

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

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14

15 **Running title:** Effect of acidification on SAR11

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16 **Abstract**

17 Ubiquitous SAR11 *Alphaproteobacteria* numerically dominate marine planktonic
18 communities. Because they are excruciatingly difficult to cultivate, there is
19 comparatively little known about their physiology and metabolic responses to long-
20 and short- term environmental changes. As surface oceans take up anthropogenic,
21 atmospheric CO₂, the consequential process of ocean acidification could affect the
22 global biogeochemical significance of SAR11. Shipping accidents or inadvertent
23 release of chemicals from industrial plants can have strong short-term local effects
24 on oceanic SAR11. This study investigated the effect of 2.5 fold acidification of
25 seawater on the metabolism of SAR11 and other heterotrophic bacterioplankton
26 along a natural temperature gradient crossing the North Atlantic Ocean, Norwegian
27 and Greenland Seas. Uptake rates of the amino acid leucine by SAR11 cells as well
28 as other bacterioplankton remained similar to controls despite an instant ~50%
29 increase in leucine bioavailability upon acidification. This high physiological
30 resilience to acidification even without acclimation, suggests that open ocean
31 dominant bacterioplankton are able to cope even with sudden and therefore more
32 likely with long-term acidification effects.

33

34 **Key words**

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

37

38 **Introduction**

39 Heterotrophic bacteria, among them the ubiquitous alphaproteobacterial SAR11
40 clade (hereafter SAR11, (Morris *et al.*, 2002)), form an abundant and important
41 component of pelagic marine microbial communities, dominate the remineralisation
42 of phytoplankton-derived organic matter (Martin *et al.*, 1987, Boyd *et al.*, 1999) and
43 constitute a nutrient-rich food source for the smallest eukaryotes (Sherr & Sherr,
44 2002, Hartmann *et al.*, 2013). SAR11 are present in surface waters throughout the
45 world's oceans (Brown *et al.*, 2012) but experience unique environmental conditions
46 in the polar oceans, including a highly seasonal radiation regime, low water
47 temperature and perennial or continuous sea ice-cover.

48 Human activities were shown to have a large, cumulative impact on the majority of
49 the global ocean (~66%), ocean acidification and sea surface temperature being the
50 major factors followed by ocean-based pollution, shipping and destructive fishing
51 (Halpern *et al.*, 2015). Bacteria of polar surface waters may be subject to more rapid
52 and intense ocean acidification due to the higher solubility of carbon dioxide (CO₂) at
53 low water temperatures leading to reduced pH values and a decreased buffering
54 capacity (Sabine *et al.*, 2004, Fabry *et al.*, 2009). Apart from the slow process of
55 ocean acidification, shipping activities can locally decrease pH due to NO_x and SO_x
56 emissions (Hassellöv *et al.*, 2013) or by accidental release of acidifying agents into
57 the water (Mamaca *et al.*, 2009). Acidification may have indirect effects on the
58 speciation of trace metals, e.g. increasing the availability of iron and copper because
59 of their higher solubility at lower pH (Morel *et al.*, 2003). However, culture studies
60 have shown that despite the higher solubility the bioavailability of iron could
61 decrease as a result of acidification (Shi *et al.*, 2010). The effect of lowered pH on
62 inorganic nutrient speciation is less well known. Using theoretical speciation
63 diagrams Zeebe and Wolf-Gladrow (2001) showed significant differences for

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

70 Abundance of the SAR11 clade has been shown to co-vary negatively with
71 particulate organic carbon but positively with turnover rates of dissolved free amino
72 acids (Giebel *et al.*, 2011). Transcriptional responses of SAR11 from the North
73 Pacific subtropical gyre indicate that these organisms acquire nitrogen from
74 dissolved organic matter in addition to its usage as an energy source (Sharma *et al.*,
75 2014). Besides organic matter, temperature and salinity have been proposed to drive
76 distribution and abundance of different SAR11 groups (Brown *et al.*, 2012,
77 Herlemann *et al.*, 2014).

