Production and transformation of dissolved neutral sugars and amino acids by bacteria in seawater

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Abstract

Dissolved organic matter (DOM) in the ocean consists of a heterogeneous mixture of molecules, most of which are of unknown origin. Neutral sugars and amino acids are among the few recognizable biomolecules in DOM, and the molecular composition of these biomolecules is shaped primarily by biological production and degradation processes. This study provides insight into the bioavailability of biomolecules as well as the chemical composition of DOM produced by bacteria. The molecular compositions of combined neutral sugars and amino acids were investigated in DOM produced by bacteria and in DOM remaining after 32 days of bacterial degradation. Results from bioassay incubations with natural seawater (sampled from water masses originating from the surface waters of the Arctic Ocean and the North Atlantic Ocean) and artificial seawater, indicate that the molecular compositions following bacterial degradation are not strongly influenced by the initial substrate or bacterial community. The molecular composition of neutral sugars released by bacteria was characterized by a high glucose content (47 mol%) and heterogeneous contributions from other neutral sugars (3-14 mol%). DOM remaining after bacterial degradation was characterized by a high galactose content (33 mol%), followed by glucose (22 mol%) and the remaining neutral sugars (7-11 mol%). The ratio of D-amino acids to L-amino acids increased during the experiments as a response to bacterial degradation, and after 32 days the D/L ratios of aspartic acid, glutamic acid, serine and alanine reached around 0.79, 0.32, 0.30 and 0.51 in all treatments, respectively. The striking similarity in neutral sugar and amino acid compositions between natural (representing marine semi-labile and refractory DOM) and artificial (representing bacterially-produced DOM) seawater samples, suggests that microbes transform bioavailable neutral sugars and amino acids into a common more persistent form.

35 Keywords: Dissolved organic matter, DOM, neutral sugars, amino acids, glucose, galactose, D/L
36 ratio, bioavailability, semi-labile, refractory

1. Introduction

Approximately 700 petagrams (10^{15} g) of carbon in the ocean is in the form of dissolved organic matter (DOM) and consist of a broad range of different chemical compounds spanning a continuum of sizes and reactivities (Hansell, 2013; Siegenthaler and Sarimento, 1993). Although all organic matter originates from organisms, only about 6.6 % of surface DOM and 2 % of deep ocean DOM is identified as specific biomolecules such as neutral sugars and amino acids (Benner, 2002). Despite their low concentrations, the rapid turnover of simple biomolecules suggests that they play an important role in the cycling of carbon and nitrogen in the ocean (Rich et al., 1997, 1996; Skoog et al., 1999). However, several studies have also indicated that some biomolecules can resist bacterial degradation over year long timescales (Kirchman et al., 2001; Ogawa et al., 2001). Heterotrophic bacteria are well known sources of semi-labile and refractory DOM (Kaiser and Benner, 2008; McCarthy et al., 1998; Ogawa et al., 2001), and ~23 % of the entire DOC pool is estimated to derive from bacteria via the microbial carbon pump (microbial transformation of bioavailable DOM to refractory DOM) (Benner and Herndl, 2011; Jiao et al., 2010). However, still only little is known about microbial production of specific semi-labile and refractory biomolecules. This is in part due to the low concentration of individual biomolecules in seawater and the consequent analytical challenge involved.

The most common biomolecules in the oceanic DOM pool are carbohydrates (Benner et al., 1992), with neutral sugars constituting up to half of the total carbohydrate pool (Biersmith and Benner, 1998). Seven different neutral sugars are commonly detected (fucose, rhamnose, arabinose, galactose, glucose, mannose and xylose) and the amount of each neutral sugar relative to the total amount of neutral sugars (the molecular composition, mol%) differs among DOM released by different groups of organisms (Lazareva and Romankevich, 2012). The molecular composition can therefore be used as a tracer of different organisms or processes. E.g. freshly released neutral sugars

from different algal species exhibit great variation in mol% but generally have high contributions of glucose and galactose, each sometimes reaching values above 50 mol% (Amon and Benner, 2003; Biersmith and Benner, 1998; Hama and Yanagi, 2001). Surface ocean DOM is also rich in glucose and galactose with many studies reporting ~20 mol% for each of the two compounds (Goldberg et al., 2009; Kaiser and Benner, 2009; Skoog and Benner, 1997). With depth, glucose and fucose increase in relative abundance, accounting for about 20-40 % and 16-19 % in the deep ocean, respectively (Kaiser and Benner, 2009; McCarthy et al., 1996). The molecular composition of neutral sugars directly released by heterotrophic bacteria during growth in the ocean, however, remains unknown. The molecular composition found in the ocean reflects both the neutral sugars released by different organisms and the neutral sugars persisting in organic matter after long-term bacterial degradation, and the sources and sinks are difficult to distinguish. Knowledge of the composition of neutral sugars produced and transformed by heterotrophic bacteria is key to understanding the origin and fate of these specific DOM components in the ocean.

D-enantiomers of amino acids are useful as bacterial biomarkers, since bacteria are the predominant source of D-amino acids in seawater (Kaiser and Benner, 2008). Four different pairs of L- and D-amino acids are important in DOM (aspartic acid, glutamic acid, serine and alanine) and the amount of D relative to L (the D/L ratio) has been used as an indicator of amino acid bioavailability (Jørgensen et al., 1999). Freshly released DOM from phytoplankton has a low D/L ratio, but the ratio increases during bacterial degradation of DOM (Amon et al., 2001) due to direct release of D-amino acids by bacteria during growth (Kawasaki and Benner, 2006); viral lysis of cells and the subsequent release of cell wall D-amino acids (Middelboe and Jørgensen, 2006); and a presumably higher bioavailability of L-amino acids (Amon et al., 2001; Hopkins et al., 1994; Pérez et al., 2003). The presence of grazers such as flagellates can also increase the bacterial uptake of D-amino acids, possibly due to a higher release from bacterivory and subsequent microbial uptake (Pérez et al.,

85 2003). The D/L ratio does not follow a certain pattern with depth in the ocean. Both a decrease, an
86 increase and no change has been observed with depth in different studies (Jørgensen et al., 1999;
87 Kaiser and Benner, 2008; McCarthy et al., 1998; Pérez et al., 2003), making it difficult to distin-
88 guish between different sources and sinks and the balance between these. Knowledge of the D/L
89 ratio in freshly produced DOM and in DOM remaining after long-term microbial degradation is
90 necessary to understand the origin and fate of amino acids in the ocean.

91 In the present study, we investigated the concentration, composition and bioavailability of
92 neutral sugars and amino acids in two seawater samples collected between Greenland and Iceland
93 representing cold seawater originating from the Arctic Ocean and warm seawater originating from
94 the Atlantic Ocean, respectively. During 32 days bioassay incubations of seawater samples and par-
95 allel glucose enriched artificial seawater samples, the bacterial production and decomposition of
96 individual neutral sugars and amino acids were investigated. The aim was to compare the molecular
97 composition of biomolecules in DOM produced by bacteria and in DOM remaining after long-term
98 bacterial degradation and to use these molecular signatures of bacterial activity to further under-
99 stand the origin of neutral sugars and amino acids in the ocean.

100

101 **2. Methods**

102 *2.1. Sampling site and incubation experiments*

103 Seawater for 32 day incubations was collected from two different locations in the Denmark Strait:
104 at 10 m depth in the warm (9.8 °C) north going North Icelandic Irminger Current (henceforth re-
105 ferred to as the Atlantic sample) and at 80 m depth in the cold (-1.6 °C) south going East Greenland
106 Current (henceforth referred to as the Arctic sample, Fig. 1). The Arctic Ocean receives a large
107 amount of freshwater, supplying 25-36 Tg (10^{12} g) terrigenous dissolved organic carbon (DOC) per
108 year (Raymond et al., 2007), and up to 41 % of this DOC is exported through the Fram Strait

(Opsahl et al., 1999). The East Greenland Current transports cold Arctic water from the Fram Strait south along the Greenland shelf where it mixes with water from the North Atlantic Ocean (Stein, 1988). The seawater collected for bioassay incubations included terrigenous DOM from the Arctic Ocean as well as marine DOM from the Atlantic Ocean.

Six different treatments all consisting of 90 % 0.2 µm filtered seawater and 10 % GF/C filtered inoculum were incubated for 32 days in the dark at 18°C. Three treatments of Arctic and three treatments of Atlantic samples were incubated: a natural seawater sample (NSW), an artificial seawater sample with 60 µM glucose C as the only carbon source (ASW_{glu}) and a natural seawater sample spiked with 60 µM glucose C (NSW_{glu}). Hence, the carbon pool consisted of either natural DOM, glucose or both. Only two different bacterial inoculums were used: one from the Arctic and one from the Atlantic sampling site. All samples were amended with inorganic N and P to have 10 µM KNO_3 and 2 µM Na_2HPO_4 at the beginning of the incubations. N and P were added in excess to reduce the risk of nutrient limitation during the incubations.

