Resilience of SAR11 bacteria to rapid acidification in the high latitude open ocean

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Abstract

Ubiquitous SAR11 *Alphaproteobacteria* numerically dominate marine planktonic communities. Because they are excruciatingly difficult to cultivate, there is comparatively little known about their physiology and metabolic responses to long- and short- term environmental changes. As surface oceans take up anthropogenic, atmospheric $CO_{2,}$ the consequential process of ocean acidification could affect the global biogeochemical significance of SAR11. Shipping accidents or inadvertent release of chemicals from industrial plants can have strong short-term local effects on oceanic SAR11. This study investigated the effect of 2.5 fold acidification of seawater on the metabolism of SAR11 and other heterotrophic bacterioplankton along a natural temperature gradient crossing the North Atlantic Ocean, Norwegian and Greenland Seas. Uptake rates of the amino acid leucine by SAR11 cells as well as other bacterioplankton remained similar to controls despite an instant ~50% increase in leucine bioavailability upon acidification. This high physiological resilience to acidification even without acclimation, suggests that open ocean dominant bacterioplankton are able to cope even with sudden and therefore more likely with long-term acidification effects.

Key words

CARD-FISH / flow cytometric cell sorting / isotopic tracer labelling / pCO2 perturbation

Introduction

Heterotrophic bacteria, among them the ubiquitous alphaproteobacterial SAR11 clade (hereafter SAR11, (Morris *et al.*, 2002)), form an abundant and important component of pelagic marine microbial communities, dominate the remineralisation of phytoplankton-derived organic matter (Martin *et al.*, 1987, Boyd *et al.*, 1999) and constitute a nutrient-rich food source for the smallest eukaryotes (Sherr & Sherr, 2002, Hartmann *et al.*, 2013). SAR11 are present in surface waters throughout the world's oceans (Brown *et al.*, 2012) but experience unique environmental conditions in the polar oceans, including a highly seasonal radiation regime, low water temperature and perennial or continuous sea ice-cover.

Human activities were shown to have a large, cumulative impact on the majority of the global ocean (~66%), ocean acidification and sea surface temperature being the major factors followed by ocean-based pollution, shipping and destructive fishing (Halpern *et al.*, 2015). Bacteria of polar surface waters may be subject to more rapid and intense ocean acidification due to the higher solubility of carbon dioxide (CO_2) at low water temperatures leading to reduced pH values and a decreased buffering capacity (Sabine *et al.*, 2004, Fabry *et al.*, 2009). Apart from the slow process of ocean acidification, shipping activities can locally decrease pH due to NO_x and SO_x emissions (Hassellöv *et al.*, 2013) or by accidental release of acidifying agents into the water (Mamaca *et al.*, 2009). Acidification may have indirect effects on the speciation of trace metals, e.g. increasing the availability of iron and copper because of their higher solubility at lower pH (Morel *et al.*, 2003). However, culture studies have shown that despite the higher solubility the bioavailability of iron could decrease as a result of acidification (Shi *et al.*, 2010). The effect of lowered pH on inorganic nutrient speciation is less well known. Using theoretical speciation diagrams Zeebe and Wolf-Gladrow (2001) showed significant differences for

phosphate, silicate, iron and ammonia speciation if pH drops from 8.1 to 7.8. Bulk dissolved organic matter concentrations have been shown to be uninfluenced by acidification in short-term (MacGilchrist *et al.*, 2014) and long-term experiments (Zark *et al.*, 2015). However, dissolved organic matter is highly complex and differences due to acidification could be masked in bulk analyses resulting in chances of metabolic responses and community compositions.

Abundance of the SAR11 clade has been shown to co-vary negatively with particulate organic carbon but positively with turnover rates of dissolved free amino acids (Giebel *et al.*, 2011). Transcriptional responses of SAR11 from the North Pacific subtropical gyre indicate that these organisms acquire nitrogen from dissolved organic matter in addition to its usage as an energy source (Sharma *et al.*, 2014). Besides organic matter, temperature and salinity have been proposed to drive distribution and abundance of different SAR11 groups (Brown *et al.*, 2012, Herlemann *et al.*, 2014).

Studies of acidification are currently mainly focused on ocean acidification where small or large volumes of samples are incubated for comparatively short (days) or long (up to one year) under increased pCO_2 usually mimicking predicted end of the century, i.e. 1000ppm, levels of pCO_2 (Riebesell, 2004, Engel *et al.*, 2005, Motegi *et al.*, 2013, Gattuso *et al.*, 2014, Richier *et al.*, 2014, Zark *et al.*, 2015). Alas, microbial communities can react rapidly (within hours) to elevated CO_2 levels. For example, the nitrogen fixation rates of *Trichodesmium* can increase by up to 41% within 4-6 hours after exposure to high CO_2 conditions (Hutchins *et al.*, 2009).

It is often assumed that the effect of (rapid) acidification on marine bacterioplankton will be marginal due to the natural variability of surface ocean pH (Joint *et al.*, 2011)

and the potential of bacterioplankton to grow at a wide range of pH, e.g. an isolates of the Roseobacter group exhibit optimal growth rates between pH 7.2 And 7.8, but stopped growing at pH 8.2 (Giebel *et al.*, 2011). Indeed, in estuarine or upwelling regions pH can change significantly in a short period of time (up to 1 unit) (Hofmann *et al.*, 2011). However, measurements of sites in the Pacific Ocean and Antarctica were considerably more stable, e.g. showing only 0.024-0.096 units variability (Hofmann *et al.*, 2011). Therefore, they could be more sensitive to abrupt pH changes.