78 Studies of acidification are currently mainly focused on ocean acidification where
79 small or large volumes of samples are incubated for comparatively short (days) or
80 long (up to one year) under increased pCO₂ usually mimicking predicted end of the
81 century, i.e. 1000ppm, levels of pCO₂ (Riebesell, 2004, Engel *et al.*, 2005, Motegi *et*
82 *al.*, 2013, Gattuso *et al.*, 2014, Richier *et al.*, 2014, Zark *et al.*, 2015). Alas, microbial
83 communities can react rapidly (within hours) to elevated CO₂ levels. For example,
84 the nitrogen fixation rates of *Trichodesmium* can increase by up to 41% within 4-6
85 hours after exposure to high CO₂ conditions (Hutchins *et al.*, 2009).

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

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

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

113

114 **Materials and Methods**

115 **Seawater collection and acidification**

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

126 Acidification (i.e. the manipulation of seawater to increase the ambient pCO₂ to a
127 target level of 1000 ppm, resulting in a pH of 7.79±0.07) was achieved by the
128 addition of ultra-clean hydrochloric acid and sodium bicarbonate; an approach which
129 increases CO₂ and decreases pH whilst maintaining a balanced carbonate system
130 (Riebesell *et al.*, 2010). 50ml samples were removed from control and acidified
131 bottles at the beginning and end of incubations to measure the resultant dissolved
132 inorganic carbon (DIC) and total alkalinity (TA), and to verify this remained stable
133 throughout the incubation. DIC was analysed with an Apollo SciTech DIC analyzer
134 (AS-C3) using a LI-COR (7000) CO₂ infrared detector. TA was determined using an
135 open-cell titration (Dickson *et al.*, 2007) with the Apollo SciTech's AS-ALK2 Alkalinity
136 Titrator. Analyses of Certified Reference Materials (CRMs) (A.G. Dickson, Scripps,

137 batch 117) were used at the beginning, middle and end of each analytical run to
138 ensure accuracy of the measurements. Precision was taken as the standard
139 deviation of repeated analysis of CRMs, which in this case was $\pm 1.5 \mu\text{mol kg}^{-1}$ for TA
140 and $\pm 3 \mu\text{mol kg}^{-1}$ for DIC. The remaining variables of the carbonate system, including
141 pCO_2 , were calculated using CO2SYS (MATLAB version 1.1) (Lewis & Wallace,
142 1998, van Heuven *et al.*, 2011) using the constants of Mehrbach *et al.* (1973) refitted
143 by Dickson and Millero (1987).

144 Additional triplicate samples (1.6 mL) were taken at the beginning of incubations
145 from the control and acidified seawater and fixed with 20% paraformaldehyde (PFA,
146 1% final concentration) for determination of bacterial cell abundance by flow
147 cytometry (Fig. 2a and Fig. S1), as outlined below.

148 **Microbial uptake of leucine by radioisotope dilution bioassay**

149 The effect of acidification on leucine uptake, an indicator of bacterial protein
150 synthesis, was determined using the radioisotope dilution bioassay approach
151 developed for freshwater systems (Wright & Hobbie, 1966) and subsequently
152 employed extensively in the marine environment (Hill *et al.*, 2011). [4,5- ^3H] L-Leucine
153 ($140 \text{ Ci}\cdot\text{mmol}^{-1}$, Hartmann Analytic, Germany) was added to the control and acidified
154 seawater samples at a range of concentrations (0.1, 0.2, 0.4, 0.6, 0.8, 1.0 nM) and
155 incubated in parallel at *in situ* temperature and under low-intensity, indirect artificial
156 light. Three samples (1.6 mL) were incubated for each concentration and fixed
157 sequentially after 10, 20 and 30 minutes by the addition of 20% (w/v) PFA (1% final
158 concentration) for 1 hour at room temperature (RT) in the dark.

