Primary Research Articles

TITLE:

IMBALANCED NUTRIENT RECYCLING IN A WARMER OCEAN DRIVEN BY DIFFERENTIAL RESPONSE OF EXTRACELLULAR ENZYMATIC ACTIVITIES

RUNNING TITLE

OCEAN WARMING IMBALANCES NUTRIENT RECYCLING

AUTHORS:

Begoña Ayo[1,2] (*), Naiara Abad[1], Itxaso Artolozaga[1], Iñigo Azua[1], Zuriñe Baña[1], Marian Unanue[1], Josep M. Gasol[3], Carlos M. Duarte[4,5], Juan Iriberri[1,2]

AUTHOR AFFILIATIONS:

1. Department of Immunology, Microbiology and Parasitology, Faculty of Science and Technology, University of the Basque Country UPV/EHU, Leioa-Bizkaia, Spain.

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/gcb.13779

This article is protected by copyright. All rights reserved.

Accepted Article

2. Research Centre for Experimental Marine Biology and Biotechnology PiE-UPV/EHU, Plentzia-Bizkaia, Spain.

3. Institut de Ciències del Mar, CSIC, Barcelona, Catalunya, Spain.

4. Department of Global Change Research, Instituto Mediterráneo de Estudios Avanzados (CSIC-UIB), Esporles, Illes Balears, Spain.

5. King Abdullah University of Science and Technology (KAUST), Red Sea Research Center (RSRC), Thuwal, 23955-6900, Saudi Arabia.

CORRESPONDING AUTHOR:

Dr. Begoña Ayo

Dpto. Inmunología, Microbiología y Parasitología, Facultad de Ciencia y Tecnología, Universidad del País Vasco UPV/EHU

POB 644, E-48080 Bilbao Spain

E-mail address: begona.ayo@ehu.eus

Phone number: 0034946015397

Fax number: 0034946013500

This article is protected by copyright. All rights reserved.

Accepted Article

KEYWORDS: (6-10)

Extracellular enzymatic activity, β-glucosidase, leucine aminopeptidase, alkaline phosphatase, temperature-sensitivity, C:N:P molar ratio, global warming, subtropical and tropical ocean.

TYPE OF PAPER:

Primary Research Article

ABSTRACT

Ocean oligotrophication concurrent with warming weakens the capacity of marine primary producers to support marine food webs and act as a CO_2 sink, and is believed to result from reduced nutrient inputs associated to the stabilization of the thermocline. However, nutrient supply in the oligotrophic ocean is largely dependent on the recycling of organic matter. This involves hydrolytic processes catalyzed by extracellular enzymes released by bacteria, which temperature-dependence has not yet been evaluated. Here we report a global assessment of the temperature-sensitivity, as represented by the activation energies (E_a), of extracellular β-glucosidase (βG), leucine aminopeptidase (LAP) and alkaline phosphatase (AP) enzymatic activities, which enable the uptake by bacteria of substrates rich in carbon, nitrogen and phosphorus, respectively. These E_a were calculated from two different approaches, temperature experimental manipulations and a space-for-time substitution approach, which generated congruent results. The three activities showed contrasting E_a in the

This article is protected by copyright. All rights reserved.

Accepted Article

subtropical and tropical ocean, with βG increasing the fastest with warming, followed by LAP, while AP showed the smallest increase. The estimated activation energies predict that the hydrolysis products under projected warming scenarios will have higher C:N, C:P and N:P molar ratios than those currently generated, and suggest that the warming of oceanic surface waters leads to a decline in the nutrient supply to the microbial heterotrophic community relative to that of carbon, particularly so for phosphorus, slowing down nutrient recycling and contributing to further ocean oligotrophication.

Introduction

The temperature of the upper ocean has increased by about 1 °C since 1900 and is expected to warm a further 1 to 3 °C during the 21st Century (Collins *et al.*, 2013) leading to a number of changes in the functioning of the global ocean. In particular, there is evidence that the subtropical gyres of the ocean are expanding and becoming more oligotrophic (Polovina *et al.*, 2008). Since nutrient inputs into the upper ocean depend both on turbulence, supporting the diffusive fluxes, and on nutrient remineralization by microbes, ocean oligotrophication has been attributed to reduced turbulent nutrient inputs into the upper ocean as a result of strengthening of the thermocline with warming (Sarmiento *et al.*, 2004). Regarding microbial activities, respiration rates are expected to increase with ocean warming (Vázquez-Domínguez *et al.*, 2007; Regaudie-De-Gioux & Duarte, 2012), but the temperature-sensitivity of most microbial processes has not yet been resolved, leading to uncertainties as to how ocean

This article is protected by copyright. All rights reserved.

Accepted Article

biogeochemical cycles, critically dependent on microbial mineralization of organic matter, may respond to rising temperatures.

An important fraction of the organic matter in the upper ocean is composed of high molecular weight (HMW) molecules, which can be metabolized by heterotrophic prokaryotes only after undergoing external digestion by a myriad of extracellular enzymes (Chróst, 1991; Arnosti, 2003). Hence, extracellular enzyme activities are the first step in the microbial degradation of HMW organic matter in the ocean, and therefore influence the remineralization of carbon, nitrogen and phosphorous. Specifically the extracellular enzymatic activities β-glucosidase (βG), leucine aminopeptidase (LAP) and alkaline phosphatase (AP) are responsible for the hydrolysis of a broad range of HMW organic substrates rich in carbon, nitrogen and phosphorus, respectively. Each of these enzymatic activities represent the integrated activities of an heterogeneous group of isoenzymes which are likely diverse in different oceanic conditions regarding origin, composition, molecular structure, regulation, half life, sensitivity to temperature, etc. (Christian & Karl, 1998; Arrieta & Herndl, 2002; Steen *et al.*, 2015).

Although the hydrolysis rates of β-glucosidase (βG), leucine aminopeptidase (LAP) and alkaline phosphatase (AP) extracellular enzymes in surface seawater have been widely studied, determinations of their temperature sensitivity are extremely scarce. Thus, Vetter and Deming (1994) estimated Q_{10} ranging 0.8-5.5 (E_a rough estimates of -0.15-1.15 eV) for the peptidase activities in seawater of the Arctic Northeast water polynya, (Vetter & Deming, 1994). More recently, Piontek *et al.* (Piontek *et al.*, 2014) referred high activation energies for β-glucosidase (range 2.2 - 23.3 eV) and somewhat lower

This article is protected by copyright. All rights reserved.

Accepted Article

values for leucine aminopeptidase (range: 1.5 - 15.2 eV) activities in samples from the Arctic Ocean. However, these values of sensitivity to temperature correspond to the cold-adapted communities and enzymes of Arctic seawater, and they are expected to be higher than those of temperate waters since cold-adapted enzymes tend to be more responsive to increasing temperature than warm-adapted enzymes (German *et al.*, 2012). Additional information about the sensitivity to temperature of βG activity is available from studies of terrestrial systems (E_a ranging 0.27 - 0.72 eV, (Trasar-Cepeda *et al.*, 2007; Wallenstein *et al.*, 2009; German *et al.*,2012; Stone *et al.*, 2012; Steinweg *et al.*, 2013; Koyama *et al.*, 2013; Bárta *et al.*, 2014)) while much fewer data are available for LAP (E_a ranging -0.33 - 0.62 eV, (Wallenstein *et al.*, 2009; Koyama *et al.*, 2013; Bárta *et al.*, 2014)) or AP (E_a ranging 0.39 - 0.48 eV, (Bárta *et al.*, 2014)). Maybe the striking lack of data for marine systems arises from the general consideration of the temperature as a sparse driver of the extracellular enzyme activity when characterizing the carbon and nutrient cycling in the marine environments (Arnosti *et al.*, 2014) while being considered as fundamental driver in terrestrial systems. However, in the present situation of global warming the responses to increasing temperatures in the limiting first step of the degradation of the organic matter, especially if they are heterogeneous, may alter organic matter biogeochemical cycling in the sea surface.