The aim of the present study was to investigate the effect of acidification on protein synthesis of natural bacterioplankton from ocean waters along a latitudinal gradient from the North Atlantic to the Arctic. Importantly the study examined, for the first time, the immediate (≤2 h) and therefore direct, group-specific response of SAR11, to pH perturbation, thereby avoiding the indirect effects resulting from pH-induced changes to other components of the natural community. In addition, comparison of bacterioplankton response along the natural temperature gradient offered insights into the relationship between acidification and temperature; both of which are predicted to increase in future years. Seawater samples were acidified using a combined approach of acid and sodium bicarbonate addition in order to maintain alkalinity levels (Riebesell *et al.*, 2010). Bacterial activity was assessed by measuring the uptake and turnover of tritiated leucine, the assimilation of which is routinely used to study bacterial biomass production (Kirchman *et al.*, 1985). Leucine assimilation is a ubiquitous and obligate metabolic pathway which is most likely to react immediately to artificial acidification. This approach therefore offered a highly sensitive method to detect positive or negative effects of acidification on SAR11 and other heterotrophic bacterioplankton.

Materials and Methods

Seawater collection and acidification

The study was conducted between the 6th and 17th June 2012 as part of the UK Ocean Acidification research programme's Arctic research cruise on the RRS *James Clark Ross* (cruise JR271). Eleven open sea stations were sampled along a latitudinal transect crossing the North Atlantic Ocean, Norwegian and Greenland Seas, including ice-covered waters of the Fram Strait (Table S1; Fig. 1). At each station a 10 L seawater sample was collected from 10-20 m depth using a Niskin bottle mounted on a stainless steel frame with a Sea-Bird 9/11 plus conductivity, temperature and density (CTD) profiler. The sample was decanted into an acid cleaned polycarbonate carboy and subsequently divided into an acidified and a control treatment.

Acidification (i.e. the manipulation of seawater to increase the ambient pCO_2 to a target level of 1000 ppm, resulting in a pH of 7.79±0.07) was achieved by the addition of ultra-clean hydrochloric acid and sodium bicarbonate; an approach which increases CO_2 and decreases pH whilst maintaining a balanced carbonate system (Riebesell *et al.*, 2010). 50ml samples were removed from control and acidified bottles at the beginning and end of incubations to measure the resultant dissolved inorganic carbon (DIC) and total alkalinity (TA), and to verify this remained stable throughout the incubation. DIC was analysed with an Apollo SciTech DIC analyzer (AS-C3) using a LI-COR (7000) CO_2 infrared detector. TA was determined using an open-cell titration (Dickson *et al.*, 2007) with the Apollo SciTech's AS-ALK2 Alkalinity Titrator. Analyses of Certified Reference Materials (CRMs) (A.G. Dickson, Scripps,

batch 117) were used at the beginning, middle and end of each analytical run to ensure accuracy of the measurements. Precision was taken as the standard deviation of repeated analysis of CRMs, which in this case was ±1.5 μmol kg^{-1} for TA and ±3 μmol kg^{-1} for DIC. The remaining variables of the carbonate system, including pCO_2, were calculated using CO2SYS (MATLAB version 1.1) (Lewis & Wallace, 1998, van Heuven *et al.*, 2011) using the constants of Mehrbach et al (1973) refitted by Dickson and Millero (1987).

Additional triplicate samples (1.6 mL) were taken at the beginning of incubations from the control and acidified seawater and fixed with 20% paraformaldehyde (PFA, 1% final concentration) for determination of bacterial cell abundance by flow cytometry (Fig. 2a and Fig. S1), as outlined below.

Microbial uptake of leucine by radioisotope dilution bioassay

The effect of acidification on leucine uptake, an indicator of bacterial protein synthesis, was determined using the radioisotope dilution bioassay approach developed for freshwater systems (Wright & Hobbie, 1966) and subsequently employed extensively in the marine environment (Hill *et al.*, 2011). [4,5-^{3}H] L-Leucine (140 Ci·$mmol^{-1}$, Hartmann Analytic, Germany) was added to the control and acidified seawater samples at a range of concentrations (0.1, 0.2, 0.4, 0.6, 0.8, 1.0 nM) and incubated in parallel at *in situ* temperature and under low-intensity, indirect artificial light. Three samples (1.6 mL) were incubated for each concentration and fixed sequentially after 10, 20 and 30 minutes by the addition of 20% (w/v) PFA (1% final concentration) for 1 hour at room temperature (RT) in the dark.

Each fixed bioassay sample was filtered through a 0.2 μm pore-size polycarbonate filter (Nuclepore, Whatman, UK) to collect cells, washed twice with 3 mL ultrapure

161 water (Purelab, Elga Process Water, Marlow, UK) and the filters placed into plastic
162 vials to which 3 mL Goldstar scintillation cocktail (Meridian, UK) were added. The
163 radioactivity retained by particulate material was then measured on ship by liquid
164 scintillation counting (Tri-Carb 3100, Perkin Elmer, UK) to assess microbial leucine
165 uptake. ^{3}H-leucine uptake rates were determined for each concentration of ^{3}H-
166 leucine addition from the regression of uptake versus incubation time (Fig. S2a). The
167 ambient concentration of the leucine pool and its turnover time were determined from
168 the x-axis and y-axis intercepts of the regression of leucine uptake rate versus
169 concentration, respectively (Fig. S2b).