159 Each fixed bioassay sample was filtered through a $0.2 \mu\text{m}$ pore-size polycarbonate
160 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. ^3H -leucine uptake rates were determined for each concentration of ^3H -
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 ^3H -
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).

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

192 **Identification of dominant bacterial phyla and clades by CARD-FISH**

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

210 Flow cytometric cell sorting was carried out as described above and at least 50 000-
211 100 000 cells were collected onto each filter. At four stations samples had to be pre-
212 concentrated in order to achieve these cell numbers. Therefore, 60 ml of PFA-fixed
213 (1% v/v) sample was concentrated at a flow rate of 2.5 ml min⁻¹ onto a 0.1 µm pore-
214 size Teflon filter (M-Tech Diagnostics, UK) using a syringe pump (KD Scientific,
215 USA).

216 Overall hybridisation efficiency was determined using the Eub338I-III probe mix
217 (Amann *et al.*, 1990) targeting Eubacteria. To identify LNA, which have estimated
218 low ribosomal content due to their small cell size (Kemp *et al.*, 1993, Morris *et al.*,
219 2002), a mix of six different probes and one unlabelled helper probe targeting SAR11
220 *Alphaproteobacteria* (Morris *et al.*, 2002, Gomez-Pereira *et al.*, 2013) were used in
221 order to enhance hybridisation signal. Additionally, a probe targeting the SAR86
222 *Gammaproteobacteria* core cluster (Zubkov *et al.*, 2001) was applied. The majority of
223 HNA were identified by probes targeting the *Bacteroidetes* (Manz *et al.*, 1996),
224 *Alphaproteobacteria* (Neef, 1997), and *Gammaproteobacteria* (Manz *et al.*, 1992)
225 phyla. Information on each probe used, including target bacterial groups, nucleic acid
226 sequence and percentage of formamide used for hybridisation, can be found in the
227 supplementary material (Table S2).

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

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

238 **Data analysis**

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

244

245 **Results and Discussion**

246 **Bacterial abundance and identification of dominant populations**

247 Bacterial abundances ranged from $2 \cdot 10^5$ to $2 \cdot 10^6$ cells ml^{-1} . Although LNA
248 represented occasionally a significant proportion of the bacterial population (up to
249 45%, station 8), HNA were dominant at all stations (on average $72 \pm 10\%$, Fig. 2a).
250 CARD-FISH analyses on flow cytometrically sorted LNA using a mix of seven
251 different SAR11 probes to enhance the signal (Gomez-Pereira *et al.*, 2013) revealed
252 that the LNA were almost exclusively composed of SAR11 *Alphaproteobacteria*
253 ($90 \pm 3\%$, $n=10$, Fig. 2b). Therefore, we will refer to LNA as SAR11 from this point
254 onward. If single probes are used on LNA, the hybridisation efficiency can be very
255 low, e.g. only $18 \pm 8\%$ of the sorted population positively hybridised with the general
256 eubacterial probe (Eub338I-III, Fig. 2b) and the resulting signal was very weak.
257 Although slightly higher, perhaps attributable to the use of a probe mix, the here
258 measured total abundance of SAR11 ($25 \pm 9\%$) is comparable to a study carried out

259 in the Western Arctic Ocean in May-June, where FISH using a single probe revealed
260 that SAR11 comprised up to $15\pm 11\%$ of the bacterioplankton community (Malmstrom
261 *et al.*, 2007). In addition to the SAR11 probe mix, a SAR86 targeted probe was
262 applied to the sorted LNA cells (SAR86_1245, Fig. 2b) in order to identify the minute
263 remaining part of the population. However, SAR86 target cells were detected in only
264 4 out of 9 samples and then just a minute fraction exhibited positive hybridisation
265 signals (0.2-1% of the cells). Similar to SAR11, SAR86 *Gammaproteobacteria* have
266 a small genome size and could hence be expected within the LNA (Dupont *et al.*,
267 2012). Potentially these values underestimate the presence of SAR86 in the samples
268 due to the above-mentioned limitations of using a single probe (Fig. 2c).