The temperature-dependence of a specific extracellular enzyme activity can be characterized through the activation energy (E_a), the slope of the Arrhenius equation describing the intrinsic temperature-sensitivity of a biochemical reaction (Arrhenius, 1889). In order to calculate this activation energy, several approaches can be used, including experimental temperature manipulations or space-for-time substitution, each of them having both advantages and disadvantages (Elmendorf *et al.*, 2015). The space-

This article is protected by copyright. All rights reserved.

Accepted Article

for-time substitution approach measures the variability of the studied process under the natural temperature gradients existing in the ecosystem over space, and assumes that this variability will be equivalent when temperature changes over time. However, the obtained results may be biased by environmental heterogeneity, co-varying abiotic factors, or adaptations to local climatic conditions (Dunne *et al.*, 2004). This approach has been widely used in biodiversity modeling (Blois *et al.*, 2013), as well as in marine microbial ecology to determine the temperature dependence of plankton metabolism (Regaudie-De-Gioux & Duarte, 2012; García-Corral *et al.*, 2014) or heterotrophic prokaryotic production (Lønborg *et al.*, 2016). Experimental temperature manipulations, on the other hand, allow a controlled approach to determine the response of a process to temperature changes when all the other factors remain unaltered, yet the results obtained through these short-term experiments may not adequately reflect the changes that the microbial communities will develop under temperatures increasing slowly during long periods of time. The level of congruence between the results obtained through the two approaches will indicate the robustness of the predictions about the response of the studied activities to warming.

Here we determined the E_a for various extracellular enzymatic activities along the track of the Malaspina 2010 Circumnavigation Expedition (Duarte, 2015) providing a global survey of the subtropical and tropical Pacific, Atlantic, and Indian oceans. In particular, we assessed variability in the E_a for three extracellular enzymatic activities: β-glucosidase (βG), leucine aminopeptidase (LAP) and alkaline phosphatase (AP), and determined the mean value of sensitivity to temperature of each group of enzymes (isoenzymes) able to perform a specific hydrolytic activity. In order to obtain a more consistent evaluation of the temperature effect on the rates of these biological processes

This article is protected by copyright. All rights reserved.

Accepted Article

(Elmendorf *et al.*, 2015), we estimated the E_a for these extracellular enzymatic activities with two approaches: we performed temperature manipulations to experimentally estimate the E_a for each activity in surface and Deep Chlorophyll Maximum (DCM) layers for 29 stations sampled along the subtropical and tropical Oceans, and we also carried out a space-for-time substitution approach to obtain average E_a for each activity from the relationship between changes in the *in situ* hydrolysis rates across space and seawater temperature.

Materials and Methods

Seawater samples were collected between 32.1° N and 37.2° S in the Pacific (7 stations), Atlantic (16 stations) and Indian (6 stations) Oceans during the Circumnavigation Expedition Malaspina 2010 (www.expeditionMalaspina.es), carried out onboard R/V *Hespérides* from December 2010 until July 2011 (Duarte, 2015) (Fig. S1 and Table S1). All stations were sampled at 07:00-10:00 am, at 3 m ("surface") with 30 L Niskin bottles and, in 16 stations, the Deep Chlorophyll Maximum layer was also sampled ("DCM", depth ranging from 23 to 160 m) using a rosette sampling system (24x12 L Niskin bottles) fitted with a SeabirdSBE9 CTD instrument.

Three enzymatic activities were assayed following a modified fluorogenic substrate method, initially proposed by Hoppe in 1983 (Hoppe, 1983). These activities were selected to represent the hydrolysis of C-, N- and P-rich organic substrates commonly found in natural seawater. β-glucosidases (βG) hydrolyse β-glycosidic linkages in combined carbohydrates, and they were assayed with the analogue 4-methyl-umbelliferyl-β-D-glucopyranoside. Leucine aminopeptidases (LAP) hydrolyse peptidic

This article is protected by copyright. All rights reserved.

Accepted Article

bonds in proteinaceous materials, and this activity was assayed with the analogue L-Leucine-4-methyl-coumarinyl-7-amide, a model substrate for the hydrolysis of a great variety of peptides and proteins. Alkaline phosphatases (AP) hydrolyse phosphate groups from monophosphate esters, and this activity was assayed with the analogue 4-methyl-umbelliferyl phosphate, which has been reported to present hydrolysis rates comparable to natural substrates (Berman, 1988). The fluorogenic substrates were obtained from Biosynth (Switzerland). These analogues are artificial substrates for a broad-specificity β-glucosidases, peptidases and phosphatases (Chróst, 1991; Christian & Karl, 1998; Steen *et al.*, 2015), and therefore the results here do not refer to the hydrolysis rates and activation energies of specific enzymes but of the whole of isoenzymes present in the samples.

Frozen (-20 °C) and concentrated (x1000) stock solutions of the model substrates in 40% methanol were brought to ambient temperature and diluted. Ten µl of these working solutions were added to 240 µl of samples in 96 well microplates, in order to achieve saturating concentrations of 1 mM for L-Leucine-4-methyl-coumarinyl-7-amide and 350 µM for the other two fluorogenic substrates. Three plates were filled in this way, each containing 4 replicates for each activity. One of the plates was incubated at *in situ* temperature, and the other two plates were incubated at temperatures ranging 8-29 °C.

Fluorescence in the plates was measured immediately after substrate addition and several times during the next 48 h, although in most of the cases incubations shorter than 18 h were enough to obtain a significant increase in fluorescence caused by the enzymatic cleavage of the fluorogenic substrates. Fluorescence was measured with a spectrofluorometer (Synergy 2, Bio Tek) equipped with a microplate reader and fitted

This article is protected by copyright. All rights reserved.

Accepted Article

with top fluorescence probes, with excitation and emission wavelengths of 365 and 445 nm respectively, and operated through Gen 5 data analysis software (Bio Tek). The fluorescence increased linearly over the course of the incubation period. Relative fluorescence units were calibrated with 4-methylumbelliferone (MUF) and 4-methylcoumarin (MCA) standards (SIGMA) in the range of 0-96 nM final concentration. Subsamples without substrate were used as blanks to determine the background fluorescence of the samples. Previous experiments performed on heat-killed coastal water showed that abiotic hydrolysis of the substrate was not significant.

The effect of temperature on the enzymatic activity was determined by two approaches, the space-for-time substitution approach and the experimental manipulation approach. For the space-for-time substitution approach, data on potential hydrolysis rates for the βG, LAP and AP enzymatic activities were grouped and averaged within bins covering 1 °C, to ensure the same weight for each bin with independence of the number of samples. The activation energy (E_a, eV) of the hydrolysis process was determined from the negative slope of the Arrhenius plot of natural logarithm of the hydrolysis rate *versus* the reciprocal of K_BT, where K_B is the Boltzmann constant ($8.62x10^{-5}$ eV K^{-1}) and T is the absolute temperature (K).