170 **Leucine uptake rates of different bacterial clusters by flow cytometry**

171 Additional control and acidified seawater samples were incubated with 0.4 nM ^{3}H-
172 leucine (final concentration) for 2 hours at ambient temperature and fixed with PFA
173 (1% final concentration) for subsequent flow cytometric sorting of different bacterial
174 clusters for stations 6 to 15 (Table S1). Samples were stained with SYBR Green I
175 nucleic acid stain (Sigma-Aldrich, UK) in the presence of tri-potassium citrate
176 (Sigma-Aldrich, UK) at RT (Marie *et al.*, 1997). Stained samples were analysed, and
177 bacterial cells enumerated and sorted by flow cytometry (FACSort, Becton
178 Dickinson, UK) according to the light-scattering properties (90° or side light scatter)
179 and relative concentration of SYBR Green I stain per particle (green fluorescence;
180 FL1, 530 ± 30 nm). The total bacterioplankton population could be divided into two
181 distinct clusters differentiated by their nucleic acid content: low nucleic acid and high
182 nucleic acid containing bacteria (hereafter LNA and HNA respectively, Fig. S1). The
183 HNA and LNA cells were enumerated by the addition of an internal standard
184 comprising a mixture of 0.5 µm and 1.0 µm multifluorescent latex beads
185 (Polysciences, USA) of known concentration (Zubkov & Burkill, 2006).

Radioisotopically labelled HNA and LNA cells were sorted at low flow rate (~12 µl min^{-1}) for 1, 2, 3 and 4 minutes, filtered directly onto 0.2 µm pore-size polycarbonate filters (Nuclepore, Whatman, UK), washed three times with 3 mL ultrapure water and transferred into plastic vials to which 8 mL of Goldstar scintillation cocktail were added. Radioactivity within the sorted cells was assayed using an ultra-low level liquid scintillation counter (1220 Quantulus, Wallac).

Identification of dominant bacterial phyla and clades by CARD-FISH

CAtalysed Reporter Deposition Fluorescence *In Situ* Hybridisation (CARD-FISH) on flow cytometrically sorted LNA and HNA cells was performed to determine the taxonomic composition of the bacterial populations at all stations. Before sorting for CARD-FISH, the flow cytometer was extensively cleaned with Decon (5%, Fisher, UK), commercially available thin bleach and ultra-pure water (Millipore, UK). Subsequently, a new sheath fluid filter with a pore-size of 0.1 µm was inserted, and the flow cytometer run for 1 hour at high flow rate (~200 µl·sec^{-1}). In order to validate cleanliness of the flow cytometer a 60 ml water sample was taken off the sorting line, filtered onto a 0.2 µm pore-sized polycarbonate filter (Nuclepore, Whatman, UK), dried and counterstained with Vectashield® DAPI (4',6-diamidino-2-phenylindole, Vector Laboratories Ltd., UK) for 10 min at RT. The filtered volume of 60 ml equalled approximately the volume filtered during cell sorts. At least two transects of the filter were inspected to detect potential contaminants using an epifluorescence microscope (Axioscope, Zeiss, Germany) equipped with a LED light source pE-300 (CoolLED, UK). If more than 20 cells were counted per transect, the cleaning process was repeated. The cleanliness of the instrument was checked repeatedly during the cell sorting for CARD-FISH.

Flow cytometric cell sorting was carried out as described above and at least 50 000-100 000 cells were collected onto each filter. At four stations samples had to be pre-concentrated in order to achieve these cell numbers. Therefore, 60 ml of PFA-fixed (1% v/v) sample was concentrated at a flow rate of 2.5 ml min^{-1} onto a 0.1 µm pore-size Teflon filter (M-Tech Diagnostics, UK) using a syringe pump (KD Scientific, USA).

Overall hybridisation efficiency was determined using the Eub338I-III probe mix (Amann *et al.*, 1990) targeting Eubacteria. To identify LNA, which have estimated low ribosomal content due to their small cell size (Kemp *et al.*, 1993, Morris *et al.*, 2002), a mix of six different probes and one unlabelled helper probe targeting SAR11 *Alphaproteobacteria* (Morris *et al.*, 2002, Gomez-Pereira *et al.*, 2013) were used in order to enhance hybridisation signal. Additionally, a probe targeting the SAR86 *Gammaproteobacteria* core cluster (Zubkov *et al.*, 2001) was applied. The majority of HNA were identified by probes targeting the *Bacteroidetes* (Manz *et al.*, 1996), *Alphaproteobacteria* (Neef, 1997), and *Gammaproteobacteria* (Manz *et al.*, 1992) phyla. Information on each probe used, including target bacterial groups, nucleic acid sequence and percentage of formamide used for hybridisation, can be found in the supplementary material (Table S2).

CARD-FISH was performed as described in Gomez-Pereira et al. (2013). Briefly, cells were permeabilised with lysozyme (10 $mg \cdot ml^{-1}$, Sigma Aldrich, UK) and achromopeptidase (60 $U \cdot ml^{-1}$, Sigma Aldrich, UK) at 37°C for 1 h and 30 min, respectively. Filters were hybridised overnight (SAR11 probe mix) or for 3 h (remaining probes) at 46°C at varying formamide concentrations (Table S2). Hybridisation buffer and probes (50 $ng \cdot ml^{-1}$) were mixed at a 300:1 ratio. Positive hybridisation of the probe was detected using fluorescently labelled tyramide

Alexa488 (Life Technologies, Carlsbad, CA, USA) at a ratio of 1:1000 in amplification buffer. Finally cells were counterstained with DAPI (4',6-diamidino-2-phenylindole), mounted in Vectashield® antifading reagent and enumerated microscopically.

Data analysis

All statistical analyses were performed using SigmaPlot. For normally distributed data Student's t-tests were used to compare treatments, while for non-normally distributed data Wilcoxon signed rank tests were applied. P values below 0.05 were considered significant. Errors were calculated according to standard error propagation procedures.