269 The hybridisation signal for the HNA was very strong and 84-97% of cells were
270 positively hybridised with probe EUB338 I-III (Fig. 2c). In contrast to LNA, HNA
271 represented a very heterogeneous mix of bacteria. The selected probes in this study
272 allowed identification of the majority of the cells ($61\pm 18\%$). *Bacteroidetes* were
273 present at all studied stations where, with the exception of one of the southernmost
274 stations (Station 6, 10%), they represented a major fraction of the total sorted HNA
275 cells (on average $42\pm 12\%$) (Fig. 2c). At one of the northernmost stations (Station 15)
276 *Bacteroidetes* could not be determined due to a lack of sample material. *Alpha*- and
277 *Gammaproteobacteria* abundances were more variable and significantly lower (4-
278 26% and 1-17% respectively) (Fig. 2c).

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

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

286 Flow cytometric analyses of relevant microbial populations (bacterioplankton, LNA,
287 HNA and small eukaryotes) exhibited no significant fluctuations in cell abundance in
288 relation to acidification (Fig. S3). Turnover times of mRNA in bacteria suggest that
289 they are able to react in a matter of minutes to environmental change (Selinger *et al.*,
290 2003, Steglich *et al.*, 2010). Hence, the experimental set-up of ≤ 2 hours allowed
291 sufficient time to determine a direct effect of acidification.

292 Acidification did not affect the bulk community leucine uptake rates in offshore
293 waters of the North Atlantic Ocean or Norwegian and Greenland Seas (t-test,
294 $p=0.73$; Fig. 3a). Effects of acidification on different parts of the bacterial community
295 could be potentially masked in the bulk community analyses. However, average
296 cellular leucine uptake rates of SAR11 and HNA separated by flow cytometrical cell
297 sorting across eight stations (Mann-Whitney, $p=0.97$ and 0.66 respectively, Fig. 4)
298 were not affected by acidification either. These two bacterial groups are the major
299 consumers of dissolved leucine in these waters, as shown by comparing the sum of
300 the uptake rates of SAR11 and HNA versus the total uptake measured (Fig. S4).
301 Presumably due to their larger size, HNA usually consumed significantly more
302 leucine $\text{cell}^{-1} \text{h}^{-1}$ than SAR11 (Mann-Whitney, $p<0.001$, Fig. S5) and accounted for
303 $83 (\pm 16\%)$ of the total microbial leucine uptake, therefore dominating bacterial
304 production in these waters whilst, owing to their strong latitudinal variation in
305 abundance (Fig. 2a), SAR11 contributed 3-45% of total bacterial production.

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

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

325 The above results suggest that SAR11 and other heterotrophic bacterioplankton
326 encountered in this study are tolerant to pH changes, thereby supporting the
327 conclusions of Joint *et al.* (2011) who deduced theoretically that microorganisms will
328 adapt to ocean acidification. However, acidification is just one parameter in the
329 currently changing ocean, so cumulative effects of for example increases in sea
330 surface temperature and UV radiation will have to be taken into consideration.
331 Moreover, other organisms might be not able to adapt to pH change, therefore
332 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-

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

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

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

377 **Conclusions**

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

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

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403 The bathymetry in Fig. 1 is reproduced from the GEBCO Digital Atlas published by
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406 The authors declare that there are no conflicts of interest.