In the experimental manipulation approach, we used the rates measured at different incubation temperatures. For each sample and enzymatic activity, the activation energy (E_a, eV) of the hydrolysis process was determined from the negative slope of the Arrhenius plot of natural logarithm of the hydrolysis rate *versus* the reciprocal of K_BT, where K_B is the Boltzmann constant ($8.62x10^{-5}$ eV K^{-1}) and T is the absolute temperature (K).

This article is protected by copyright. All rights reserved.

Accepted Article

The predicted hydrolysis rate (R_2) for each activity at any temperature (T_2) was calculated from the temperature coefficient Q_{10} and the hydrolysis rate (R_1) at the current temperature (T_1), by means of the equations:

$$Q_{10} = \left(\frac{R_1}{R_2}\right)^{\frac{10}{T_1 - T_2}}$$

$$Q_{10} = e^{\frac{10E_a}{RT^2}}$$

where Q_{10} is defined as the factor by which a biological rate is increased for a 10 °C rise in temperature, E_a is the activation energy (eV), R is the gas constant (8.314472 mol^{-1} K^{-1}) and T is the mean absolute temperature (K) used in the determination (Raven & Geider, 1988; Vaquer-Sunyer & Duarte, 2013).

The stoichiometry of the products was estimated by assuming that for an activity of one mol per hour, βG activity releases 6 mol carbon (1 mol glucose) per hour and AP activity releases 1 mol phosphorous (inorganic phosphate) per hour (Bárta *et al.*, 2014). The stoichiometry of the products of the LAP activity is more difficult to define, since the peptidases are very diverse, and they are able to hydrolyze not only leucine but also alanine (Bárta *et al.*, 2014), arginine, methionine, tyrosine, and other aminoacids (Steen *et al.*, 2015). While currently the composition of the hydrolysis products is unknown, we assumed an average stoichiometric composition of 4.5 mol carbon and 1 mol nitrogen per hour for the LAP activity. However we are aware of the conservativeness of these conversion values, since many other C and N containing substrates were not considered.

Linear regressions of LN of hydrolysis rates *versus* the reciprocal of K_B T were considered for further analysis in the two approaches only when significance was

This article is protected by copyright. All rights reserved.

Accepted Article

p<0.05. The difference between linear regression parameters for the βG and LAP enzymatic activities in the space-for-time substitution approach was analysed with ANOVA. In the experimental manipulation approach, the differences among the three activities were analysed with the non-parametric Wilcoxon test for paired samples with *post-hoc* Bonferroni correction for three comparisons (p<0.0167). Differences in the E_a between surface and the DCM were determined with the non-parametric Wilcoxon test for paired samples (p<0.05). The relations between E_a of activities were determined by correlation (Spearman´s *rho* coefficient) and type II linear regression analysis (p<0.05). The statistical analyses were performed with IBM SPSS Statistics version 23 for Windows and R version 3.1.1. (R Core Team, 2014).

RESULTS

Space-for-time substitution approach.

The potential hydrolysis rates of the three extracellular enzymatic activities (Table S2) (binned by 1 °C) at different *in situ* water temperatures were used to generate an estimate of the activation energies (E_a) through a space-for-time substitution approach. *In situ* temperatures ranged from 13.5 °C to 28.7 °C throughout the sampling stations. The linear regression plots for the three activities, where the absolute value of the slope estimates the value of E_a (eV), are presented in Fig. 1a. We detected significant temperature dependence for the βG and LAP activities, but not for the AP activity. The E_a (mean ± SE) for βG (0.39 ± 0.12 eV) was higher than that for LAP (0.24 ± 0.08 eV) (ANOVA, p<0.05).

This article is protected by copyright. All rights reserved.

Accepted Article

Experimental temperature manipulation

The measured values of E_a for the three activities are presented in Table S3, while global mean values for each activity are presented in Fig. 1b, where we observe that the pattern of EEA temperature sensitivity derived from space-for-time substitution was consistent with that derived from experimentally determined average E_a values. Global mean (± SE) E_a was highest for βG (0.34 ± 0.03 eV), followed by LAP (0.21 ± 0.01 eV), and lowest for AP (0.17± 0.01 eV) (Wilcoxon´s signed rank test *plus* Bonferroni correction, $p<0.05$).

The β-glucosidase activity showed the widest range of values for E_a, ranging from 0.168 eV (station 60, Indian Ocean) to 0.718 eV (station 51, Indian Ocean) in surface waters and from 0.126 eV (station 113, Pacific Ocean) to 0.603 eV (station 55, Indian Ocean) in waters of the Deep Chlorophyll Maximum (DCM, located at an average depth of 104 ± 9 m). In surface waters, the E_a values for LAP activity ranged from 0.081 eV (station 134, Atlantic Ocean) to 0.499 eV (station 55, Indian Ocean), and in DCM waters, the E_a values for LAP activity ranged from 0.065 eV (station 128, Atlantic Ocean) to 0.359 eV (station 55, Indian Ocean). E_a for alkaline phosphatase presented the smallest range among the three activities tested, from 0.051 eV (station 109, Pacific Ocean) to 0.359 eV (station 71, Indian Ocean) in surface waters and from 0.119 eV (stations 119, Indian Ocean and 11, Atlantic Ocean) to 0.256 eV (station 40, Atlantic Ocean) in DCM waters (Table S3). The mean (± SE) E_a in surface waters was highest for βG (0.39 ± 0.04 eV), followed by LAP (0.22 ± 0.02 eV), and was lowest for AP (0.17 ± 0.02 eV) (Wilcoxon´s signed rank test *plus* Bonferroni correction, $p<0.05$), and the same trend was observed for the E_a in the DCM, but with somewhat lower values (0.29 ± 0.04 eV for βG, 0.19 ± 0.02 eV for LAP and 0.16 ± 0.02 eV for AP) (Fig. S2).

This article is protected by copyright. All rights reserved.

Accepted Article

Since the analysis of the response of sensitivity to temperature towards absolute latitude for each activity indicated no differences in samples from surface or DCM waters, data from the two water layers were pooled for each activity. Significant and similar increases of sensitivity to temperature with the absolute latitude were observed for the three activities (Fig.2).

The E_a of βG, LAP and AP showed good correlations with each other across stations, and similarly in both surface and DCM waters (Fig. 3). The association between LAP and AP was the highest (68.5%), followed by the association between LAP and βG (58.5%) and βG and AP (47.7%). In all cases the Spearman *rho* correlation coefficient was positive, indicating global similar trends in the sensitivity to temperature for these three activities, although with different intensities.

Projections for a future warmer ocean

The type and relative quantity of the products generated under a particular warming rate were roughly estimated by applying the robust E_a coefficients (Table S3) to current potential hydrolysis rates (Table S2) in order to calculate the predicted hydrolysis rates at each sampling station under future warming scenarios in the range of +1 °C to +4 °C. These projections (Fig. 4) predict significant variations in the molar composition of the hydrolysis products both in surface and in DCM waters due to higher seawater temperatures: for each °C of warming, the mean increases of the C:N, C:P and N:P molar ratios were 0.2%, 1.2% and 0.6% in surface waters and 0.1%, 0.8% and 1.0% in DCM waters, respectively.

This article is protected by copyright. All rights reserved.

