

# Basin-scale variability of microbial methanol uptake in the Atlantic Ocean

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**Abstract.** Methanol is a climate active gas and the most abundant oxygenated volatile organic compound (OVOC) in the atmosphere and seawater. Marine methylotrophs are aerobic bacteria that utilise methanol from seawater as a source of carbon (assimilation) and/or energy (dissimilation). A few spatially limited studies have previously reported methanol oxidation rates in seawater; however the basin-wide ubiquity of marine microbial methanol utilisation remains unknown. This study uniquely combines seawater <sup>14</sup>C labelled methanol tracer studies with 16S rRNA pyrosequencing to investigate variability in microbial methanol dissimilation and known methanol utilising bacteria throughout a meridional transect of the Atlantic Ocean between 47° N to 39° S. Microbial methanol dissimilation varied between 0.05–1.68 nmol l<sup>-1</sup> h<sup>-1</sup> in the top 200 m of the Atlantic Ocean and showed significant variability between biogeochemical provinces. The highest rates of methanol dissimilation were found in the northern subtropical gyre (average 0.99±0.41 nmol l<sup>-1</sup> h<sup>-1</sup>), which were up to eight times greater than other Atlantic regions. Microbial methanol dissimilation rates displayed a significant inverse correlation with heterotrophic bacterial production (determined using <sup>3</sup>H-leucine). Despite significant depth stratification of bacterial communities, methanol dissimilation rates showed much greater variability between oceanic provinces compared to depth. There were no significant differences in rates between samples collected under light and dark environmental conditions. The variability in the numbers of SAR11 (16S rRNA gene sequences) were estimated to explain approximately 50% of the changes in microbial methanol dissimilation rates. We estimate that SAR11 cells in the Atlantic Ocean account for between 0.3-59 % of the rates of methanol dissimilation in Atlantic waters, compared to <0.01-2.3 % for temperate coastal waters. These results make a substantial contribution to our current knowledge and understanding of the utilisation of methanol by marine microbial communities, but highlight the lack of understanding of *in situ* methanol production mechanisms.

## 1. Introduction

Methanol is the most abundant oxygenated volatile organic compound (OVOC) in the background troposphere where it acts as a climate active gas, influencing the oxidative capacity of the atmosphere, concentrations of ozone and hydroxyl radicals (Carpenter et al.,

2012). Methanol has been shown to be ubiquitous in waters of the Atlantic Ocean ranging between <27–361 nM (Beale et al., 2013; Williams et al., 2004; Yang et al., 2013; Yang et al., 2014). Our knowledge of the sources and sinks of methanol is limited and often lacks consensus. For example, recent eddy covariance flux estimates demonstrated a consistent flux of atmospheric methanol into the surface waters of a meridional transect of the Atlantic Ocean (Yang et al., 2013). However, along a similar transect, 12 months earlier, Beale et al. (2013) calculated that the Atlantic Ocean represents an overall source of methanol to the atmosphere (3 Tg yr<sup>-1</sup>), which was largely attributable to an efflux from the North Atlantic gyre; where surface concentrations were as high as 361 nM. Wet deposition from rainwater has also recently been suggested to represent a supply of methanol to the ocean (Felix et al., 2014).

Although *in situ* marine photochemical production of methanol has previously been found to be insignificant (Dixon et al., 2013), there is thought to be a substantial unidentified biological source of methanol in seawater (Dixon et al., 2011a). Biological production by phytoplankton and during the breakdown of marine algal cells are possible sources (Heikes et al., 2002; Nightingale, 1991; Sieburth and Keller, 1989). Recent laboratory culture experiments suggest that methanol is produced by a wide variety of phytoplankton including cyanobacteria (*Prochlorococcus marinus*, *Synechococcus* sp. and *Trichodesmium erythraeum*) and Eukarya (*Emiliania huxleyi*, *Phaeodactylum tricornutum* and *Nannochloropsis oculata*, *Dunaliella tertiolecta*) (Mincer and Aicher, 2016, Halsey et al., 2017). The mechanisms of *in situ* methanol production and their regulation remains largely unknown, although Halsey et al. (2017) reported light-dependent rates of methanol production in cultures of the marine green flagellate *Dunaliella tertiolecta* (cell size of 10–12 µm).