Results and Discussion

Bacterial abundance and identification of dominant populations

Bacterial abundances ranged from $2 \cdot 10^5$ to $2 \cdot 10^6$ cells ml^{-1}. Although LNA represented occasionally a significant proportion of the bacterial population (up to 45%, station 8), HNA were dominant at all stations (on average 72±10%, Fig. 2a). CARD-FISH analyses on flow cytometrically sorted LNA using a mix of seven different SAR11 probes to enhance the signal (Gomez-Pereira *et al.*, 2013) revealed that the LNA were almost exclusively composed of SAR11 *Alphaproteobacteria* (90±3%, n=10, Fig. 2b). Therefore, we will refer to LNA as SAR11 from this point onward. If single probes are used on LNA, the hybridisation efficiency can be very low, e.g. only 18±8% of the sorted population positively hybridised with the general eubacterial probe (Eub338I-III, Fig. 2b) and the resulting signal was very weak. Although slightly higher, perhaps attributable to the use of a probe mix, the here measured total abundance of SAR11 (25±9%) is comparable to a study carried out

in the Western Arctic Ocean in May-June, where FISH using a single probe revealed that SAR11 comprised up to 15±11% of the bacterioplankton community (Malmstrom *et al.*, 2007). In addition to the SAR11 probe mix, a SAR86 targeted probe was applied to the sorted LNA cells (SAR86_1245, Fig. 2b) in order to identify the minute remaining part of the population. However, SAR86 target cells were detected in only 4 out of 9 samples and then just a minute fraction exhibited positive hybridisation signals (0.2-1% of the cells). Similar to SAR11, SAR86 *Gammaproteobacteria* have a small genome size and could hence be expected within the LNA (Dupont *et al.*, 2012). Potentially these values underestimate the presence of SAR86 in the samples due to the above-mentioned limitations of using a single probe (Fig. 2c).

The hybridisation signal for the HNA was very strong and 84-97% of cells were positively hybridised with probe EUB338 I-III (Fig. 2c). In contrast to LNA, HNA represented a very heterogeneous mix of bacteria. The selected probes in this study allowed identification of the majority of the cells (61±18%). *Bacteroidetes* were present at all studied stations where, with the exception of one of the southernmost stations (Station 6, 10%), they represented a major fraction of the total sorted HNA cells (on average 42±12%) (Fig. 2c). At one of the northernmost stations (Station 15) *Bacteroidetes* could not be determined due to a lack of sample material. *Alpha*- and *Gammaproteobacteria* abundances were more variable and significantly lower (4-26% and 1-17% respectively) (Fig. 2c).

The homogeneity and methodological accessibility of SAR11 as revealed by our above analyses (Fig. 2b) makes it an ideal candidate to directly and quantitatively study the effect of acidification on a particular group of heterotrophic bacteria instead of bulk bacterial matter, where potential signals could be masked by the presence of other bacterial populations.

Effect of acidification on bioavailable concentrations, microbial uptake rates and turnover time of leucine

Flow cytometric analyses of relevant microbial populations (bacterioplankton, LNA, HNA and small eukaryotes) exhibited no significant fluctuations in cell abundance in relation to acidification (Fig. S3). Turnover times of mRNA in bacteria suggest that they are able to react in a matter of minutes to environmental change (Selinger *et al.*, 2003, Steglich *et al.*, 2010). Hence, the experimental set-up of ≤ 2 hours allowed sufficient time to determine a direct effect of acidification.

Acidification did not affect the bulk community leucine uptake rates in offshore waters of the North Atlantic Ocean or Norwegian and Greenland Seas (t-test, p=0.73; Fig. 3a). Effects of acidification on different parts of the bacterial community could be potentially masked in the bulk community analyses. However, average cellular leucine uptake rates of SAR11 and HNA separated by flow cytometrical cell sorting across eight stations (Mann-Whitney, p=0.97 and 0.66 respectively, Fig. 4) were not affected by acidification either. These two bacterial groups are the major consumers of dissolved leucine in these waters, as shown by comparing the sum of the uptake rates of SAR11 and HNA versus the total uptake measured (Fig. S4). Presumably due to their larger size, HNA usually consumed significantly more leucine $cell^{-1}$ h^{-1} than SAR11 (Mann-Whitney, p=<0.001, Fig. S5) and accounted for 83 (±16%) of the total microbial leucine uptake, therefore dominating bacterial production in these waters whilst, owing to their strong latitudinal variation in abundance (Fig. 2a), SAR11 contributed 3-45% of total bacterial production.

In contrast to leucine uptake, acidification did significantly increase leucine bioavailability (Wilcoxon signed rank, p=0.005; Fig. 3b) which, in turn, increased the

turnover time (Wilcoxon signed rank, p=<0.001; Fig. 3c). Because cell numbers in the acidified and control sample were comparable (Fig. S3), the increased leucine bioavailability could not be attributed to organic matter release by cell death due to the acidification of the sample. It is more likely that pH-induced dissolution of colloidal material resulted in higher concentrations of bio-available amino acids in the water (Hansell & Carlson, 2014). On average, concentration of leucine in acidified samples was 1.8 times higher with up to three times higher concentrations at two stations. Increase organic matter is often considered to have a negative impact on members of the SAR11. However, the stable leucine uptake rates suggest that acidification and the measured release of amino acids does not have a detrimental effect on this group. Despite the comparatively more stable pH in the surface open ocean (Hofmann *et al.*, 2011), the results are in accordance with the ability of bacterioplankton in estuaries, upwelling zones and other aquatic systems, such freshwater lakes, to cope with very large (order of magnitude) and rapid (within hours) pH changes. The ubiquitous distribution of SAR11 (Brown et al 2012), already suggested that they are adapted over a wide pH range and our study confirmed that they can persist under rapidly changing conditions as well.