Accepted Article

DISCUSSION

The estimated values of activation energy (E_a) revealed a positive feedback between temperature and potential hydrolysis rates for the extracellular βG, LAP and AP activities as predicted by the metabolic theory of ecology (Brown *et al.*, 2004). The space-for-time-substitution approach allowed the estimation of E_a for βG and LAP activities, but it failed to deliver a significant estimate of E_a for AP activity, which also showed the lowest experimentally-determined response to temperature. Whereas the consistency of the space-for-time substitution with the experimentally-determined E_a for the extracellular enzymatic activities provides additional confidence on the robustness of our findings, the space-for-time substitution has many sources of variability and, thus, has limited power to detect low E_a values, such as those for AP activity. These empirical and experimental observations indicate that in the subtropical and tropical ocean, seawater temperature is likely a key driver of the activity of the βG and LAP enzymatic activities, but not so for AP activity, which might be more affected by other variables, such as the composition of the microbial communities (Labry *et al.*, 2005; Ivančić *et al.*, 2010; Peacock & Kudela, 2013) or the concentration of substrates (Vidal *et al.*, 2003; Tanaka *et al.*, 2006; Duhamel *et al.*, 2010). In the space-for-time substitution approach, the E_a for βG (0.39 ± 0.12 eV) was higher than the E_a for LAP (0.24 ± 0.08 eV), evidencing the different temperature sensitivity of the hydrolysis of carbon and nitrogen organic polymers across a large spatial scale. This difference occurred despite major differences in community composition among locations in the epipelagic layer of the ocean (Sunagawa *et al.*, 2015), which probably affect the enzyme production as well as the nature of the enzymes generated. This trend of higher temperature sensitivity in βG than in LAP observed in space-for-time substitution was

This article is protected by copyright. All rights reserved.

Accepted Article

consistent with that observed in experimentally determined average E_a values, which supports the robustness of these estimates, and suggests that the results obtained in short-term manipulation experiments are robust enough as to be extrapolated to medium- and long-term projections. In addition, this overall trend underlines a higher effect of global warming on the hydrolysis on polysaccharides than on the hydrolysis of nitrogen- and phosphorous-containing organic compounds. Similar trends of higher temperature-sensitivity of enzymes degrading C-rich compounds compared to those degrading N-rich compounds have been reported for soils (Koch *et al.*, 2007; Wallenstein *et al.*, 2009) and the Arctic marine ecosystem (Piontek *et al.*, 2014).

The experimental temperature manipulations allow a more detailed analysis of the variability of E_a among extracellular enzymatic activities in the subtropical and tropical ocean. Both in surface and DCM waters, the E_a were highest for βG, followed by LAP and lowest for AP. E_a were in general higher for surface waters than DCM for glucosidase and leucine aminopeptidase, which may be a consequence of different microbial communities in these photic domains (Treusch *et al.*, 2009). The activation energies reported here are below those previously reported for the Arctic Ocean (Vetter & Deming, 1994; Piontek *et al.*, 2014), where microbial communities seem to be particularly sensitive to warming (Vaquer-Sunyer *et al.*, 2010). Indeed, the three activities examined showed similar increases in E_a with absolute latitude, a pattern consistent with reports indicating that soil extracellular enzymatic activities from colder locations were more temperature-sensitive than enzymes from warmer sites (Somero, 2004; Koch *et al.*, 2007; Dong & Somero, 2009; German *et al.*, 2012). The lower values of E_a observed for the three activities near the equator, together with the relatively high potential hydrolysis rates detected, may reflect that the microbial communities in these

This article is protected by copyright. All rights reserved.

Accepted Article

locations are acclimated to high temperatures and therefore their activities are less responsive to temperature shifts (Wei *et al.*, 2014), perhaps because their enzymes are near an upper threshold of activity. On the contrary, since warming at high latitudes is expected to be more intense than that at low latitudes, especially in the Northern Hemisphere (Collins *et al.*, 2013), the higher E_a in high latitudes suggest a steep poleward acceleration of marine organic matter hydrolysis rates with warming.

The activation energies of the three activities were positively correlated with each other across stations both in surface and in DCM waters. This indicates that the greater increase in βG hydrolytic activity compared to LAP and AP activities is an inherent characteristic of the enzymes, and consistent across the subtropical and tropical ocean, likely associated to the chemical nature of the three hydrolyzed bonds, the glycosidic, peptide and ester bonds, respectively.

The different temperature-sensitivity of the extracellular enzymatic activities examined here has profound implications for the biogeochemical functioning of a warmer ocean. In particular, the results predict that warming of the upper ocean, where primary producers release fresh organic matter, should generate differential increases in the rate of hydrolysis of the distinct HMW components of the organic matter. The projections about the type and relative quantity of the products generated under future warming scenarios across the range of +1 °C to +4 °C show a significant variation in the molar composition of the products of the hydrolysis: warming is expected to generate products enriched in C and depleted in N and particularly P, as represented by higher C:N and especially C:P and N:P molar ratios. Hence, 21st century warming may yield a significant shift in the C:N:P molar ratio of the products generated during the extracellular digestion of HMW compounds, involving an increase in the C:P and N:P

This article is protected by copyright. All rights reserved.

molar ratio by 1.2% and 0.6 (surface) and 0.8 and 1.0% (DCM) for each °C of warming. This apparent small difference would be amplified across multiple cycles of organic matter production and recycling, as nutrients turnover very rapidly in the subtropical ocean (Dore *et al.*, 2008). In an scenario of reduced turbulent nutrient inputs due to strengthening of the thermocline with ocean warming (Sarmiento *et al.*, 2004), the differential nutrient remineralization by microbes through extracellular enzymatic activities reported here would lead to a reduced supply of inorganic phosphate relative to those of C and N. Provided that inorganic C is predicted to be in ample supply in a CO_2-enriched world (Riebesell, 2004) and that reactive N can be fixed from atmospheric N_2 and is also supplied in increasing amounts from anthropogenic sources (Duce *et al.*, 2008), the resulting outlook is for a P-limited, more oligotrophic future ocean.

The tendency toward a nutrient depleted (particularly in P) warmer ocean is further enhanced by a likely depletion in N and P content of the organic matter released by primary producers in a warmer ocean. Mesocosm experiments have shown that warming induces an increase in the biological release of DOC *versus* that of DON (Engel *et al.*, 2011; Taucher *et al.*, 2012; Biermann *et al.*, 2014). Wohlers-Zöllner *et al* (Wohlers-Zöllner *et al.*, 2011), in turn, observed that warming favored the accumulation of DOC compounds by a mixed plankton community under N-replete conditions. Moreover, there is evidence for an increase in the C:P and N:P molar ratios of cyanobacteria (Levitan *et al.*, 2010) and C:N molar ratios of diatoms (Berges *et al.*, 2002) with warming, which presumably could trigger similar shifts in the composition of their exudation products. Lastly, an expansion of oligotrophic regions due to future warming of the ocean surface (Sarmiento *et al.*, 2004) may lead to an increase in the

Accepted Article

This article is protected by copyright. All rights reserved.

Accepted Article

abundance of small-sized primary producers *versus* larger sized ones (Morán *et al.*, 2010; Martiny *et al.*, 2013). Considering that for coexisting plankton lineages cyanobacteria are characterized by a much higher C:N:P ratio than diatoms (235:35:1 for *Prochlorococcus*, 161:25:1 for *Synechococcus* and 107:16:1 for diatoms) (Martiny *et al.*, 2013), and assuming that the released organic matter broadly matches the composition of the primary producers (Thornton, 2014), the stoichiometric ratios of cyanobacteria provide an approximation of the stoichiometric ratio of the organic material to be generated in a future, warmer ocean. Under these future scenarios, the hydrolytic activity of extracellular enzymes on these C-rich and N- and/or P-depleted organic substrates would result in low molecular weight organic material even more depleted in N and mainly in P, further favoring the shift toward picocyanobacteria.