Onboard the ship, the majority of the seawater was filtered through a 0.2 µm cartridge filter (Millipore Opticap) to obtain the DOM fraction, and a small bacterial inoculum was filtered through a GF/C filter (Whatman, ~1.2 µm). Both filters were cleaned with ample amounts of seawater before the filtrates were collected. The filtrates were acclimated to 18°C in the dark for approximately 24 hours and the incubations were initiated by addition of bacterial inoculum to the 0.2 µm filtered water in acid washed amber glass bottles. Each of the six treatments consisted of 9 amber glass bottles (200 mL) of seawater and three bottles were sacrificed during each subsampling. The artificial seawater for ASW_{glu} samples was prepared as described by Kester et al. (1967) and adjusted to the salinities of the NSW samples (35.1 and 33.5 for Atlantic and Arctic seawater, respectively) by further addition of MilliQ water. Subsamples for bacterial abundance (all heterotrophic prokaryotes), DOC concentration, dissolved combined neutral sugars and amino acids were taken on days 0, 6

and 32. Bacterial abundance and DOC were measured from all triplicate bottles while neutral sugars and amino acids were measured from a single bottle. All samples from day 0 were taken approximately 12 hours after the addition of inoculum, glucose and nutrients.

2.2. Sample analysis

Subsamples for determination of bacterial abundance were fixed with glutaraldehyde (1.3 % final concentration) and stored frozen (-80°C) until measurement. Bacteria were counted by flow cytometry on a BD FACS Canto II flow cytometer using the nucleic acid stain SYBR Green to stain the fixed cells (Marie et al., 1997). Subsamples for measurement of DOC concentration were 0.2 µm filtered (Acrodisc) and collected in acid cleaned high-density polyethylene (HDPE) bottles. The samples were acidified with 2 M HCl to a pH of 2 and stored cold (5°C) until analysis on a Shimadzu TOC-V_{CPH} analyzer. The instrument was calibrated using a standard series made from acetoanilide and performance was evaluated using deep-sea water reference material made available by the Hansell CRM Program. The measured concentration of DOC in the deep-sea reference (41-43 µM) corresponded well with values from the Hansell CRM Program (41-44 µM).

Neutral sugars and amino acids were measured from 0.2 µm filtered subsamples collected in acid cleaned HDPE bottles that were stored frozen (-20°C) until analysis. The concentration of free and combined hydrolyzable neutral sugars (fucose, rhamnose, arabinose, galactose, glucose, mannose and xylose) was measured on a Dionex 500 ion chromatography system with pulsed amperometric detection (PAD) as described by Skoog and Benner (1997) and Kaiser and Benner (2009). The relative deviation from the mean of replicate hydrolysis (excluding replicate experiments) was 29-40 % for concentrations <20 nM and 10-33 % for concentrations >20 nM of individual neutral sugars. For total hydrolyzable neutral sugars the relative deviation from the mean was 15 %. Free and combined hydrolyzable amino acids were analyzed according to Kaiser and Benner

(2005) on an Agilent 1260 UPLC using a fluorescence detector and corrected for hydrolysis induced racemization (Kaiser and Benner 2005). The concentration of the following amino acids was measured: L- and D-asparagine (asparagine and aspartic acid), L- and D-glutamine (glutamine and glutamic acid), L- and D-serine, L-histidine, L-threonine, glycine, L-arginine, β-alanine, L- and D-alanine, γ-aminobutyric acid, L-tyrosine, L-valine, L-methionine, L-phenylalanine, L-isoleucine, L-leucine and L-lysine. The relative deviation from the mean of duplicate hydrolysis (excluding replicate experiments) was 4-23 % for concentrations <10 nM and 3-8 % for concentrations >10 nM of individual amino acids. For total hydrolyzable amino acids the relative deviation from the mean was 8 %.

2.3. Terminology and calculations

The sum of all neutral sugars and the sum of all amino acids measured are termed total hydrolyzable neutral sugars (THNS) and total hydrolyzable amino acids (THAA), respectively. The contributions of THNS and THAA to total DOM (the yields) were calculated as the ratio of carbon bound in either THNS or THAA to the total concentration of DOC. For THAA, the calculations excluded the non-protein amino acids β-alanine and γ-aminobutyric acid. The mol% of a specific compound was calculated as the molar ratio of the compound concentration to the total concentration of compounds, THNS or THAA.

To investigate the neutral sugars produced by bacteria after utilization of the added glucose, the concentration and composition of neutral sugars on day 6 in glucose enriched treatments were compared to the corresponding values of NSW samples. For NSW_{glu} samples, it was necessary to take into account that a fraction of neutral sugars were bound in natural oceanic DOM. Assuming that bacterial transformation of natural oceanic DOM was similar in NSW and NSW_{glu} samples, a subtraction of the concentration of individual sugars (NS) in NSW samples from the corresponding

concentrations in the NSW_{glu} samples allowed an estimation of neutral sugars produced by bacteria in NSW_{glu} samples:

$$NS_{bacterially\ produced} = NS_{NSW_{glu}} - NS_{NSW}.$$

For ASW_{glu} samples, only the neutral sugars bound in the 10 % inoculum had to be subtracted, assuming similar concentrations and compositions in the inoculum and the NSW samples:

$$NS_{bacterially\ produced} = NS_{ASW_{glu}} - 0.1 \cdot NS_{NSW}.$$

3. Results

3.1. Bacterial abundance and dissolved organic carbon

The abundance of bacteria was low at the beginning of the experiments, with less than $0.4 \cdot 10^6$ cells/mL in Atlantic samples and less than $0.2 \cdot 10^6$ cells/mL in Arctic samples (Fig. 2a, b and c). During the first 6 days of the experiment, the abundance increased considerably in all treatments, reaching $1.3 \cdot 10^6$ and $0.4 \cdot 10^6$ cells/mL in Atlantic and Arctic NSW samples and between $3.7 \cdot 10^6$ and $5.2 \cdot 10^6$ cells/mL in glucose enriched treatments. From day 6 to day 32, the bacterial abundance stayed constant or decreased slightly in all samples. The initial DOC concentration in Atlantic and Arctic samples was approximately 60, 115 and 70 µM in NSW, NSW_{glu} and ASW_{glu} samples, respectively (Fig. 2d, e and f). DOC concentrations decreased in all treatments during the first 6 days and stayed approximately constant from day 6 to day 32. The net DOC consumption in the Atlantic samples was 4±3.6, 52±3.6 and 58±1.4 µM in NSW, NSW_{glu}, ASW_{glu} samples, respectively. The corresponding values for the Arctic samples were 2±1.5, 52±0 and 56±2.1 µM DOC. The DOC consumption after 32 days corresponded to 7 and 1 % of total DOC in the Atlantic and Arctic NSW samples, respectively.

3.2. Neutral sugars

205 The initial concentrations of THNS ranged from 210 to 339 nM in NSW samples and from 7041 to
206 7850 nM in the glucose enriched samples. THNS decreased in all treatments over time, particularly
207 during the first 6 days of the incubations (Fig. 3a, b and c). After 32 days, the net consumption of
208 THNS in the Atlantic samples was 130, 7585 and 6992 nM in NSW, NSW_{glu}, ASW_{glu}, respectively.
209 The corresponding values for Arctic samples were 84, 6990 and 6943 nM. Initially, THNS consti-
210 tuted 3.5 and 2.4 % of total DOC in Atlantic and Arctic NSW samples, respectively. The yields
211 decreased to 2.5 and 1.7 % on day 6 and continued to decrease at a slower pace during the remain-
212 ing of the incubations (Fig. 3d). In the glucose enriched treatments, the yields were approximately
213 40 and 63 % in NSW_{glu} and ASW_{glu} samples, respectively, decreasing to ~3 and 9 % after 6 days
214 (Fig. 3e and f). The molecular composition (mol%) of neutral sugars changed over time but varied
215 little between treatments (Table 1). Galactose and glucose were the most abundant neutral sugars in
216 all samples, together comprising between 38 and 74 mol%. The glucose mol% increased from day 0
217 to day 6 in all samples (disregarding glucose treatments, day 0) followed by a decrease from day 6
218 to day 32. The galactose mol% followed the opposite pattern: decreased during the first 6 days fol-
219 lowed by an increase from day 6 to day 32. At the end of the experiments, galactose was the most
220 abundant neutral sugar comprising between 21 and 47 %. At the same time, glucose only comprised
221 12 to 32 %.

222 Due to the high concentration of glucose on day 0 in the glucose enriched treatments, these
223 samples were diluted 100 times prior to analysis. Consequently, no other neutral sugars were deter-
224 mined on day 0. The molecular composition of neutral sugars produced by bacteria in the glucose
225 enriched treatments during the first 6 days was calculated as described in the Methods section. It
226 was characterized by a high glucose mol% (47±12) and a relatively low mol% of the remaining
227 neutral sugars, ranging from 3±2 to 14±9 (Table 1). After 32 days, the molecular composition in all

228 samples was characterized by a high galactose mol% (33±11), a lower glucose mol% (22±8) and an
229 even lower mol% of the remaining neutral sugars, ranging from 7±5 to 11±3.
230
231 *3.3. Amino acids*
232 The initial concentration of THAA ranged from 170 to 244 nM in all treatments (Fig. 4a, b and c).
233 During the incubations, the concentration of THAA decreased in the Arctic and Atlantic NSW sam-
234 ples. The glucose enriched samples showed an increased concentration of THAA on day 6 followed
235 by a decrease. The only exception was the Arctic ASW_{glu} sample, which like NSW samples showed
236 a decreasing THAA concentration throughout the incubation. The yield of THAA in NSW samples
237 decreased from 1.4 to 1.1 % and from 1 to 0.8 % for Atlantic and Arctic treatments, respectively
238 (Fig. 4d). The yield in NSW_{glu} samples increased from 0.7 and 0.6 % on day 0 to 1.5 and 1.8 % on
239 day 6 and ended at 1.5 and 0.8 % on day 32 in Atlantic and Arctic samples, respectively (Fig. 4e).
240 The yield in ASW_{glu} samples increased from 1.1 and 1.0 % on day 0 to 7.3 and 2.1 % on day 6 and
241 decreased to 2.3 and 0.7 % on day 32 in Atlantic and Arctic samples, respectively (Fig. 4f).
242 The molecular composition (mol%) of amino acids varied little from sample to sample and
243 did also not show any notable changes over time (Table 2). Glycine was the most abundant amino
244 acid comprising between 26 and 40 mol%. The four amino acids from which both the L- and the D-
245 enantiomers have been measured (asparagine, glutamine, serine and alanine) were the second-most
246 abundant amino acids comprising between 6 and 18 mol%. The concentration of D-amino acids
247 only changed slightly while L-amino acids generally decreased in concentration during the time
248 course of the experiments, leading to an increased D/L ratio with time (Table 2).
249
250 **4. Discussion**
251 *4.1. Concentration and bioavailability*