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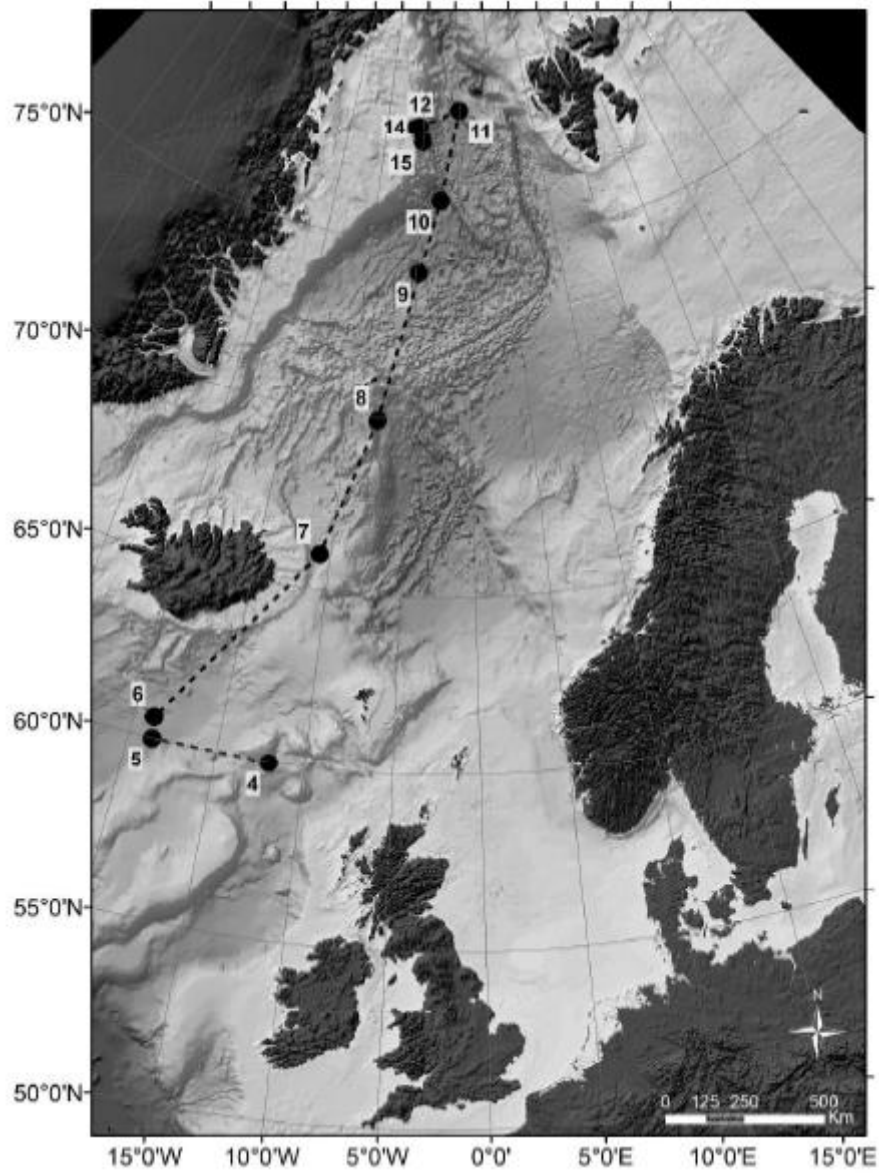
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Fig. 1. Location of stations sampled during this study in the North Atlantic Ocean, Norwegian and Greenland Seas.