However, we must also consider the capability of microbial communities to cope with this projected environmental imbalance in C:N:P ratios through different mechanisms (revised in Mooshammer *et al.*, 2014), such as the production of higher amounts of extracellular AP (Vidal *et al.*, 2003), the reduction of the cellular needs for N or P (Bertilsson et al, 2003; Levitan et al 2010) or even the regulation of the efficiencies of elements use (del Giorgio & Cole, 1998).

In summary, the greater temperature-sensitivity of the enzymes processing carbon compared to those breaking down nitrogen and phosphorous-rich organic molecules indicates that warming of the upper ocean is expected to lead to reduced nutrient recycling, particularly that of phosphorus, contributing to the tendency toward oligotrophication of a warmer ocean. We predict that ocean warming will cause an increase in the hydrolysis rates of organic matter along with a shift in the composition of the products generated by the extracellular enzymes toward higher C:P and N:P

This article is protected by copyright. All rights reserved.

Accepted Article

molar ratios. The ensuing increase in nutrient limitation can generate a cascade of effects on key ecosystem processes, as the effect of temperature on microbial respiration (López-Urrutia *et al.*, 2006; Hoppe *et al.*, 2008), growth rates (Degerman *et al.*, 2013), bacterial growth efficiency (Vázquez-Domínguez *et al.*, 2007) and microbial community composition (Degerman *et al.*, 2013) are dependent on the availability of limiting nutrients. An increase in the nutrient export with organic matter, resulting from slower rates of N and P remineralization compared to that of C, is likely to lead to a more oligotrophic ocean, dominated by cyanobacteria and a weaker biological pump in a warmer world.

ACKNOWLEDGEMENTS

This is a contribution to the MALASPINA Expedition 2010 project, funded by the CONSOLIDER-Ingenio 2010 program from the Spanish Ministry of Economy and Competitiveness (Ref. CSD2008-00077). NA was supported by a grant from the Basque Government (Ref. BFI-2010-130). We thank one anonymous reviewer for helpful comments on an earlier version of this manuscript. We thank the participants in the Malaspina Expedition and the crew of BIO Hespérides and the personnel of the Marine Technology Unit of CSIC (UTM) for their invaluable support.

REFERENCES

Arnosti C (2003) Microbial extracellular enzymes and their role in dissolved organic matter cycling. In: *Aquatic ecosystems: Interactivity of dissolved organic matter,*

This article is protected by copyright. All rights reserved.

Accepted Article

(eds Findlay SEG, Sinsabaugh RL), pp. 315–342, Elsevier Science Academic Press, San Diego, USA. doi: 10.1016/B978-012256371-3/50014-7.

Arnosti C, Bell C, Moorhead DL, Sinsabaugh RL, Steen AD, Stromberger M, Wallenstein M, Weintraub MN (2014) Extracellular enzymes in terrestrial, freshwater and marine environments: perspectives on system variability and common research needs. *Biogeochemistry*, **117**, 5–21. doi: 10.1007/s10533-013-9906-5.

Arrhenius S (1889) Über die Reaktionngeschwindigkeit bei des Inversion von Rohrzucker durch Säuren. *Zeitschrift für Physikalische Chemie*, **4**, 226–248.

Arrieta JM, Herndl GJ (2002) Changes in bacterial β-glucosidase diversity during a coastal phytoplankton bloom. *Limnology and Oceanography*, **47**, 594–599. doi: 10.4319/lo.2002.47.2.0594.

Bárta J, Šlajsová P, Tahovská K, Picek T, Šantrůčková H (2014) Different temperature sensitivity and kinetics of soil enzymes indicate seasonal shifts in C, N and P nutrient stoichiometry in acid forest soil. *Biogeochemistry*, **117**, 525–537. doi: 10.1007/s10533-013-9898-1.

Berges JA, Varela DE, Harrison PJ (2002) Effects of temperature on growth rate, cell composition and nitrogen metabolism in the marine diatom *Thalassiosira pseudonana* (Bacillariophyceae). *Marine Ecology Progress Series*, **225**, 139–146. doi: 10.3354/meps225139.

Berman T (1988) Differential uptake of orthophosphate and organic phosphorus substrates by bacteria and algae in Lake Kinneret. *Journal of Plankton Research*,

This article is protected by copyright. All rights reserved.

Accepted Article

10, 1239–1249. doi: https://doi.org/10.1093/plankt/10.6.1239.

Bertilsson S, Berglund O, Karl DM, Chisholm SW (2003) Elemental composition of marine *Prochlorococcus* and *Synechococcus*: Implications for the ecological stoichiometry of the sea. *Limnology and Oceanography*, **48**, 1721-1731. doi: 10.4319/lo.2003.48.5.1721.

Biermann A, Engel A, Riebesell U (2014) Changes in organic matter cycling in a plankton community exposed to warming under different light intensities. *Journal of Plankton Research*, **36**, 658–671. doi: 10.1093/plankt/fbu005.

Blois JL, Williams JW, Fitzpatrick MC, Jackson ST, Ferrier S (2013) Space can substitute for time in predicting climate-change effects on biodiversity. *Proceedings of the National Academy of Sciences of the United States of America*, **110**, 9374–9379. doi: 10.1073/pnas.1220228110.

Brown JH, Gillooly JF, Allen AP, Savage VM, West GB (2004) Toward a metabolic theory of ecology. *Ecology*, **85**, 1771–1789. doi: 10.1890/03-9000.

Christian JR, Karl DM (1998) Ectoaminopeptidase specificity and regulation in Antarctic marine pelagic microbial communities. *Aquatic Microbial Ecology*, **15**, 303–310. doi: 10.3354/ame015303.

Chróst R (1991) Environmental control of the synthesis and activity of aquatic microbial ectoenzymes. In: *Microbial Enzymes in Aquatic Environments* (ed Chróst R), pp. 29–59, Springer-Verlag, Berlin, Germany. doi: 10.1007/978-1-4612-3090-8_3.

Collins M, Knutti R, Arblaster J et al. (2013) Long-term climate change: Projection,

This article is protected by copyright. All rights reserved.

Accepted Article

commitments and irreversibility. In: *Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change* (eds Stocker TF, Qin D, Plattner GK et al.), pp. 1029–1136, Cambridge University Press, Cambridge. doi: 10.1017/CBO9781107415324.024.

Degerman R, Dinasquet J, Riemann L, de Luna SS, Andersson A (2013) Effect of resource availability on bacterial community responses to increased temperature. *Aquatic Microbial Ecology*, **68**, 131–142. doi: https://doi.org/10.3354/ame01609.

del Giorgio PA, Cole J (1998) Bacterial growth efficiency in natural aquatic systems. *Annual Review of Ecological Systems*, **29**, 503–41.

Dong Y, Somero GN (2009) Temperature adaptation of cytosolic malate dehydrogenases of limpets (genus *Lottia*): differences in stability and function due to minor changes in sequence correlate with biogeographic and vertical distributions. *Journal of Experimental Biology*, **212**, 169–177. doi: 10.1242/jeb.024505.