In the NSW samples, the initial DOC concentrations (61-63 µM) were similar to previous measurements from the region (e.g. Amon et al. 2003; Benner et al. 2005). After 32 days, 7 and 1 % of the DOC was consumed in Atlantic and Arctic NSW samples, respectively. These values are comparable to estimates by Amon and Benner (2003) who estimated around 10 % of Atlantic and 2 % of Arctic DOC to be labile. The initial concentrations of THNS in NSW samples (210 and 339 nM) were within the range of concentrations (60-409 nM) found in ultrafiltered surface samples (< 0.1 µm, < 100 m) from the region (Amon and Benner, 2003), and GF/F filtered surface samples from the Sargasso Sea which ranged from ~180 to 450 nM (Goldberg et al., 2009). A considerable fraction of the neutral sugars in NSW samples were labile, as indicated by the preferential removal of neutral sugars (Fig. 3D). This trend has also been observed in previous studies (Amon and Benner, 2003; Amon et al., 2001). The initial concentrations of THAA in NSW samples (189 and 244 nM) corresponded well with values from surface waters of the Sargasso Sea (~150-200 nM, Lee and Bada 1977; Kaiser and Benner 2008, 2009) and the Arctic Ocean (~150-500 nM, Dittmar et al. 2001; Shen et al. 2012). The preferential removal of amino acids in NSW samples indicated that a fraction of amino acids was labile (Fig. 4D). Our study supports the general notion that neutral sugar and amino acid yields can be used as biochemical indicators of DOC bioavailability (Amon et al., 2001), since the highest DOC consumption was observed in the Atlantic NSW sample, which was also associated with the highest yields of THNS and THAA.

In the glucose enriched samples, 49-56 µM DOC was consumed during the experiment. The DOC samples on day 0 were collected 12 hours after the addition of glucose, and the actual DOC consumption was therefore likely up to 12-17 µM higher (estimated from the missing free glucose). Taking this into account, the DOC consumptions agreed well with the amount of added glucose and possibly a small contribution from the original DOM pool. The DOC consumption estimates were calculated from the missing free glucose: glucose was added to a final concentration of 10,000 nM

276 before addition of the inoculum. However, the concentration measured at the beginning of the ex-
277 periment ranged from 7041 to 7850 nM. Sample hydrolysis possibly also altered some of the added
278 glucose, resulting in lower measured concentrations (Skoog and Benner, 1997). The DOC con-
279 sumption in NSW_{glu} samples (52 µM) was lower than in the ASW_{glu} samples (56-58 µM), despite of
280 a higher DOC concentration and an equal amount of glucose. Although this discrepancy is small, it
281 may represent a minor contribution of labile DOC associated with the inorganic salts used to pre-
282 pare the ASW_{glu} samples or an inhibition of degradation of natural DOM in the presence of a simple
283 labile substrate (Gontikaki et al., 2013). However, further studies are warranted to resolve the im-
284 portance of the latter process. The significant drop in neutral sugar yields clearly reflects the labile
285 nature of added free glucose. The amino acid yields increased at the beginning of the incubations
286 and decreased from day 6 to day 32 – a trend also observed in previous studies (Kawasaki and
287 Benner, 2006; Ogawa et al., 2001). The initial increase in amino acid yields is due to the significant
288 increase in bacterial abundance and subsequent release of amino acids.
289
290 *4.2. Bacterial production of neutral sugars during the first 6 days*
291 Bacterial production and subsequent release of neutral sugars was calculated from the glucose en-
292 riched treatments as described in the Methods section. The amount of neutral sugars produced dur-
293 ing the first 6 days of our incubation (104-208 nM) was within the range of concentrations of neu-
294 tral sugars observed in the ocean (20-800 nM, Benner (2002)). The molecular composition was
295 characterized by a high glucose mol% (47±12) and a relatively low mol% of the remaining neutral
296 sugars (Fig. 5), and are strikingly similar to the composition of bacterial DOM found in a study by
297 Ogawa et al. (2001) after 7 days of incubation. Calculations of the neutral sugars produced in the
298 NSW_{glu} samples on day 6 are associated with uncertainty since bacterial degradation of the natural
299 DOM is unknown. However, if only the ASW_{glu} samples are used when calculating the bacterially-

derived neutral sugars on day 6, the results are almost identical: a high glucose mol% of 50 and low mol% of the remaining neutral sugars ranging from 4 to 11.

Similar patterns have also been observed in algal-derived DOM (Hama and Yanagi, 2001; Lazareva and Romankevich, 2012), e.g. in DOM from a fresh diatom culture (Biersmith and Benner, 1998) and from sea ice algae (Amon and Benner, 2003). However, DOM released by other algal cultures exhibit different molecular compositions of neutral sugars with galactose or xylose being most abundant (Biersmith and Benner, 1998). In the ocean, glucose is generally the most abundant neutral sugar, however, values above 30 mol% are only observed occasionally (Table 3). Amon and Benner (2003) suggested that degradation processes rather than production processes determine the neutral sugar composition in the ocean, based on the similarities observed in samples of different origin (terrestrial and marine) and from different locations (oceanic regions and water masses). This can possibly explain the difference in molecular composition of DOM in the ocean and the bacterially-produced DOM after 6 days in the present study. Only small differences in neutral sugar composition between Atlantic and Arctic samples were observed after 6 days of incubation despite the different bacterial inocula. Moreover, there was a striking similarity in the bacteria-derived neutral sugar composition observed in the present study and in the study by Ogawa et al. (2001). Together, these results suggest that bacterially-produced neutral sugars, independent of bacterial community structure, are important in shaping the molecular composition of neutral sugars in seawater.

4.3. Bacterial degradation of neutral sugars

After 32 days, a clear degradation signature had emerged with glucose being less important and galactose being more important (Fig. 6). This trend has also been seen in studies of marine sediments (Oakes et al., 2010). The majority of the remaining DOM after 32 days was assumed to be semi-labile or refractory, and the striking similarity between treatments indicates that the molecular

composition of semi-labile or refractory neutral sugars (i.e. material persisting longer that 32 days) attains a fairly specific molecular composition, irrespective of initial DOM composition. Furthermore, the similarity between treatments suggests that bacterial degradation processes shape the composition of semi-labile or refractory neutral sugars. After 32 days, about 85 to 87 % of the neutral sugars present in ASW_{glu} samples are of bacterial origin (i.e. produced by bacteria during glucose assimilation), while only 13 to 15 % originate from the DOM added with the inoculum (excluding glucose), assuming that neutral sugars in the inoculum follows the same degradation pattern as in NSW samples and that no refractory neutral sugars were added with the inorganic salts while preparing the artificial seawater. Hence, the molecular composition observed in the ASW_{glu} treatments is primarily the result of bacterially-produced and bacterially–altered molecules containing neutral sugars. In NSW samples, however, the molecular composition after 32 days primarily reflects the natural background level of refractory and semi-labile neutral sugars present in the seawater when collected. Since this background signature is approaching the molecular composition of bacterially-produced neutral sugars in ASW_{glu} samples remaining after 32 days, we hypothesize that semi-labile and refractory neutral sugars primarily originate from bacterial processing of DOM and bacterial remains. Mesocosm studies have shown that 91-94 % of dissolved neutral sugars accumulated during an algal bloom were degraded within 15-20 days (Kragh and Søndergaard, 2009; Meon and Kirchman, 2001), and here we find that only 32-49 % of the neutral sugars produced by bacteria in ASW_{glu} samples were degraded within a period of 26 days, from day 6 to day 32. Neutral sugar containing molecules produced by bacteria appear to be less bioavailable than those produced by algae.