The above results suggest that SAR11 and other heterotrophic bacterioplankton encountered in this study are tolerant to pH changes, thereby supporting the conclusions of Joint *et al*. (2011) who deduced theoretically that microorganisms will adapt to ocean acidification. However, acidification is just one parameter in the currently changing ocean, so cumulative effects of for example increases in sea surface temperature and UV radiation will have to be taken into consideration. Moreover, other organisms might be not able to adapt to pH change, therefore community structure and thus biogeochemistry will perhaps change dramatically to

333 which bacteria cannot adapt that easily. Indeed, some bacterial groups, e.g.
334 Gammaproteobacteria and Flavobacteriaceae, seem to be more susceptible to pH
335 changes as seen by subtle shifts in bacterial community composition in long-term
336 microcosm studies (Krause *et al.*, 2012).

337 **Bacterioplankton activity along the latitudinal transect and temperature**
338 **gradient**

339 A positive linear correlation was observed between total microbial community leucine
340 uptake rate and temperature (R^2=0.53, p=0.001, Fig. 5a) with a single high outlier at
341 3.45° C corresponding to the northernmost station in relatively warm Atlantic–
342 influenced water (Station 11, Fig. 1). In contrast, the bioavailable leucine
343 concentration was independent of temperature (R^2=0.006, p=0.73, Fig. 5b).

344 Average cellular leucine uptake rates of SAR11 followed the same trend of
345 increasing uptake rate with increasing temperature (R^2=0.24, p=0.05, Fig. 5c) as
346 observed for the total microbial community. The cellular uptake trend observed for
347 SAR11 was enhanced at the population level (R^2=0.86, p<0.001, Fig. 5d) reflecting
348 their increased abundance along the temperature gradient towards elevated
349 temperatures (Fig. 2a). The positive correlation of both abundance and leucine
350 uptake rates (Fig. 5) of SAR11 bacteria with temperature suggests that, owing to
351 their tolerance to acidification, SAR11 could thrive in polar regions following the
352 predicted rise in sea surface temperature (Rhein *et al.*, 2013). In addition, SAR11
353 could profit from the immediate increase in bio-available leucine following
354 acidification (Fig. 3) since it has been shown that SAR11-specific transporter gene
355 expression increases significantly after the addition of dissolved organic matter
356 (Poretsky *et al.*, 2010). Indeed, different temperature-adapted phylotypes can co-

occur within the SAR11 population indicating potential ecological niche differentiation between SAR11 subgroups (Brown *et al.*, 2012).

In contrast to LNA, the cellular and population uptake rates of the heterogeneous HNA were not significantly correlated with temperature (R^2=0.12, p=0.16, Fig. 5e, and R^2=0.11, p=0.18, Fig. 5f, respectively). Compositional similarity of the HNA population, according to our FISH analyses (Fig. 2c), across stations, could potentially indicate that temperature has limited effect on the leucine uptake rates of the HNA. No relationships were observed between cellular uptake rates and the abundance of each of the three identified HNA groups (*Bacteroidetes*, *Alphaproteobacteria* and *Gammaproteobacteria*) (Fig. 2c); however 38±18% of the HNA remained unidentified.

Considerable latitudinal variation was observed in the HNA:SAR11 ratio of average cellular leucine uptake rates (Fig S6). At lower latitudes (60-75°N) the difference in cellular uptake rate was much less pronounced with similar values recorded for both SAR11 and HNA at several stations. Interestingly, significantly higher cellular uptake rates were observed for HNA in the Fram Strait region compared to lower latitudes (Mann-Whitney, p=0.007, 77-79°N; Fig 4), despite a weak negative correlation between latitude and total microbial uptake rates (R^2=0.3, p=0.009) This could perhaps be due to a shift in community composition owing to the fact that three of these four highest latitude stations were associated with ice cover.

Conclusions

Acidification of seawater mimicking end of the century pCO_2 levels or accidental release of acidic compounds in shipping resulted in an instantaneous increase (1.8 fold on average) of bioavailability of the amino acid leucine. In contrast, it had no

effect on amino acid uptake rates of SAR11 and other heterotrophic bacteria along a natural temperature gradient suggesting a high physiological tolerance of bacterioplankton to acidification in the North Atlantic Ocean, Norwegian and Greenland Seas. A positive correlation between temperature and cell abundance as well as amino acid uptake rates of SAR11 bacteria indicated that SAR11 might benefit from anticipated future climate-induced increases in sea surface temperature by increasing their population abundance and productivity.