Methylophilic bacteria are capable of utilising one-carbon compounds including methanol as their sole source of energy (methanol dissimilation) and carbon (methanol assimilation). Methylophilic bacteria are widespread in terrestrial and aquatic systems (Kolb, 2009), but research into these bacteria in marine environments is still at an early stage. Traditionally, methylophilic bacteria were thought to utilise methanol dehydrogenase (MDH encoded by *mdhA*, McDonald and Murrell, 1997) to metabolise methanol to formaldehyde, with further oxidation to CO<sub>2</sub> or incorporation of carbon into biomass (Chistoserdova, 2011; Chistoserdova et al., 2009). However, recent progress in this field has resulted in the discovery of the *xoxF* gene, encoding an alternative MDH (Wilson et al., 2008) and

seemingly present in all known gram-negative methylotrophs to date (Chistoserdova, 2011; Chistoserdova et al., 2009). The presence of methylotrophs in seawater has been confirmed using a range of molecular approaches including functional gene primers, stable isotope probing and metaproteomics (Dixon et al., 2013; Grob et al., 2015; Neufeld et al., 2008; Neufeld et al., 2007; Taubert et al., 2015). There are also bacterial cells that utilise methanol and other C<sub>1</sub> compounds for the production of energy but not biomass e.g. SAR11 for which Sun et al. (2011) proposed the new term ‘methylovores’, distinct from true methylotrophs which use C<sub>1</sub> compounds as sources of carbon and energy.

Limited studies of microbial methanol assimilation in the Atlantic Ocean have previously shown rates up to 0.42 nmol l<sup>-1</sup> h<sup>-1</sup> in recently upwelled coastal waters of the Mauritanian Upwelling (Dixon et al., 2013). However, open ocean waters of the Atlantic were substantially lower ranging between 0.002–0.028 nmol l<sup>-1</sup> h<sup>-1</sup> (Dixon et al., 2013). Microbial methanol dissimilation rates are generally up to 1000-fold higher than rates of assimilation; ranging between 0.70–11.2 and <0.001–0.026 nmol l<sup>-1</sup> h<sup>-1</sup> respectively for coastal waters (Sargeant et al., 2016; Dixon et al., 2011b). Methanol dissimilation rates ranging between 0.08–6.1 nmol l<sup>-1</sup> h<sup>-1</sup> have also been found in open ocean Atlantic waters (Dixon et al., 2011a). However, despite the ubiquity of methanol in seawater, the spatial extent or quantification of microbial methanol utilisation for energy production on a basin scale has not been previously investigated. Therefore, the objective of this research was to simultaneously characterise the spatial variability in microbial methanol dissimilation rates (at depths to 200 m) and in microbial community groups throughout contrasting biogeochemical regions of the Atlantic Ocean. This study represents the first basin-wide approach to investigating methanol as a source of reducing power and energy for microbes.

## **2. Materials and Methods**

### *2.1. Sampling strategy*

Sampling was carried out during an Atlantic Meridional Transect (AMT) (<http://www.amt-uk.org>). The research cruise (JC039, RRS James Cook, 13/10/09–01/12/09) departed from Falmouth, UK (50.15° N, 05.07° W) and arrived in Punta Arenas, Chile (53.14° S, 70.92° W). Water samples were collected daily from pre-dawn (97, 33, 14 and 1 % photosynthetically active radiation (PAR) equivalent depths and 200 m) and solar noon (97

1 %) conductivity-temperature-depth (CTD) casts. The PAR equivalent depths were 5 m, 10-31  
2 m, 15-54 m and 38-127 m for the 97, 33, 14, 1 % light levels respectively and typically varied  
3 with oceanic province. The pre-dawn and solar noon sampling periods were approximately  
4 45-65 nautical miles apart (sampling locations are shown in Fig. 1). The Atlantic Ocean was  
5 divided into five oceanic provinces, following the approach of Dixon et al. (2013), according  
6 broadly to chlorophyll *a* concentrations ( $<0.15 \text{ mg m}^{-3}$  gyre regions,  $>0.15 \text{ mg m}^{-3}$  temperate  
7 or upwelling regions, Fig. 1) with the northern gyre sub-divided into northern subtropical  
8 gyre (NSG) and northern tropical gyre (NTG). Measurements of the concentration of  
9 methanol in seawater (Beale et al. 2013) and of methanol assimilation rates (Dixon et al.  
10 2013) made during this transect have been reported previously.

## 12 2.2. Microbial methanol uptake

13 The oxidation of methanol to  $\text{CO}_2$  (dissimilation) was determined using  $^{14}\text{C}$ -labelled  
14 methanol (American Radiolabelled Chemicals Inc, Saint Louis, MO, USA) seawater  
15 incubations as previously described in Dixon et al. (2011b). Seawater samples of 1 ml were  
16 incubated with  $\sim 10 \text{ nM}$  (final concentration)  $^{14}\text{C}$ -labelled methanol to measure rates of  
17 microbial methanol dissimilation. Seawater methanol concentrations ranged between 48-361  
18 nM (Beale et al., 2013) thus the radiotracer additions represent 3-21 % of *in situ*  
19 concentrations in Atlantic waters. Incubations were conducted in triplicate, with 'killed'  
20 controls (5 % trichloroacetic acid, TCA, final concentration), at *in situ* temperatures and in  
21 the dark. Incubation temperatures were determined by the sea surface temperature recorded  
22 by the corresponding CTD casts. Sample counts of  $^{14}\text{CO}_2$ , captured in the precipitate as  
23  $\text{Sr}^{14}\text{CO}_3$  ( $\text{nCi ml}^{-1} \text{ h}^{-1}$ ), were divided by the total  $^{14}\text{CH}_3\text{OH}$  added to the sample ( $\text{nCi ml}^{-1}$ ) to  
24 calculate the apparent rate constants,  $k$  ( $\text{h}^{-1}$ ).