The molecular composition of neutral sugars in the deep ocean is significantly different from that observed at the end of our incubation experiments, although both reflect a high degree of bacterial degradation and transformation. Our results indicate that bacterially degraded neutral sugars

348 have a high galactose mol% (Table 1, Fig. 6), but measurements in the deep ocean reveal a high
349 glucose mol% (Table 3). This discrepancy has been seen in other studies as well (e.g. Amon et al.
350 2001) and can be due to fundamental environmental differences between the ocean interior and bio-
351 assay incubations and the different time scales of carbon cycling. However, the difference can pos-
352 sibly also be due to differences in diagenetic state of the neutral sugars in the deep ocean and in the
353 surface waters sampled for this study. In addition, it is possible that bacterial degradation of particu-
354 late organic material (POM) plays a major role in shaping the molecular composition in the deep
355 ocean. POM supports a major fraction of the respiratory carbon demand below the photic layer
356 (Arístegui et al., 2002) and POM is known to have a high glucose content (Hernes et al., 1996;
357 Panagiotopoulos and Sempéré, 2007). In our incubations, only the 10 % inoculum contained POM,
358 and it is likely that our results mainly reflect bacterial degradation of DOM, either DOM produced
359 by bacteria from glucose or DOM initially present in the samples, while deep ocean observations
360 mainly reflect bacterial degradation of POM. Finally, the high glucose mol% in the deep ocean pos-
361 sibly reflects bacterially-produced neutral sugars with a high glucose content (Fig. 5), or other neu-
362 tral sugar sources also having a high glucose mol%, e.g. submarine vent microbes (Skoog et al.,
363 2007). In the Pacific Ocean, the glucose mol% is significantly higher than in the Atlantic Ocean
364 (Kaiser and Benner, 2009), supporting the hypothesis that the bacterially-produced neutral sugars
365 become more important with time in the deep ocean. However, further studies are needed to fully
366 understand bacterial degradation of neutral sugars bound in DOM and POM and the connection
367 between *in situ* measurements and incubation studies.
368
369 *4.4. Bacterial degradation of amino acids*
370 The molecular composition of amino acids did not vary considerably among samples nor change
371 over the time course of the experiments (Table 2). The most abundant amino acid was glycine with

a mol% between 26 and 40 followed by aspartic acid, glutamic acid, serine and alanine, which all typically ranged from 10 to 15 mol%. The same trends are seen in the ocean, except that the mol% of glycine is somewhat lower (15-32) and the mol% of γ-aminobutyric acid increases with depth (Table 4). A high glycine content is generally associated with highly degraded organic matter (Dauwe et al., 1999), however, the high mol% observed in our incubations are rarely seen in the ocean (Table 4). The amino acid γ-aminobutyric acid is known to increase with depth in the ocean and has therefore been used as an indicator of organic matter diagenesis (Dauwe and Middelburg, 1998; Davis et al., 2009). No consistent increase in γ-aminobutyric acid is seen during our incubations, suggesting that the increase observed in the ocean could result from long-term degradation. This hypothesis is consistent with results from a study by Davis et al. (2009) who found an increase in γ-aminobutyric acid of only 0-7 mol% during 20-33 days incubation experiments.

The four measured amino acids with L- and D-enantiomers exhibited a clear degradation pattern (Fig. 7). Despite of very different D/L ratios at the beginning of the experiment, all treatments ended up having almost identical ratios after only 32 days (Table 2). The D/L ratios of amino acids in NSW samples generally remained constant or increased slightly during the incubation. In the glucose enriched samples, however, the ratio increased significantly and after 32 days all treatments had almost similar D/L ratios. This trend was especially clear for aspartic acid (except the Arctic ASW_{glu} sample indicated on the figure) and serine, where the variability after 32 days was minimal. As an exception, Atlantic and Arctic samples seemed to have slightly different D/L ratios of glutamic acid after 32 days, with Atlantic samples being below 0.27 and Arctic samples above 0.35. Hence, the amount of D-amino acids produced relative to L-amino acids utilized was higher in Arctic samples than in Atlantic samples. Previous studies have also reported increased D/L ratios with time in degradation experiments (Amon et al., 2001; Jørgensen et al., 1999) and with depth in the ocean (Dittmar et al., 2001; Kaiser and Benner, 2008). The common endpoints in D/L ratios ob-

served here have not previously been observed and suggest that a degradation signature exists. It appears that DOM source (Arctic DOM, Atlantic DOM and glucose) and bacterial community (Arctic versus Atlantic) have a minor influence on D/L amino acid ratios, except for glutamic acid where bacterial community structure to some extent might influence the ratio. The fact that D/L amino acid ratios in NSW samples equals bacterially-produced D/L ratios in ASW_{glu} samples after 32 days indicates that bacteria are the dominant source of amino acids in semi-labile and refractory DOM.

The D/L ratios observed at the end of the incubation experiments were considerably higher than D/L ratios in the deep ocean (Kaiser and Benner, 2008; McCarthy et al., 1998; Pérez et al., 2003). In our experiments as well as in the ocean, the highest D/L ratio is that of aspartic acid, followed by the D/L ratio of alanine and the (almost equal) D/L ratios of glutamic acid and serine. However, the D/L ratios observed after 32 days in our incubations were about 0.1 to 0.3 units higher than in the deep ocean (Fig. 7, McCarthy et al. 1998). The time scale of the incubation experiments was much shorter than the time scale of ocean circulation, and it is possible that deep ocean amino acids exhibit a different D/L ratio due to a higher degree of diagenesis. The observed difference could also reflect the fact that the incubations included a larger fraction of DOM of bacterial origin and therefore had a higher D-amino acid content than DOM and POM in the ocean. The main sources of D-amino acids are bacterial cell wall-membrane components including peptidoglycans, lipopolysaccharides and lipopeptides (Kaiser and Benner, 2008). The ASW_{glu} samples demonstrated that during bacterial utilization of glucose and subsequent degradation of bacterial remnants, DOM with high D/L amino acid ratios is produced. The DOM and POM available for bacterial degradation in the ocean, however, is probably derived from many different sources (Kaiser and Benner, 2008).

420 *4.5. Implications*

421 Bacteria play an important role in shaping the concentration and composition of neutral sugars and
422 amino acids in the ocean. Our results show that bacteria are capable of changing the composition of
423 biomolecules bound in DOM significantly within short time scales and that biomolecule yields can
424 be used as indicators of DOC bioavailability. Bacterial transformation of labile DOM to refractory
425 DOM via the microbial carbon pump has been suggested as an important production pathway of
426 refractory DOM (Jiao et al., 2010; Ogawa et al., 2001). Results from the present incubations indi-
427 cate that the microbial carbon pump also applies for neutral sugars and amino acids: the molecular
428 composition of biomolecules produced by bacteria and biomolecules remaining after degradation of
429 bacterial remnants in ASW_{glu} samples was strikingly similar to the composition of semi-labile or
430 refractory biomolecules remaining in NSW samples after 32 days, suggesting that bacterially-
431 produced biomolecules can persist for long periods in the ocean. The present study provides prelim-
432 inary indications of microbial production of refractory biomolecules via the microbial carbon pump,
433 but further studies are needed to test this hypothesis and better understand its quantitative im-
434 portance.

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Table 1: Molecular composition of neutral sugars in the six different treatments during the incubations. The neutral sugars produced by bacteria in the glucose enriched samples during the first 6 days (n=4, see Methods for explanation of the calculations) and the neutral sugars remaining after 32 days (n=6) are given as means and standard deviations. Nd = not determined.

Day	THNS (nM)	Fuc (mol%)	Rha (mol%)	Ara (mol%)	Gal (mol%)	Glc (mol%)	Man (mol%)	Xyl (mol%)
NSW, Atlantic								
0	339	11	10	13	23	17	14	11
6	239	14	14	9	20	20	13	9
32	209	12	12	9	27	15	12	12
NSW, Arctic								
0	211	7	9	17	16	22	13	16
6	135	12	13	15	11	29	11	10
32	126	11	14	12	21	20	10	13
NSW_{glu}, Atlantic								
0	7850	nd	nd	nd	nd	100	nd	nd
6	447	10	9	8	18	24	13	17
32	264	12	10	14	32	12	10	9
NSW_{glu}, Arctic								
0	7212	nd	nd	nd	nd	100	nd	nd
6	240	7	12	7	15	42	9	9
32	220	9	12	6	25	32	9	8
ASW_{glu}, Atlantic								
0	7127	nd	nd	nd	nd	100	nd	nd
6	192	6	7	14	7	47	7	11
32	136	4	7	8	46	28	5	2
ASW_{glu}, Arctic								
0	7041	nd	nd	nd	nd	100	nd	nd
6	183	4	12	8	13	48	6	9
32	98	0	14	13	47	25	0	0
Bacterially-produced neutral sugars		**3 ± 2**	**8 ± 4**	**7 ± 8**	**14 ± 7**	**47 ± 12**	**8 ± 3**	**14 ± 9**
Composition after 32 days		**8 ± 5**	**11 ±3**	**10 ± 3**	**33 ± 11**	**22 ± 8**	**8 ± 5**	**7 ± 5**

Table 2: Molecular composition (mol%) of amino acids and D/L ratios in the six different treatments during the incubations. The amino acids His, Thr, Tyr, Val, Met, Phe, Ile and Lys all have mol% below 5 and are therefore not included in this table. The amino acids provided in the table accounted for 91-98 mol%. The molecular composition and D/L ratios after 32 days are given as means and standard deviations calculated from all six treatments. The only exception is the D/L ratio of Asx which did not include ASW_{glu} Arctic.