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405

406 The authors declare that there are no conflicts of interest.

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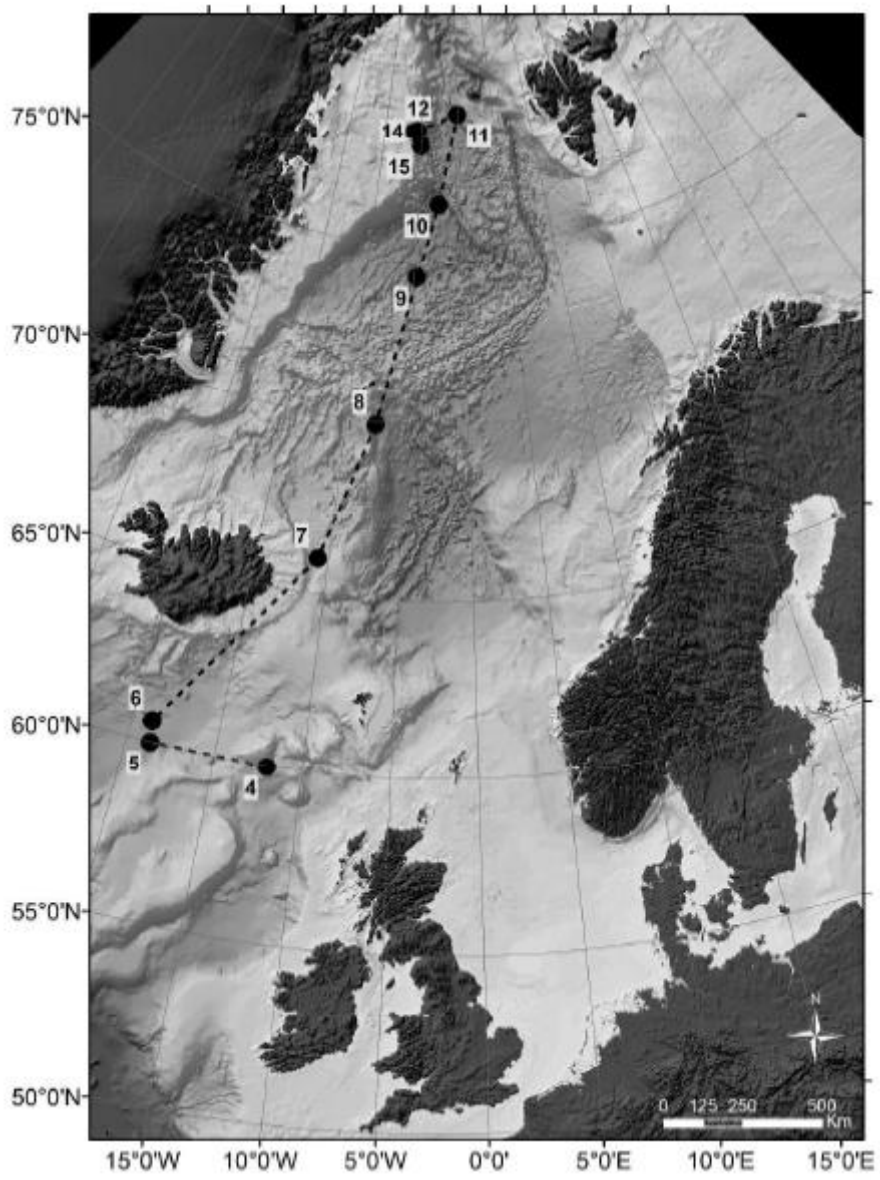

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Fig. 1. Location of stations sampled during this study in the North Atlantic Ocean, Norwegian and Greenland Seas.

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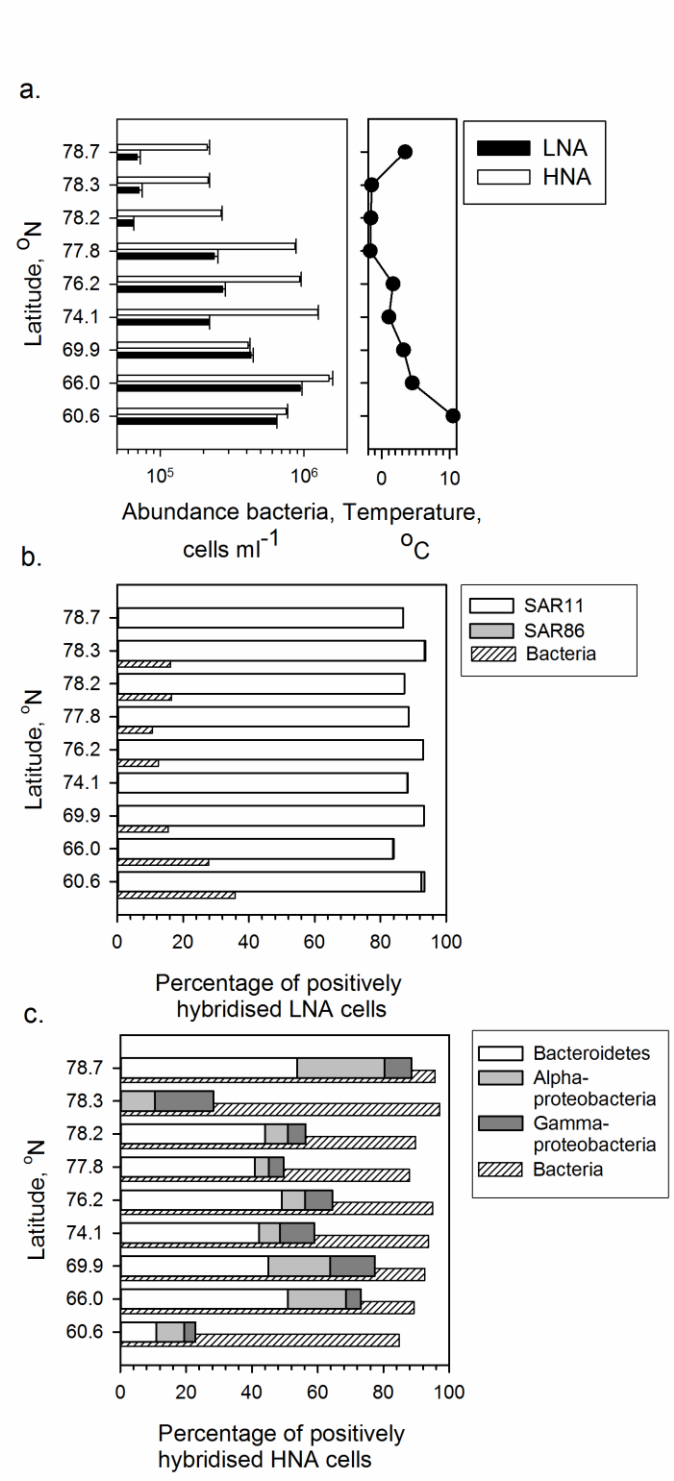

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Fig. 2. Abundance (a) and community composition (b, c) of low nucleic acid and high nucleic acid bacteria (LNA and HNA, respectively) in offshore waters along a latitudinal transect crossing the North Atlantic Ocean, Norwegian and Greenland Seas. To simplify temperature and latitudinal correlation, the temperature measured along the latitudinal transect is shown in (a) next to the abundance. CARD-FISH, using probes targeting the main bacterial phyla, was performed on flow cytometrically sorted LNA and HNA cells (b, c) in order to establish community composition of the bacterial populations. Abbreviations in Fig2b: SAR11 *Alphaproteobacteria* (SAR11), SAR86 *Gammaproteobacteria* (SAR86). The error bars in (a) represent standard difference of mean (n=2). Note that due to technical problems data were unavailable for the Bacteria (stations 9 and 11, b) and the Bacteroidetes (station 12, c) as assessed by CARD-FISH.