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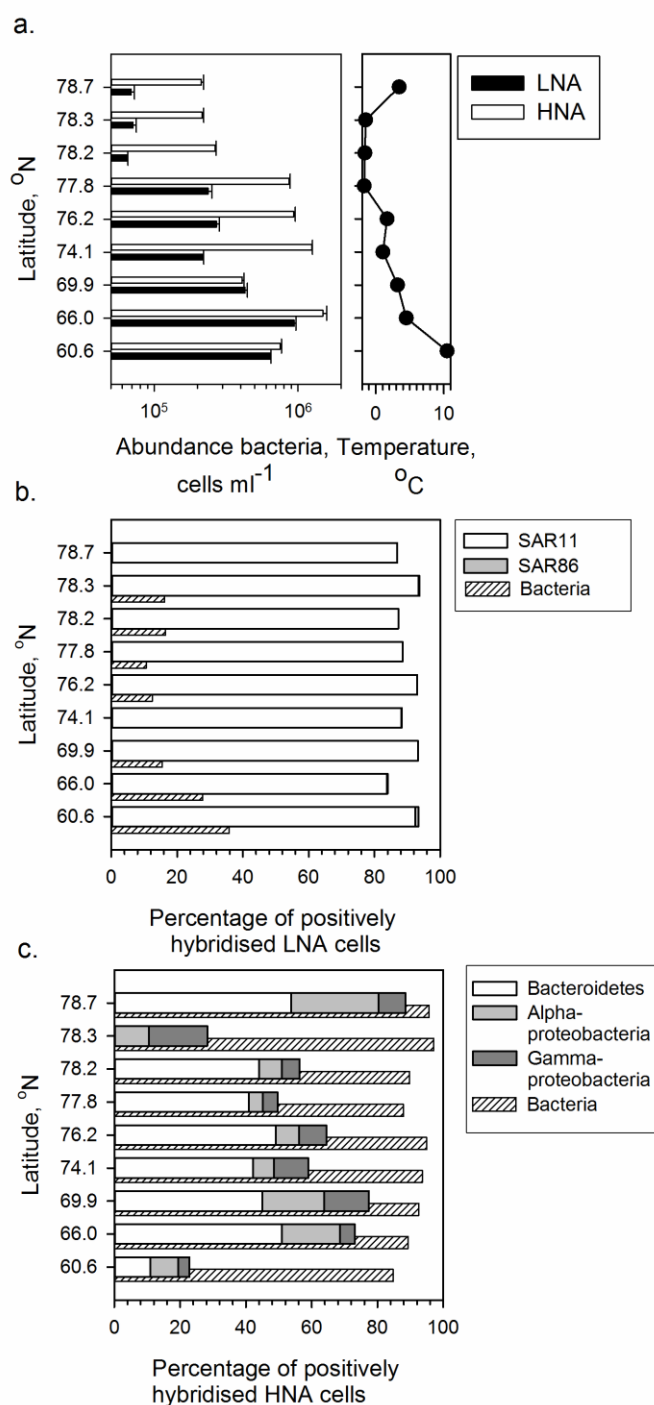
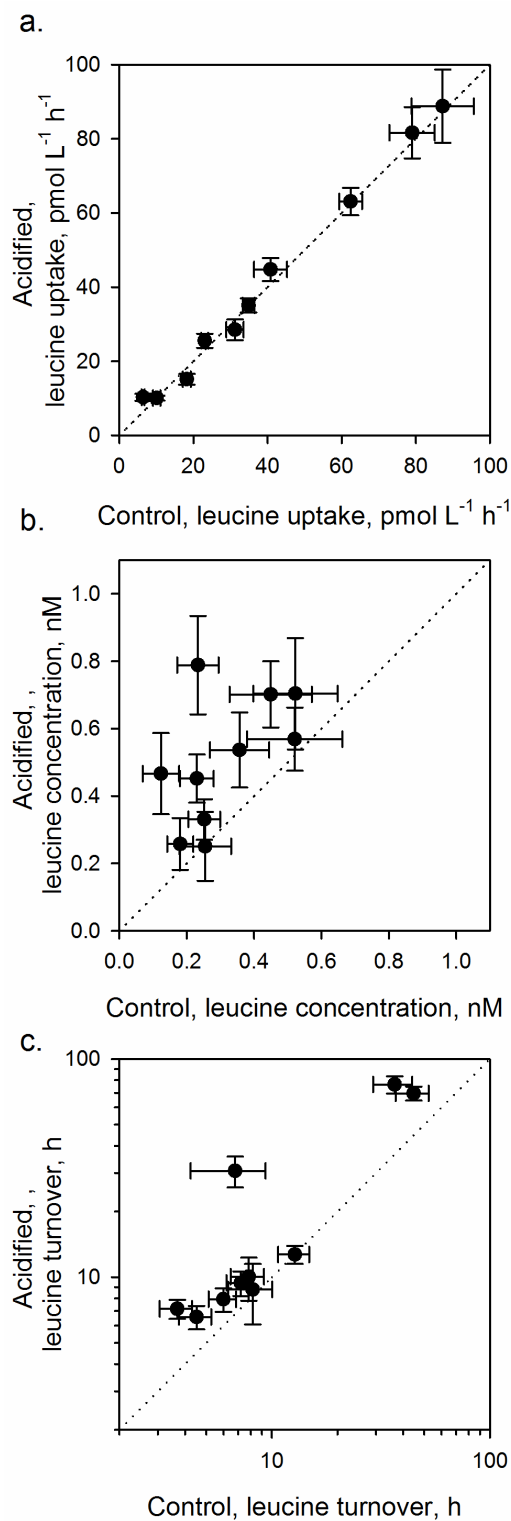