		Molecular composition (mol%)									D/L ratio			
Day	THAA (nM)	Asx	Glx	Ser	Gly	Arg	Ala	Leu	β-ala	γ-aba	Asx	Glx	Ser	Ala
NSW, Atlantic														
0	244	12	11	9	33	3	15	3	7	1	0.77	0.27	0.26	0.45
6	210	13	11	10	34	3	16	3	5	1	0.76	0.27	0.26	0.52
32	179	14	10	8	34	3	16	3	6	2	0.78	0.25	0.30	0.55
NSW, Arctic														
0	189	12	8	9	39	3	15	3	6	3	0.71	0.21	0.18	0.58
6	181	13	10	9	37	3	16	3	5	2	0.79	0.27	0.28	0.56
32	154	13	8	8	38	3	17	3	6	2	0.82	0.40	0.31	0.63
NSW_{glu}, Atlantic														
0	239	13	11	11	31	3	15	3	7	2	0.32	0.10	0.12	0.41
6	286	11	11	15	33	2	14	3	3	2	0.53	0.15	0.13	0.36
32	250	13	13	10	30	3	16	4	4	1	0.76	0.22	0.26	0.44
NSW_{glu}, Arctic														
0	220	12	11	13	34	2	15	3	5	2	0.37	0.09	0.10	0.46
6	307	12	12	13	29	5	15	4	3	1	0.43	0.10	0.12	0.29
32	174	12	7	9	40	2	17	3	5	1	0.8	0.41	0.30	0.54
ASW_{glu}, Atlantic														
0	208	10	16	9	34	4	10	6	1	2	0.28	0.04	0.05	0.11
6	254	9	15	15	35	4	9	4	1	2	0.54	0.11	0.15	0.15
32	87	12	16	11	26	5	15	6	2	2	0.81	0.27	0.31	0.41
ASW_{glu}, Arctic														
0	170	9	14	11	34	4	10	6	1	2	0.34	0.06	0.07	0.11
6	65	9	17	11	26	6	14	5	3	2	0.44	0.18	0.13	0.36
32	31	12	8	6	36	0	18	4	4	8	1.24	0.35	0.30	0.49
Composition and ratio after 32 days														
		13±1	**10±4**	**9±2**	**34±5**	**3±2**	**17±1**	**4±1**	**5±2**	**3±3**	**0.79±0.02**	**0.32±0.08**	**0.30±0.02**	**0.51±0.08**

606 Table 3: Molecular composition (mol%) of neutral sugars found in the oceans' surface, mesopelagic and deep layers. HMW DOM: 1 nm to
607 0.2 µm; GF/F DOM: < ~0.7µm.

Location	Depth (m)	DOM size	Fuc	Rha	Ara	Gal	Glc	Man	Xyl	Reference
Surface ocean, 0-200 m										
North Pacific	10	HMW DOM	16	12	9	19	17	13	12	McCarthy et al. (1996)
Sargasso Sea	2	HMW DOM	15	13	9	20	15	13	13	McCarthy et al. (1996)
Gulf of Mexico	10	HMW DOM	17	14	10	19	13	14	10	McCarthy et al. (1996)
North Pacific	10-40	0.2 µm	13	8	4	22	36	9	9	Borch and Kirchman (1997)[a]
Equatorial Pacific	2-200	HMW DOM	16	14	8	18	20	12	13	Skoog and Benner (1997)
Greenland Sea	20-50	GF/F	1	13	7	13	52	13	1	Engbrodt and Kattner (2005)
North Pacific	3-5	HMW DOM	17	11	7	23	17	14	10	Repeta and Aluwihare (2006)
Sargasso Sea	0-140	GF/F	13	10	7	22	21	13	13	Goldberg et al. (2009)[a]
Sargasso Sea	5-200	Unfiltered	14	12	11	21	17	12	13	Kaiser and Benner (2009)
North Pacific	20-200	Unfiltered	15	11	12	20	19	11	11	Kaiser and Benner (2009)
Mesopelagic ocean, 200-1000 m										
North Pacific	765	HMW DOM	18	14	9	10	29	8	11	McCarthy et al. (1996)
Sargasso Sea	900	HMW DOM	18	19	7	14	21	11	8	McCarthy et al. (1996)
Gulf of Mexico	750	HMW DOM	19	13	11	14	20	11	9	McCarthy et al. (1996)
North Pacific	250	0.2 µm	16	0	0	42	31	6	6	Borch and Kirchman (1997)[a]
Equatorial Pacific	400	HMW DOM	17	15	7	19	22	12	10	Skoog and Benner (1997)
Sargasso Sea	250	GF/F	12	9	6	17	35	11	11	Goldberg et al. (2009)[a]
Sargasso Sea	350-900	Unfiltered	17	11	12	19	18	11	12	Kaiser and Benner (2009)
North Pacific	250-750	Unfiltered	17	11	10	19	23	10	10	Kaiser and Benner (2009)
Deep ocean, 1000-5200 m										
North Pacific	4000	HMW DOM	16	9	7	10	26	13	5	McCarthy et al. (1996)
Sargasso Sea	2400	HMW DOM	19	17	10	13	17	10	11	McCarthy et al. (1996)
Equatorial Pacific	4000	HMW DOM	16	14	3	19	19	13	12	Skoog and Benner (1997)
Greenland Sea	1800-4500	GF/F	19	16	22	11	21	6	6	Engbrodt and Kattner (2005)
North Pacific	5200	HMW DOM	25	15	8	19	12	12	9	Repeta and Aluwihare (2006)
Sargasso Sea	1360-4300	Unfiltered	16	9	11	19	26	10	9	Kaiser and Benner (2009)
North Pacific	2000-4000	Unfiltered	19	8	7	21	41	0	6	Kaiser and Benner (2009)

608 [a]Mannose and xylose were stated as one value, and for simplicity, this value has been split up into two identical mol%.

609 Table 4: Molecular composition (mol%) of amino acids found in the oceans' surface, mesopelagic and deep layers. HMW DOM: 1 nm to 0.2
610 µm; GF/F DOM: < ~0.7µm. nd = no data.

Location	Depth (m)	DOM size	Asx	Glx	Ser	Gly	Arg	Ala	Leu	β-ala	γ-aba	Reference
Surface ocean, 0-200 m												
North Pacific	20-200 m	unfiltered	10	14	8	26	2	15	2	5	2	Kaiser and Benner (2009)
North Pacific	10 m	HMW DOM	11	18	10	16	4	13	5	1	0	McCarthy et al. (1996)
North Pacific	0-400 m	GF/F	10	11	12	19	9	15	4	nd	nd	Yamashita and Tanoue (2003)[a]
Chukchi Sea	0-200 m	GF/F	12	8	5	29	2	13	1	8	8	Davis and Benner (2005)
Sargasso Sea	20-100 m	unfiltered	8	9	6	23	6	14	2	5	7	Kaiser and Benner (2009)
Sargasso Sea	2 m	HMW DOM	10	15	9	15	6	16	5	1	2	McCarthy et al. (1996)
Gulf of Mexico	10 m	HMW DOM	12	16	11	17	3	16	3	2	0	McCarthy et al. (1996)
Mesopelagic ocean, 200-1000 m												
North Pacific	250-750 m	unfiltered	11	12	7	28	3	17	0	6	4	Kaiser and Benner (2009)
North Pacific	765	HMW DOM	9	12	6	21	2	15	5	4	0	McCarthy et al. (1996)
Chukchi Sea	201-1000 m	unfiltered	12	6	4	32	1	13	1	7	14	Davis and Benner (2005)[b]
Sargasso Sea	350-500 m	unfiltered	8	18	5	16	4	15	3	3	8	Kaiser and Benner (2009)
Sargasso Sea	900	HMW DOM	10	18	7	16	8	14	5	1	0	McCarthy et al. (1996)
Gulf of Mexico	750	HMW DOM	11	18	7	16	7	12	5	1	0	McCarthy et al. (1996)
Deep ocean, 1000-4300 m												
North Pacific	2000-4000 m	unfiltered	11	10	3	27	3	20	0	6	9	Kaiser and Benner (2009)
North Pacific	4000 m	HMW DOM	10	15	12	18	5	10	1	2	1	McCarthy et al. (1996)
Chukchi Sea	1000 m	unfiltered	11	5	5	30	2	13	1	6	19	Davis and Benner (2005)
Sargasso Sea	1360-4300 m	unfiltered	11	9	6	22	6	10	2	5	13	Kaiser and Benner (2009)
Sargasso Sea	2400 m	HMW DOM	13	17	8	20	5	18	3	1	nd	McCarthy et al. (1996)

611 [a]Most sampling sites were shallower than 200 m.
612 [b]Samples collected <300 m were GF/F filtered and samples collected >300 m were unfiltered.

613 **Figure Legends**

614

615 Figure 1: Temperature profiles at the Atlantic and Arctic sampling sites. Seawater for 32 day incu-
616 bations was collected at 10 and 80 m depth at the Atlantic and Arctic stations, respectively (indicat-
617 ed by a ○).

618

619 Figure 2: Bacterial abundance and DOC concentration. The data points and error bars represent
620 means and standard deviations calculated from triplicate measurements. ● represents Atlantic
621 treatments and Δ represents Arctic treatments. Note the different scales of the axes.

622

623 Figure 3: Concentration and yield of total hydrolyzable neutral sugars (THNS). ● represents Atlan-
624 tic treatments and Δ represents Arctic treatments. Note the different scales of the axes and the bro-
625 ken axes on the plots of the glucose enriched treatments.