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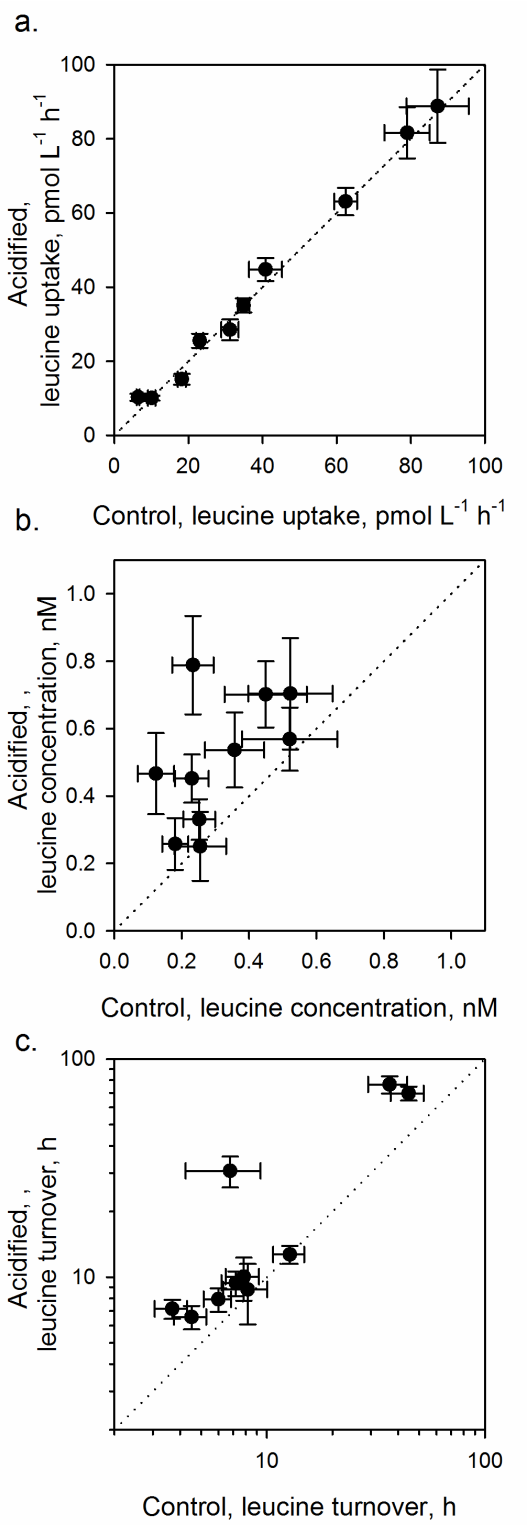

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Fig. 3. Microbial uptake rate (a), bioavailable concentration (b) and turnover time (c) of leucine in control versus acidified experimental treatments from offshore waters along a latitudinal transect crossing the North Atlantic Ocean, Norwegian and Greenland Seas. The dotted line represents a 1:1 relationship with the distribution of points on or near this line indicating no impact of acidification relative to the control. Error bars indicate propagated error of regression slope, x-axis intercept and y-axis intercept in (a), (b) and (c) respectively.

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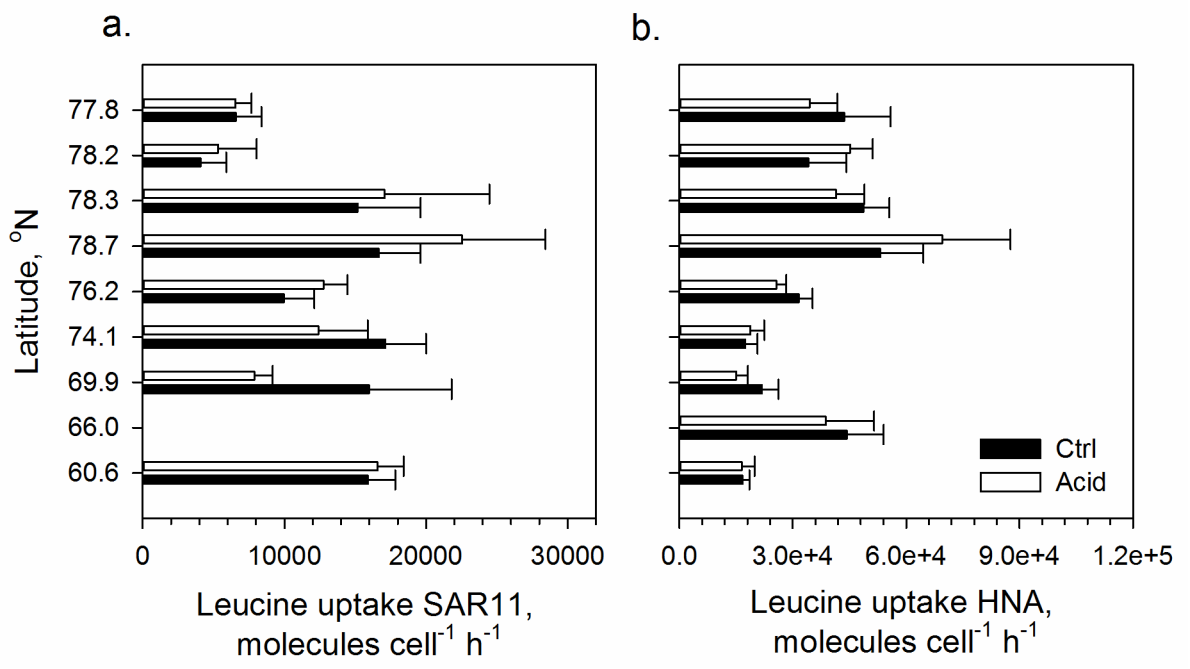