26 The incorporation of methanol carbon into microbial biomass (assimilation) was determined  
27 using sample volumes of 320 ml to increase the total sample counts (Dixon et al., 2011b)  
28 following procedures outlined in Dixon et al. (2011b, 2013). Filter sample counts were  
29 divided by the total  $^{14}\text{CH}_3\text{OH}$  added to the sample ( $\text{nCi ml}^{-1}$ ) to calculate the apparent rate  
30 constants,  $k$  ( $\text{h}^{-1}$ ). For both methanol assimilation and dissimilation, the specific activity of  
31  $^{14}\text{C}$ -labelled methanol ( $57.1 \text{ mCi mmol}^{-1}$ ) was multiplied by the apparent rate constants to  
32 calculate rates of microbial methanol uptake ( $\text{nmol l}^{-1} \text{ h}^{-1}$ ) following the approach of Dixon et

al. (2013). Evaluation of control samples suggests that  $\leq 0.3$  % of the added  $^{14}\text{CH}_3\text{OH}$  is recovered on the filters and  $\leq 2$  % in the resultant precipitate for methanol assimilation and dissimilation respectively.

### 2.3. Bacterial leucine incorporation

Rates of bacterial leucine incorporation were measured using the incorporation of  $^3\text{H}$ -leucine into bacterial protein in seawater samples using the method described by Smith and Azam (1992). A final concentration of 25 nM (6.8  $\mu\text{l}$ ) of  $^3\text{H}$ -leucine (calculated using the specific activity of 161 Ci  $\text{mmol}^{-1}$ , concentrations 1 mCi  $\text{ml}^{-1}$ , American Radiolabelled Chemicals Inc, Saint Louis, MO, USA) was incubated with 1.7 ml seawater samples. Incubations were conducted in triplicate with 'killed' controls (5 % TCA, final concentrations), at *in situ* temperature and in the dark.

### 2.4. Bacterial community composition

Seawater samples of approximately twenty litres were collected for bacterial DNA analysis from 97, 33, 1 and  $<1$  % (200 m) PAR equivalent depths during pre-dawn CTD casts only. Samples were filtered through 0.22  $\mu\text{m}$  Sterivex polyethersulfone filters (Millipore, Watford, UK) using a peristaltic pump. Filters were incubated with 1.6 ml of RNA Later (Life Technologies, to preserve samples during shipment) overnight at 4° C, after which the RNA Later was removed. Filters were stored immediately at -80° C before being shipped back to the UK on dry ice and subsequently stored at -20 °C.

Bacterial DNA was extracted from filters using a modified phenol:chloroform:isoamyl alcohol extraction method as previously described in Neufeld et al. (2007). Extracted DNA was cleaned using Amicon ultra-0.5 centrifugal filter devices (Millipore) to remove any RNA Later residue. The 16S rRNA gene primers 341F (Muyzer et al., 1993) and 907R (Muyzer et al., 1998) were used for PCR amplification (32 cycles) with an annealing temperature of 55° C. Purification of PCR products from agarose gels was conducted using the QIAquick gel extraction kit (Qiagen, Crawley, UK) before being sent to Molecular Research LP (MR DNA, <http://www.mrdnalab.com>) for 454 pyrosequencing using the GS-flx platform.

The 16S rRNA gene sequences were depleted of barcodes and primers, and then sequences less than 200 bp, with ambiguous bases or with homopolymer runs exceeding 6 bp, were removed. Sequences were de-noised and chimeras removed. After the removal of singleton sequences, operational taxonomic units (OTUs) were defined at 97 % 16S rRNA gene identity using Quantitative Insights Into Microbial Ecology (QIIME, <http://qiime.org>, Caporaso *et al.* 2010). The OTUs were assigned taxonomically using BLASTn (Basic Local Alignment Search Tool, NCBI) against the Silva database (<http://www.arb-silva.de>). Sequences were randomly re-sampled to the lowest number of sequences per sample (386 sequences per DNA sample) to standardise the sequencing effort.