626

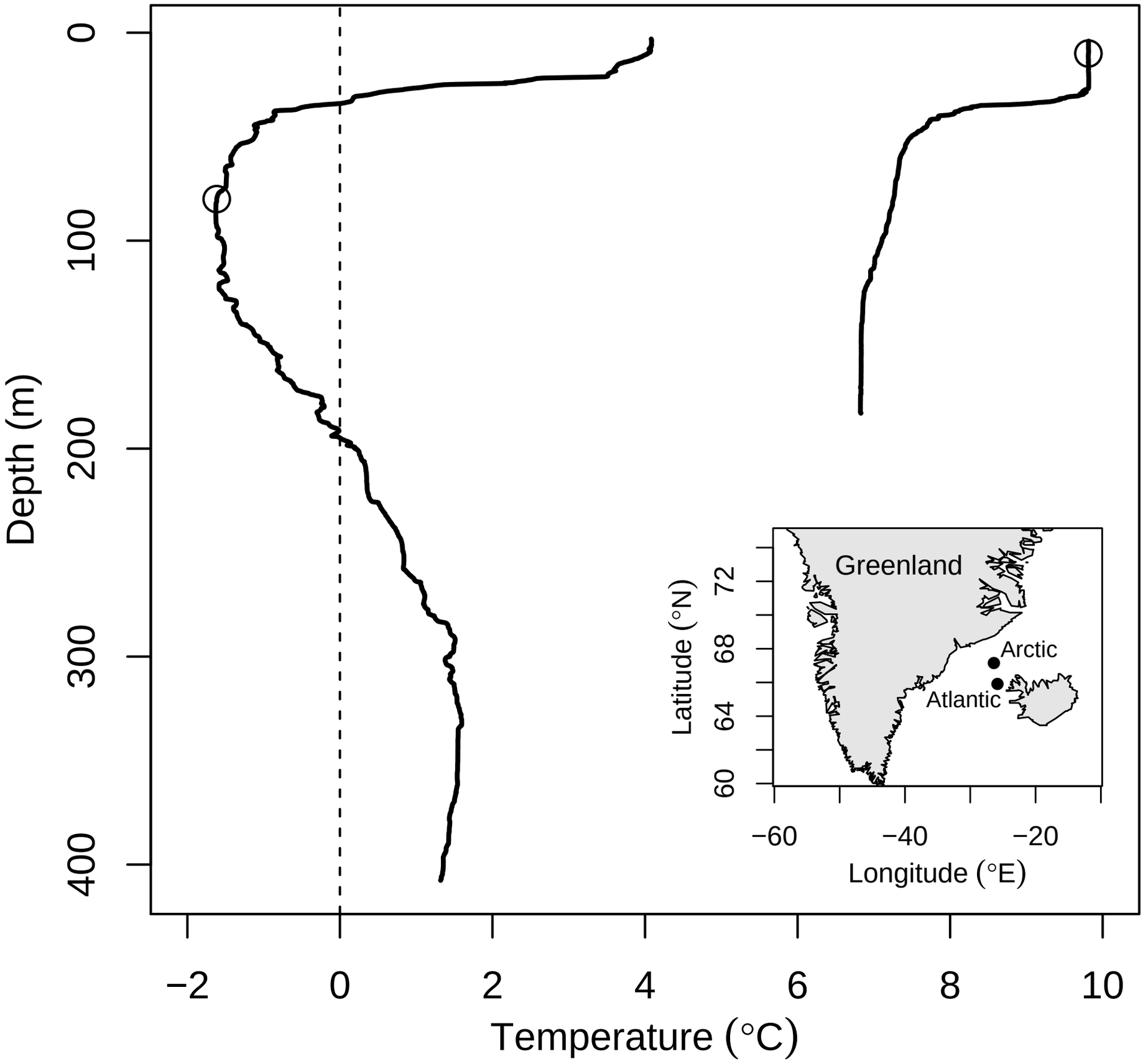
627 Figure 4: Concentration and yield of total hydrolyzable amino acids (THAA). ● represents Atlantic
628 treatments and Δ represents Arctic treatments. Note the different scales of the axes and the broken
629 axis on the yield plot of the artificial treatments.

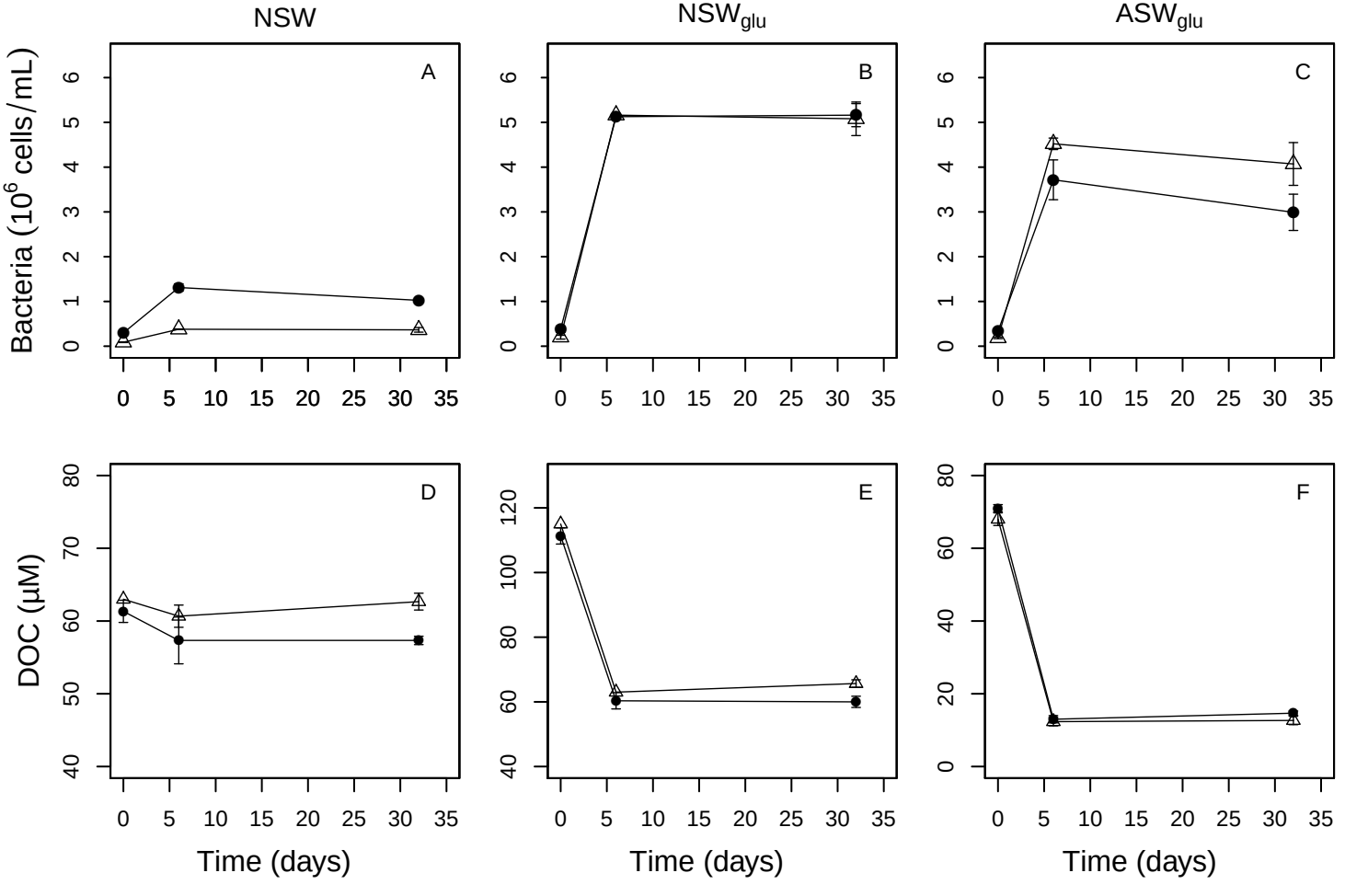
630

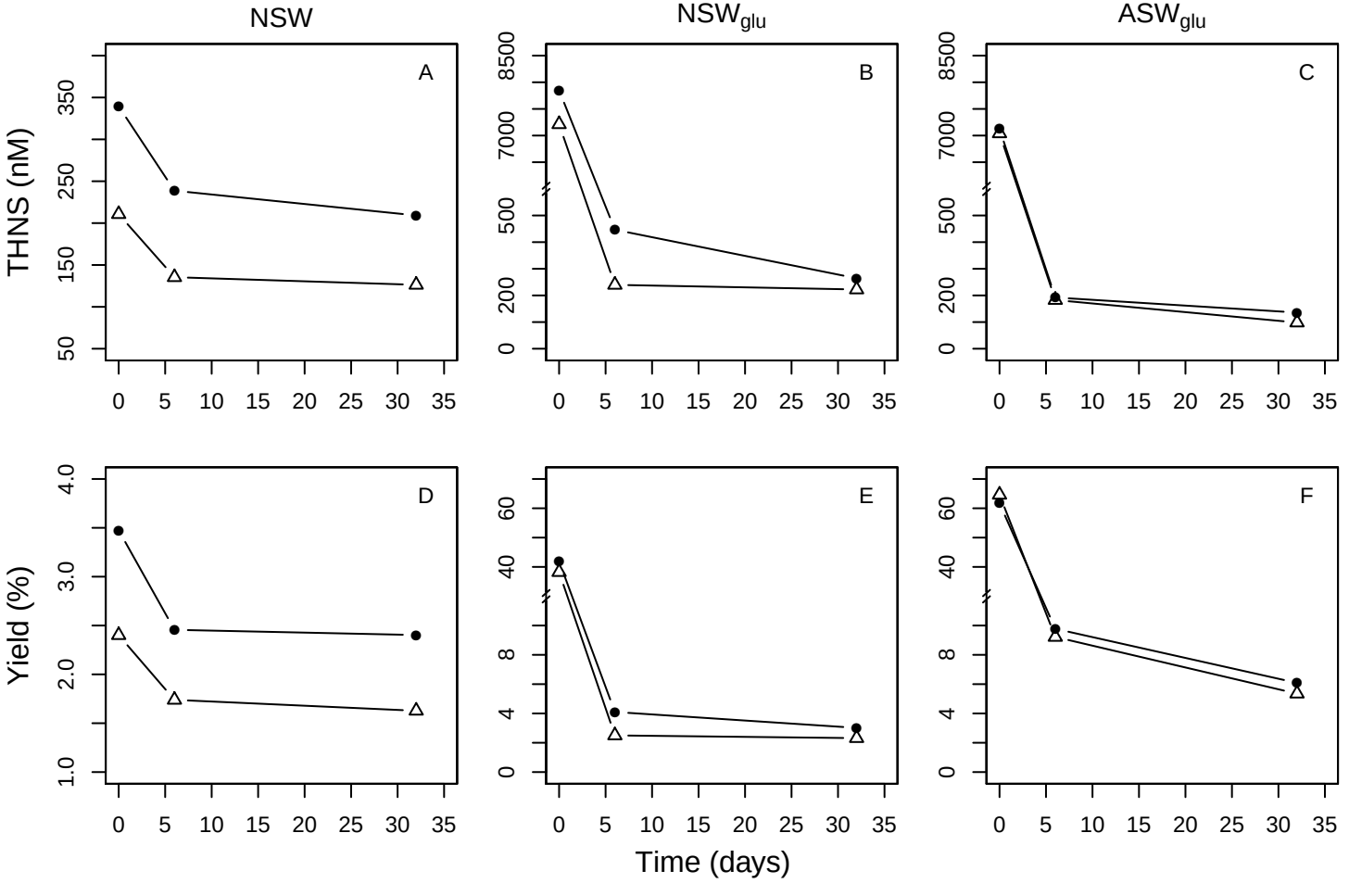
631 Figure 5: Molecular composition of neutral sugars produced by bacteria. Mean and standard devia-
632 tions were calculated from the four glucose enriched samples (n=4). The mean and standard devia-
633 tions of data from the study by Ogawa et al. (2001) were calculated from duplicate measurements of
634 bacterially-produced neutral sugars sampled on day 4 and 7 (n=4).