Dore JE, Letelier RM, Church MJ, Lukas R, Karl DM (2008) Summer phytoplankton blooms in the oligotrophic North Pacific Subtropical Gyre: Historical perspective and recent observations. *Progress in Oceanography*, **76**, 2–38. doi: 10.1016/j.pocean.2007.10.02.

Duarte CM (2015) Seafaring in the 21St Century: The Malaspina 2010 Circumnavigation Expedition. *Limnology and Oceanography Bulletin*, **24**, 11–14. doi: 10.1002/lob.10008.

This article is protected by copyright. All rights reserved.

Accepted Article

Duce RA, LaRoche J, Altieri K et al. (2008) Impacts of atmospheric anthropogenic nitrogen on the open ocean. *Science*, **320**, 893–7. doi: 10.1126/science.1150369.

Duhamel S, Dyhrman ST, Karl DM (2010) Alkaline phosphatase activity and regulation in the North Pacific Subtropical Gyre. *Limnology and Oceanography*, **55**, 1414–1425. doi: 10.4319/lo.2010.55.3.1414.

Dunne JA, Saleska SR, Fischer ML, Harte J (2004) Integrating experimental and gradient methods in ecological climate change research. *Ecology*, **85**, 904–916. doi: 10.1890/03-8003.

Elmendorf SC, Henry GHR, Robert D et al. (2015) Experiment, monitoring, and gradient methods used to infer climate change effects on plant communities yield consistent patterns. *Proceedings of the National Academy of Sciences of the United States of America*, **112**, 448–452. doi: 10.1073/pnas.1410088112.

Engel A, Händel N, Wohlers J, Lunau M, Grossart HP, Sommer U, Riebesell U (2011) Effects of sea surface warming on the production and composition of dissolved organic matter during phytoplankton blooms:results from a mesocosm study. *Journal of Plankton Research*, **33**, 357–372. doi: https://doi.org/10.1093/plankt/fbq122.

García-Corral LS, Barber E, Regaudie-De-Gioux A et al. (2014) Temperature dependence of planktonic metabolism in the subtropical North Atlantic Ocean. *Biogeosciences*, **11**, 4529–4540. doi: 10.5194/bg-11-4529-2014.

German DP, Marcelo KRB, Stone MM, Allison SD (2012) The Michaelis-Menten kinetics of soil extracellular enzymes in response to temperature: A cross-

This article is protected by copyright. All rights reserved.

Accepted Article

latitudinal study. *Global Change Biology*, **18**,1468-1479. doi: 10.1111/j.1365-2486.2011.02615.x.

Hoppe HG (1983) Significance of exoenzymatic activities in the ecology of brackish water: measurements by means of methylumbelliferyl-substrates. *Marine Ecology Progress Series*, **11**, 299–308. doi: 10.3354/meps011299.

Hoppe HG, Breithaupt P, Walther K, Koppe R, Bleck S, Sommer U, Jürgens K (2008) Climate warming in winter affects the coupling between phytoplankton and bacteria during the spring bloom: a mesocosm study. *Aquatic Microbial Ecology*, **51**, 105–115. doi: 10.3354/ame01198.

Ivančić I, Fuks D, Radić T, Lyons DM, Šilović T, Kraus R, Precali R (2010) Phytoplankton and bacterial alkaline phosphatase activity in the northern Adriatic Sea. *Marine Environmental Research*, **69**, 85–94. doi: 10.1016/j.marenvres.2009.08.004.

Koch O, Tscherko D, Kandeler E (2007) Temperature sensitivity of microbial respiration, nitrogen mineralization, and potential soil enzyme activities in organic alpine soils. *Global Biogeochemical Cycles*, **21**, GB4017. doi: 10.1029/2007GB002983.

Koyama A, Wallenstein MD, Simpson RT, Moore JC (2013) Carbon-degrading enzyme activities stimulated by increased nutrient availability in arctic tundra soils. *PLoS One*, **8**, e77212. doi: 10.1371/journal.pone.0077212.

Labry C, Delmas D, Herbland A (2005) Phytoplankton and bacterial alkaline phosphatase activities in relation to phosphate and DOP availability within the

This article is protected by copyright. All rights reserved.

Accepted Article

Gironde plume waters (Bay of Biscay). *Journal of Experimental Marine Biology and Ecology*, **318**, 213–225. doi: 10.1016/j.jembe.2004.12.017.

Levitan O, Brown CM, Sudhaus S, Campbell D, LaRoche J, Berman-Frank I (2010) Regulation of nitrogen metabolism in the marine diazotroph *Trichodesmium* IMS101 under varying temperatures and atmospheric CO_2 concentrations. *Environmental Microbiology*, **12**, 1899–1912. doi: 10.1111/j.1462-2920.2010.02195.x.

Lønborg C, Cuevas LA, Reinthaler T et al. (2016) Depth dependent relationships between temperature and ocean heterotrophic prokaryotic production. *Frontiers in Marine Science*, **3**, 90. doi: 10.3389/fmars.2016.00090.

López-Urrutia A, San Martin E, Harris RP, Irigoien X (2006) Scaling the metabolic balance of the oceans. *Proceedings of the National Academy of Sciences of the United States of America*, **103**, 8739–44. doi: 10.1073/pnas.0601137103.

Martiny AC, Pham CTA, Primeau FW, Vrugt JA, Moore JK, Levin SA, Lomas MW (2013) Strong latitudinal patterns in the elemental ratios of marine plankton and organic matter. *Nature Geoscience*, **6**, 279–283. doi: 10.1038/ngeo1757.

Morán XAG, López-Urrutia A, Calvo-Díaz A, Li WKW (2010) Increasing importance of small phytoplankton in a warmer ocean. *Global Change Biology*, **16**, 1137–1144. doi: 10.1111/j.1365-2486.2009.01960.x.

Mooshammer M, Wanek W, Zechmeister-Boltenstern S, Richter A (2014) Stoichiometric imbalances between terrestrial descomposer communitites and their resources: mechanisms and implications of microbial adaptations to their

This article is protected by copyright. All rights reserved.

Accepted Article

resources. *Frontiers in Microbiology*, **5**, 22. doi: 10.3389/fmicb.2014.00022.

Peacock M, Kudela R (2013) Alkaline phosphatase activity detected in distinct phytoplankton communities in the northern Gulf of Alaska. *Marine Ecology Progress Series*, **473**, 79–90. doi: 10.3354/meps10069.

Piontek J, Sperling M, Nöthig EM, Engel A (2014) Regulation of bacterioplankton activity in Fram Strait (Arctic Ocean) during early summer: The role of organic matter supply and temperature. *Journal of Marine Systems*, **132**, 83–94. doi: 10.1093/plankt/fbr069.

Polovina JJ, Howell EA, Abecassis M (2008) Ocean's least productive waters are expanding. *Geophysical Research Letters*, **35**, L03618. doi: 10.1029/2007GL031745.

R Core Team (2014) R Core Team. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/.

Raven JA, Geider RJ (1988) Temperature and algal growth. *New Phytologist*, **110**, 441–461. doi: 10.1111/j.1469-8137.1988.tb00282.x.

Regaudie-De-Gioux A, Duarte CM (2012) Temperature dependence of planktonic metabolism in the ocean. *Global Biogeochemical Cycles*, **26**, GB 1015. doi: 10.1029/2010GB003907.

Riebesell U (2004) Effects of CO_2 enrichment on marine phytoplankton. *Journal of Oceanography*, **60**, 719–729. doi: 10.1007/s10872-004-5764-z.