Fig. 2. Abundance (a) and community composition (b, c) of low nucleic acid and high nucleic acid bacteria (LNA and HNA, respectively) in offshore waters along a latitudinal transect crossing the North Atlantic Ocean, Norwegian and Greenland Seas. To simplify temperature and latitudinal correlation, the 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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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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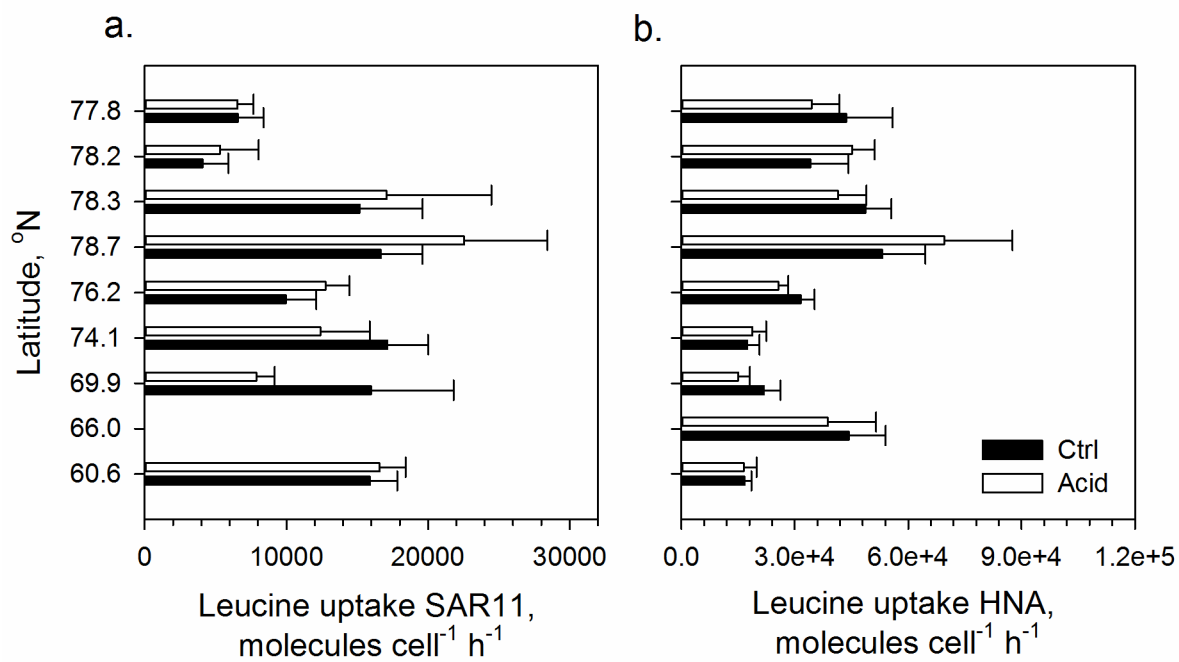