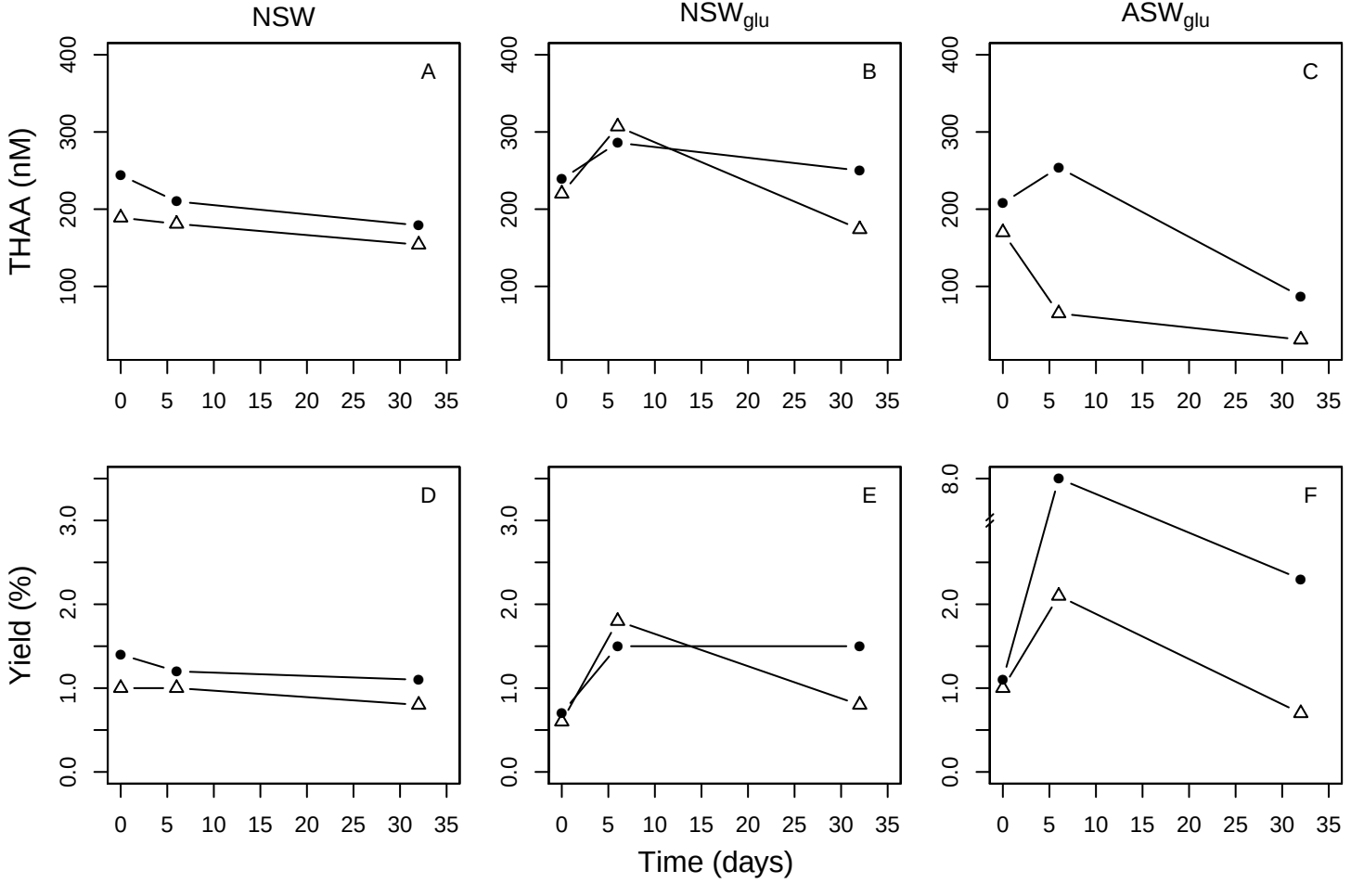
635

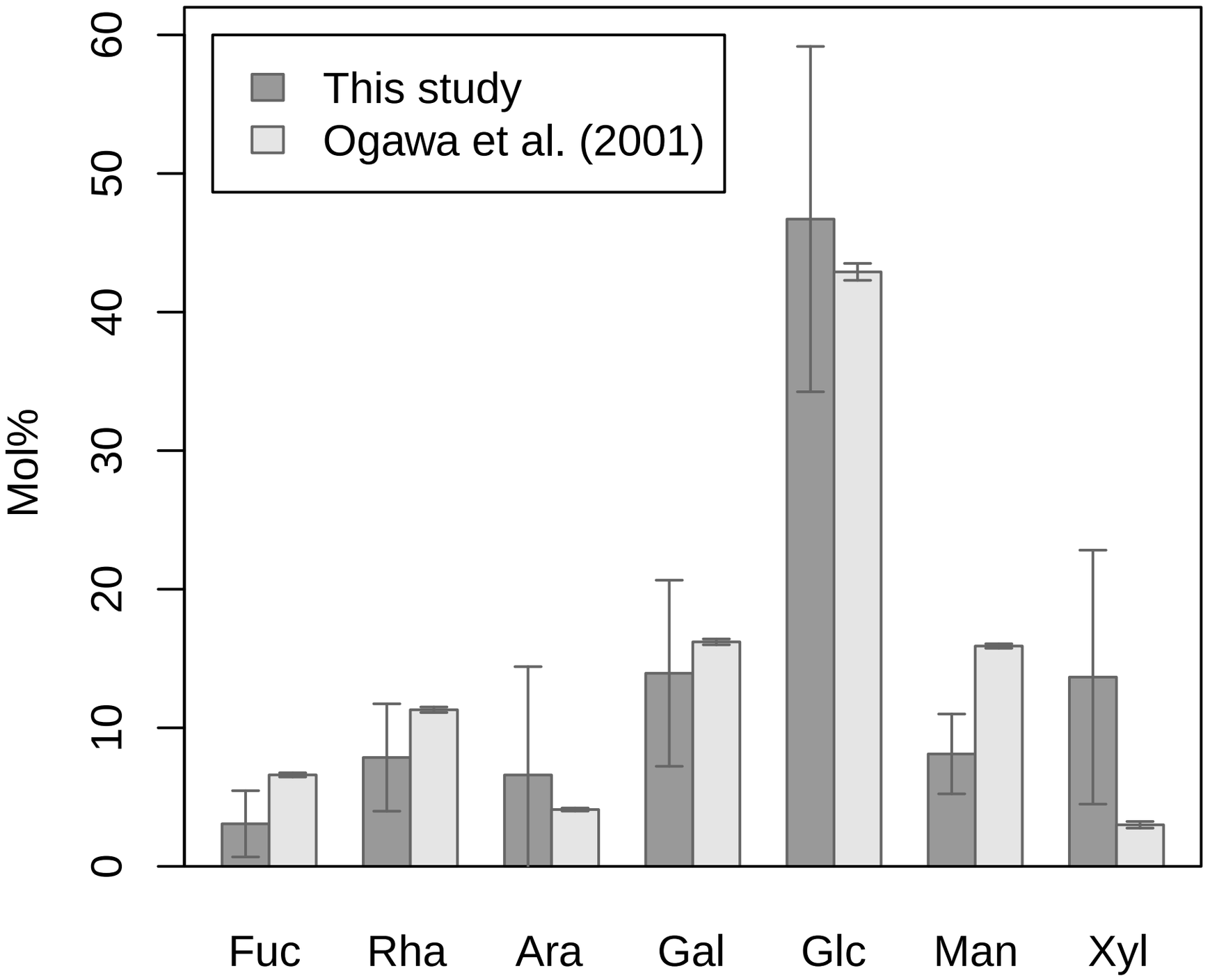
636 Figure 6: Molecular composition of neutral sugars on day 32 of the incubations. Means and stand-
637 ard deviations were calculated from all samples on day 32 (n=6).
638
639 Figure 7: Mean amino acid D/L ratios on day 32 of the incubations. Means and standard deviations
640 were calculated from all samples on day 32 (n=6). Asx did not include the artificial Arctic sample
641 which had a considerably higher D/L ratio than the rest of the samples (indicated with a star).