This article is protected by copyright. All rights reserved.

Accepted Article

Sarmiento JL, Slater R, Barber R et al. (2004) Response of ocean ecosystems to climate warming. *Global Biogeochemical Cycles*, **18**, GB3003. doi: 10.1029/2003GB002134.

Somero GN (2004) Adaptation of enzymes to temperature: Searching for basic "strategies." *Comparative Biochemistry and Physiology - B Biochemistry and Molecular Biology*, **139**, 321–333. doi: 10.1016/j.cbpc.2004.05.003.

Steen AD, Vazin JP, Hagen SM, Mulligan KH, Wilhelm SW (2015) Substrate specificity of aquatic extracellular peptidases assessed by competitive inhibition assays using synthetic substrates. *Aquatic Microbial Ecology*, **75,** 271–281. doi: 10.3354/ame01755.

Steinweg JM, Jagadanna S, Frerichs J, Mayes MA (2013) Activation energy of extracellular enzymes in soils from different biomes. *PLoS One*, **8**, e59943. doi: 10.1371/journal.pone.0059943.

Stone MM, Weiss MS, Goodale CL, Adams MB, Fernandez IJ, German DP, Allison SD (2012) Temperature sensitivity of soil enzyme kinetics under N-fertilization in two temperate forests. *Global Change Biology*, **18**, 1173–1184. doi: 10.1111/j.1365-2486.2011.02545.x.

Sunagawa S, Coelho LP, Chaffron S et al. (2015) Ocean plankton. Structure and function of the global ocean microbiome. *Science*, **348**, 1261359. doi: 10.1126/science.1261359.

Tanaka T, Henriksen P, Lignell R, Olli K, Seppälä J, Tamminen T, Thingstad TF (2006) Specific affinity for phosphate uptake and specific alkaline phosphatase activity as

This article is protected by copyright. All rights reserved.

Accepted Article

diagnostic tools for detecting phosphorus-limited phytoplankton and bacteria. *Estuaries and Coasts*, **29**, 1226–1241. doi: 10.1007/BF02781823.

Taucher J, Schulz KG, Dittmar T, Sommer U, Oschlies A, Riebesell U (2012) Enhanced carbon overconsumption in response to increasing temperatures during a mesocosm experiment. *Biogeosciences*, **9**, 3531–3545. doi: 10.5194/bg-9-3531-2012.

Thornton DCO (2014) Dissolved organic matter (DOM) release by phytoplankton in the contemporary and future ocean. *European Journal of Phycology*, **49**, 20–46. doi: 10.1080/09670262.2013.875596.

Trasar-Cepeda C, Gil-Sotres F, Leirós MC (2007) Thermodynamic parameters of enzymes in grassland soils from Galicia, NW Spain. *Soil Biology & Biochemistry*, **39**, 311-319. doi: 10.1016/j.soilbio.2006.08.002.

Treusch A, Vergin KL, Finlay LA, Donatz MG, Burton RM, Carlsson CA, Giovannoni SJ (2009) Seasonality and vertical structure of Microbial communities in an ocean gyre. *The ISME Journal*, **3**, 1148-1163. doi: 10.1038/ismej.2009.60.

Vaquer-Sunyer R, Duarte CM (2013) Experimental evaluation of the response of coastal mediterranean planktonic and benthic metabolism to warming. *Estuaries and Coasts*, **36**, 697–707. doi: 10.1007/s12237-013-9595-2.

Vaquer-Sunyer R, Duarte CM, Santiago R, Wassmann P, Reigstad M (2010) Experimental evaluation of planktonic respiration response to warming in the European Arctic Sector. *Polar Biology*, **33**, 1661–1671. doi: 10.1007/s00300-010-0788-x.

This article is protected by copyright. All rights reserved.

Accepted Article

Vázquez-Domínguez E, Vaqué D, Gasol JM (2007) Ocean warming enhances respiration and carbon demand of coastal microbial plankton. *Global Change Biology*, **13**, 1327–1334. doi: 10.1111/j.1365-2486.2007.01377.x.

Vetter YA, Deming JW (1994) Extracellular enzyme activity in the Arctic Northeast Water polynya. *Marine Ecology Progress Series*, **114**, 23–34. doi: 10.3354/meps114023.

Vidal M, Duarte CM, Agustí S, Gasol JM, Vaqué D (2003) Alkaline phosphatase activities in the central Atlantic Ocean indicate large areas with phosphorous deficiency. *Marine Ecology Progre ss Series*, **262**, 43-53. doi: 10.3354/meps262043.

Wallenstein MD, Mcmahon SK, Schimel JP (2009) Seasonal variation in enzyme activities and temperature sensitivities in Arctic tundra soils. *Global Change Biology*, **15**, 1631–1639. doi: 10.1111/j.1365-2486.2008.01819.x.

Wei H, Guenet B, Vicca S, Nunan N, AbdElgawad H, Poteau V, Shen W, Janssens I (2014) Thermal acclimation of organic matter decomposition in an artificial forest soil is related to shifts in microbial community structure. *Soil Biology and Biochemistry*, **71**, 1–12. doi: 10.1016/j.soilbio.2014.01.003.

Wohlers-Zöllner J, Breithaupt P, Walther K, Jürgens K, Riebesell U (2011) Temperature and nutrient stoichiometry interactively modulate organic matter cycling in a pelagic algal-bacterial community. *Limnology and Oceanography*, **56**, 599–610. doi: 10.4319/lo.2011.56.2.0599.

This article is protected by copyright. All rights reserved.

Accepted Article

FIGURE CAPTIONS

Figure 1. Global estimations of the activation energies (E_a) of the three extracellular enzymatic activities. a) Arrhenius plots of the relationship between binned (1 °C) potential hydrolysis rates (mean±SE) of the β-glucosidase (βG, squares), leucine aminopeptidase (LAP, circles) and alkaline phosphatase (AP, triangles) enzymatic activities and the reciprocal of K_BT, where T is the *in situ* water temperature (space-for-time substitution approach). b) Comparison of the activation energies obtained by the space-for-time substitution approach (slope±SE) and the average activation energies (mean±SE) obtained by experimental temperature manipulation (Table S3) for the three enzymatic activities.

Figure 2. Relationship between the absolute latitude and the activation energy (E_a) of the β-glucosidase (squares), leucine aminopeptidase (circles) and alkaline phosphatase (triangles) activities. Shown for each extracellular enzymatic activity are the type I regression lines and equations ($p<0.05$).

Figure 3. Spearman´s *rho* correlations between the activation energies (E_a) of the three extracellular enzymatic activities. Only paired data were considered for this analysis. Closed circles and black lines indicate data from surface waters; open circles and grey lines indicate data from DCM waters. Type II regression analysis ($p<0.05$) for E_a of pairs of activities for each water layer were: a) surface: E_a (βG) = 1.84 E_a (LAP) -0.06; DCM: E_a (βG) = 1.81 E_a (LAP) -0.05; b) surface: E_a (βG) = 3.66 E_a (AP) -0.22; DCM: E_a (βG) = 4.01 E_a (AP) -0.4; c) surface: E_a (LAP) = 1.47 E_a (AP) -0.03; DCM: E_a (LAP) = 1.81 E_a (AP) -0.09.