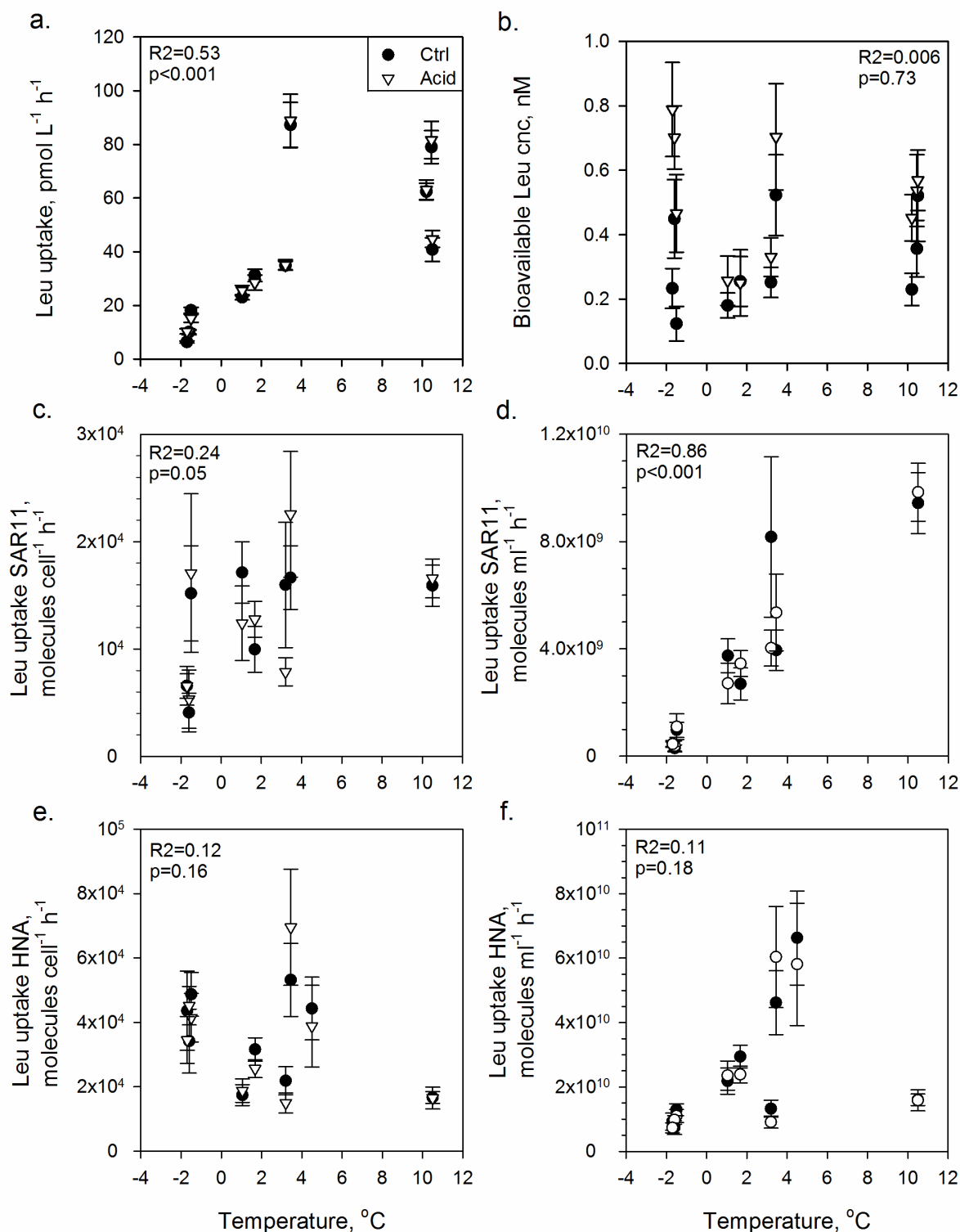


Fig. 4. Average ($n=4$) cellular leucine uptake rates of SAR11 Alphaproteobacteria (SAR11, a) and high nucleic acid (HNA, b) bacteria in control (Ctrl) versus acidified (Acid) experimental treatments from offshore waters along a latitudinal transect crossing the North Atlantic Ocean, Norwegian and Greenland 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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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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