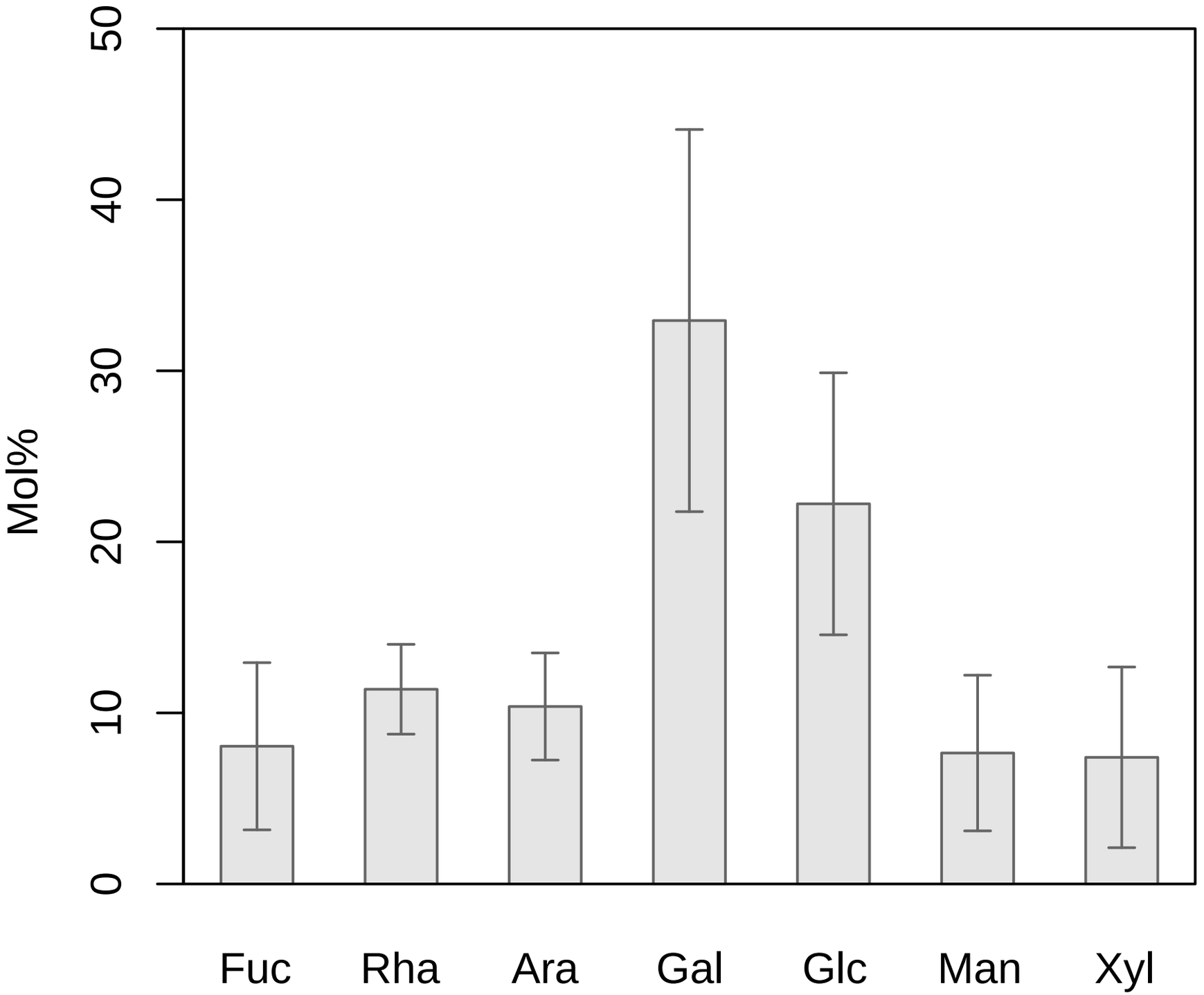

Depth (m)
0
100
200
300
400
−2
0
2
4
6
8
10
Temperature (°C)
Greenland
Arctic
Atlantic
Latitude (°N)
60
64
68
72
−60
−40
−20
Longitude (°E)

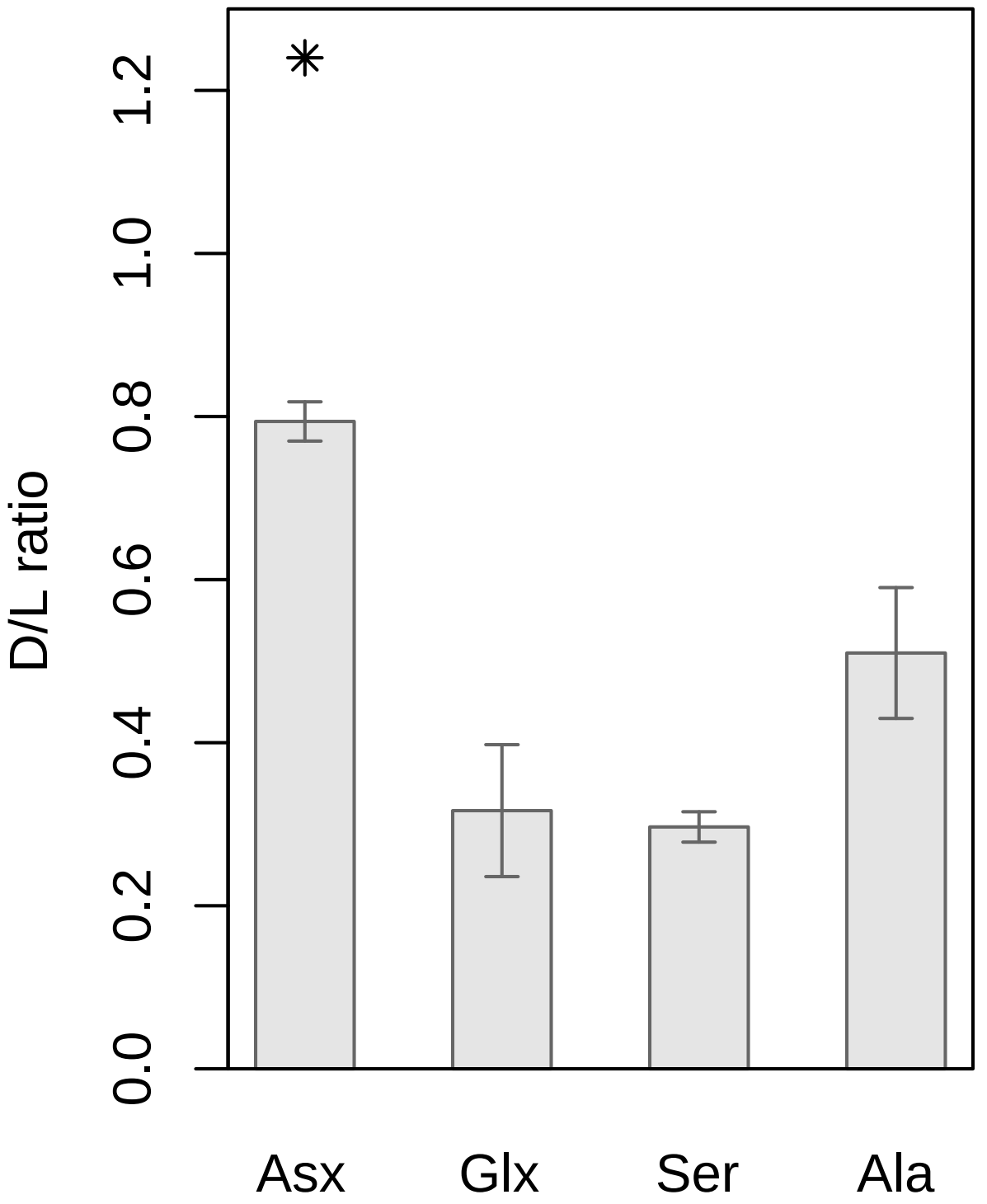

NSW
NSWglu
ASWglu
A
B
C
D
E
F
Bacteria (10^6 cells/mL)
DOC (µM)
Time (days)
Time (days)
Time (days)


NSW
NSWglu
ASWglu
A
B
C
D
E
F
THNS (nM)
Yield (%)
Time (days)
50
150
250
350
0
200
500
7000
8500
1.0
2.0
3.0
4.0
0
4
8
40
60
0
5
10
15
20
25
30
35


NSW
NSWglu
ASWglu
A
B
C
D
E
F
THAA (nM)
Yield (%)
Time (days)
100
200
300
400
0.0
1.0
2.0
3.0
8.0
0
5
10
15
20
25
30
35


This study
Ogawa et al. (2001)
Mol%
0
10
20
30
40
50
60
Fuc
Rha
Ara
Gal
Glc
Man
Xyl


Mol%
0
10
20
30
40
50
Fuc
Rha
Ara
Gal
Glc
Man
Xyl


D/L ratio
0.0
0.2
0.4
0.6
0.8
1.0
1.2
*
Asx
Glx
Ser
Ala