Figure 4. Box plot projections of the expected changes, with regard to the current situation (0 level at Y axis), driven by different levels of warming (0-4 °C) on the C:N (a-b), C:P (c-d) and N:P (e-f) molar ratios of the hydrolysis products generated by the extracellular enzymatic activities, both at the surface (left) and in the DCM (right) layers. Note the different scale for the C:N ratio.

SUPPORTING INFORMATION CAPTIONS

This article is protected by copyright. All rights reserved.

Accepted Article

Figure S1. Location of the 29 stations sampled for this study. Surface water (3 m) was sampled in all stations; the Deep Chlorophyll Maxima depth (DCM, 23-160 m) was also sampled in 16 stations (black circles).

Figure S2. Activation energies (E_a) of β-glucosidase (βG), leucine aminopeptidase (LAP) and alkaline phosphatase (AP) in surface (closed figures) and DCM (open figures). Only samples with paired data were used to perform this comparison. Significant differences (Wilcoxon signed rank test, $p<0.05$) between the two layers were detected only for β-glucosidase and leucine aminopeptidase activities.

Table S1. Geographic coordinates of the 29 sampling stations used for this study.

Table S2. Potential hydrolysis rates (nM h^{-1}) of the β-glucosidase (βG), leucine aminopeptidase (LAP) and alkaline phosphatase (AP) activities measured at *in situ* temperatures.

Table S3. E_a (eV) of the β-glucosidase (βG), leucine aminopeptidase (LAP) and alkaline phosphatase (AP) activities.

Author Contributions

JI, CMD, JMG, BA, MU, IAz and IAr designed the study. BA, IAz and ZB collected the samples and performed *in situ* research. BA, NA and ZB carried out the data analysis. BA and CMD wrote the paper. All authors provided intellectual input and made substantial contributions to the manuscript.

Conflict of Interest

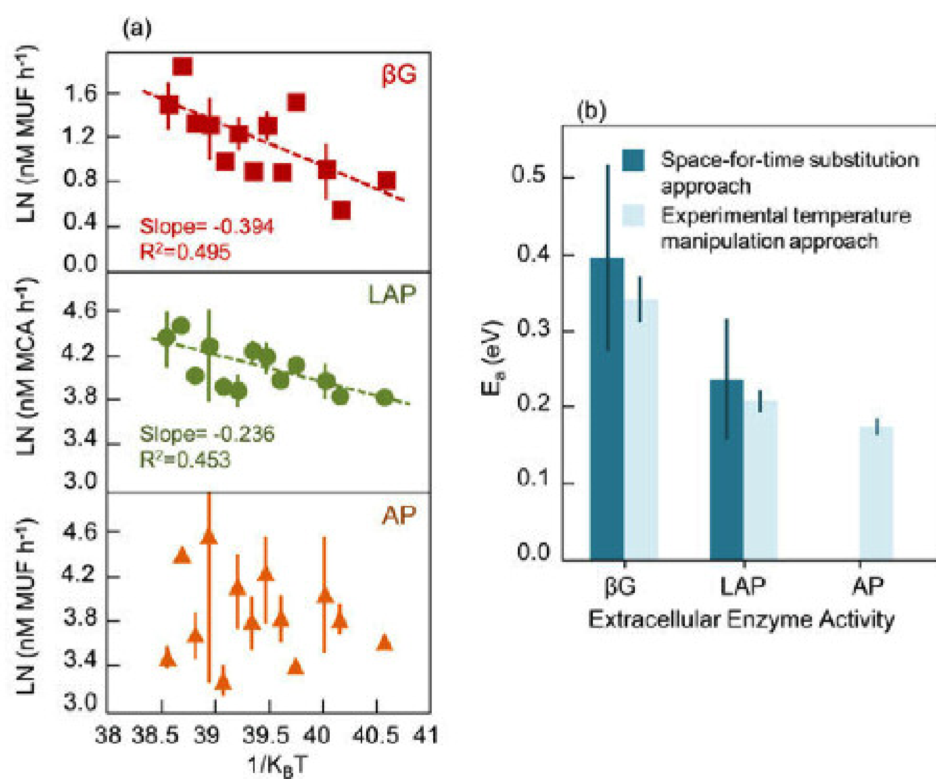
The authors declare no conflict of interest.

This article is protected by copyright. All rights reserved.

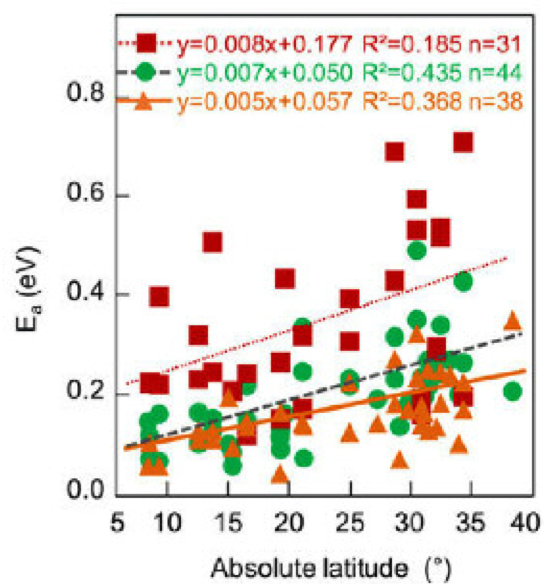
Accepted Article

Data accessibility

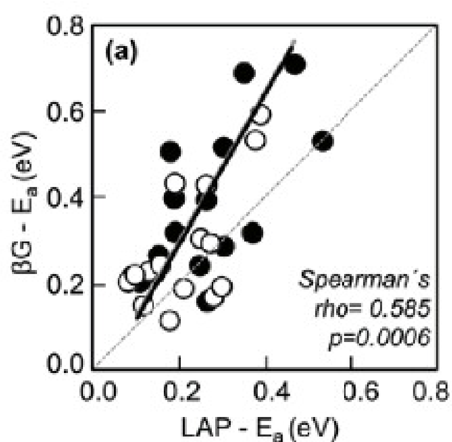
The database is available in DOI: 10.5281/zenodo.583989.

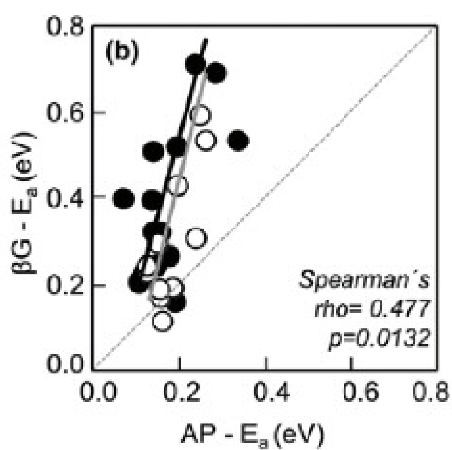


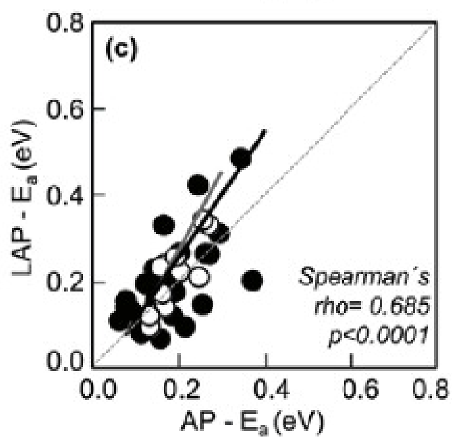
Accepted Article



Accepted Article







Accepted Article