Fig. 4. Average (n=4) cellular leucine uptake rates of SAR11 Alphaproteobacteria (SAR11, a) and high nucleic acid (HNA, b) bacteria in control (Ctrl) versus acidified (Acid) experimental treatments from offshore waters along a latitudinal transect crossing the North Atlantic Ocean, Norwegian and Greenland Seas. Error bars indicate propagated error of bioassay regression slope and standard deviation of different flow cytometric sorts (n=4). Arrows indicate stations under ice cover. Note that data for leucine uptake by SAR11 were unavailable from station 7.

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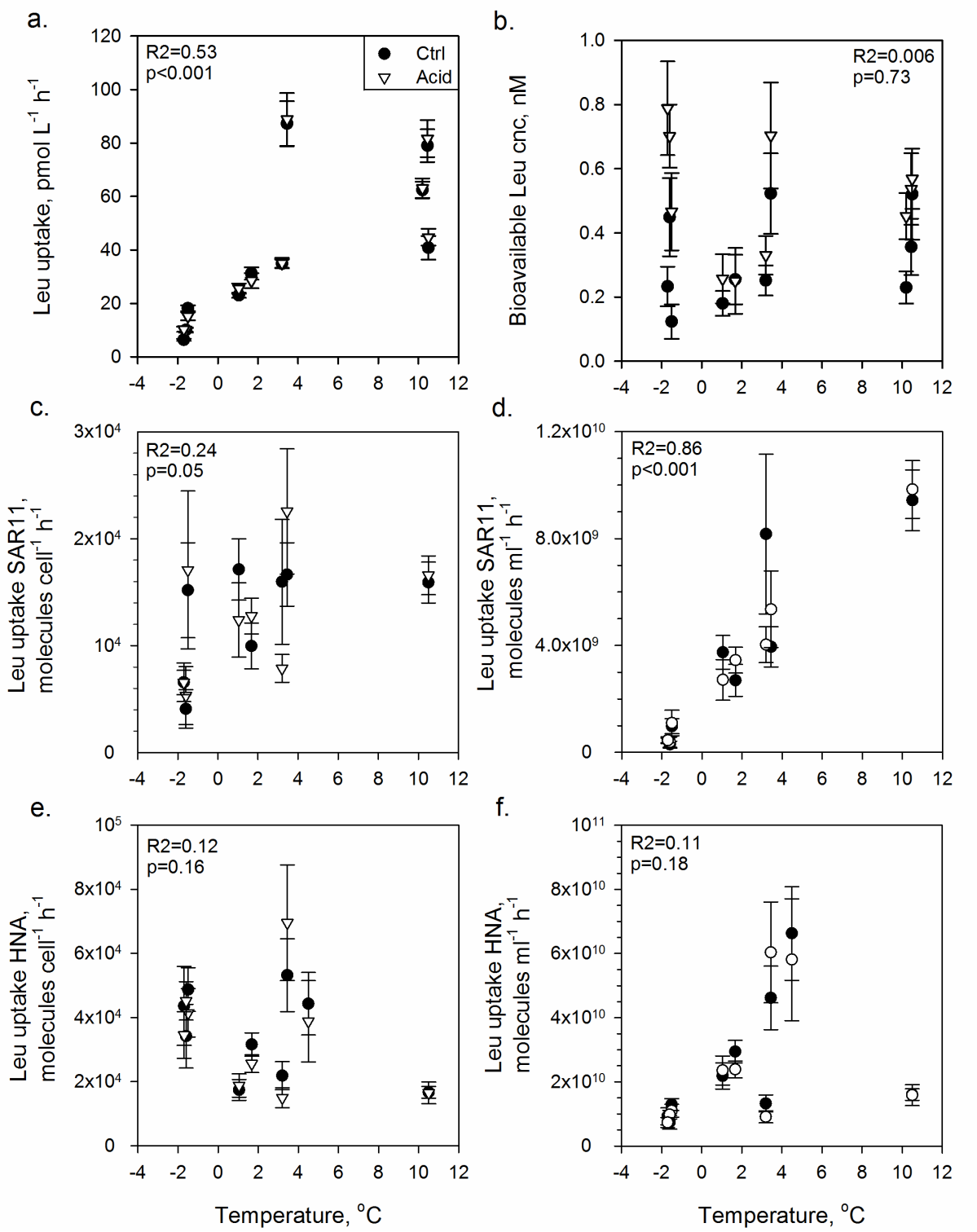

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Fig. 5. Microbial uptake rates and bioavailable concentration of leucine versus temperature in control (Ctrl) and acidified (Acid) experimental treatments from offshore waters along a latitudinal transect crossing the North Atlantic Ocean, Norwegian and Greenland Seas. (a) Total microbial community uptake rate; (b) Bioavailable leucine concentration; (c) SAR11 *Alphaproteobacteria* (SAR11) average cellular uptake rate; (d) SAR11 population uptake rate; (e) High nucleic acid bacteria (HNA) average cellular uptake rate; (f) HNA population uptake rate. R^2 and p values of the linear regression are given for each panel. Please note the different scales on the y-axis of the panels. Error bars in (a) and (b) indicate propagated error of regression slope and intercept. In (c-f) error bars show the propagated error of regression slope, standard deviation of different flow cytometric sorts (n=4) and standard difference of counts (n=2). Note that data on sorted bacterial cells were unavailable from stations 4 and 5.

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