### 3. Results

#### 3.1. Microbial methanol dissimilation

##### 3.1.1 Surface

Pre-dawn surface rates of microbial methanol dissimilation ranged between 0.05–1.49 nmol l<sup>-1</sup> h<sup>-1</sup> throughout the transect of the Atlantic Ocean (Fig. 2a). Maximum variability in surface rates of methanol dissimilation (average of  $0.96 \pm 0.45$  nmol l<sup>-1</sup> h<sup>-1</sup>, n=10) were observed north of 25° N in NT and NSG regions. At the southern limit of the NSG, rates of methanol dissimilation decreased sharply from 1.48 to 0.34 nmol l<sup>-1</sup> h<sup>-1</sup>. Generally, surface rates continued to decrease in a southward direction throughout the NTG and EQU regions, reaching a minimum of 0.05 nmol l<sup>-1</sup> h<sup>-1</sup> in Equatorial upwelling waters. Interestingly, surface rates started to gradually increase to 0.39 nmol l<sup>-1</sup> h<sup>-1</sup> in waters of the oligotrophic SG, before declining to 0.18 nmol l<sup>-1</sup> h<sup>-1</sup> in the ST area. Methanol dissimilation rates determined at pre-dawn (dark) generally exhibited a similar latitudinal pattern to those from solar noon (light). Rates south of 25° N (NTG, EQU, SG, ST) showed a significant, almost 1:1 relationship, between light (solar noon, y) and dark (pre-dawn, x) *in situ* sampling conditions ( $y=1.06x$ ,  $r=0.6240$ ,  $n=13$ ,  $P<0.05$ ), with most variability between results from light versus dark sampling occurring north of 25° N in NT and NSG provinces. This is most likely a reflection of these waters exhibiting the greatest spatial variability, as the pre-dawn and midday stations were typically 55 nautical miles apart.

##### 3.1.2 Depth distributions

The average rates of methanol dissimilation with depth are shown in Fig. 3a for each oceanic province. Rates varied between 0.05–1.68 nmol l<sup>-1</sup> h<sup>-1</sup>, but showed no consistent statistically significant trend with depth. However, clear differences were observed in microbial methanol dissimilation in the top 200 m between contrasting provinces in the Atlantic Ocean; where NSG ≥ NT > SG ≈ ST ≥ NTG > EQU. The highest rates of methanol dissimilation in the top 200 m were observed in the most northern latitudes (0.22–1.50 and 0.15–1.68 nmol l<sup>-1</sup> h<sup>-1</sup> for NT and NSG respectively), consistent with surface trends (Fig. 2a). A strong decrease was observed between the NSG (0.99 ± 0.41 nmol l<sup>-1</sup> h<sup>-1</sup>) and the NTG (0.18 ± 0.04 nmol l<sup>-1</sup> h<sup>-1</sup>) regions. However, rates of microbial methanol dissimilation determined in the oligotrophic waters of the NTG (0.18 ± 0.04 nmol l<sup>-1</sup> h<sup>-1</sup>) and SG (0.24 ± 0.12 nmol l<sup>-1</sup> h<sup>-1</sup>) regions were comparable with rates in the ST region (0.20 ± 0.05 nmol l<sup>-1</sup> h<sup>-1</sup>), with the EQU exhibiting the lowest average rates of 0.11 ± 0.03 nmol l<sup>-1</sup> h<sup>-1</sup>.

Overall, latitudinal trends in depth profiles for methanol dissimilation rates mirrored those found in surface waters. Surface microbial methanol dissimilation rates determined from pre-dawn (x) water were compared to those from 200 m (y), which are in permanent darkness (the deepest 1 % PAR equivalent depth of 175 m was found was in the SG at ~19.50°S) and also showed a ~1:1 relationship (y=0.967x, r=0.9237, n=19, P<0.001).

### 3.2. Bacterial leucine incorporation rates

#### 3.2.1 Surface

Rates of bacterial leucine incorporation (BLI) varied between 2.9–25.2 pmol l<sup>-1</sup> h<sup>-1</sup> in the pre-dawn surface waters of the Atlantic transect (Fig. 2b). On average, surface rates of BLI were highest in the relatively more productive EQU upwelling region (18.3 ± 4.8 pmol l<sup>-1</sup> h<sup>-1</sup>), and lowest in the northern sub-tropical gyre (NSG, 5.2 ± 2.3 pmol l<sup>-1</sup> h<sup>-1</sup>). Surface rates of BLI averaged 7.8 ± 2.3 pmol l<sup>-1</sup> h<sup>-1</sup> and 7.7 ± 2.4 pmol l<sup>-1</sup> h<sup>-1</sup> in the NTG and SG regions respectively. The one measurement of BLI in the ST suggested much higher rates (25.2 pmol l<sup>-1</sup> h<sup>-1</sup>) than previously determined during the transect, even when compared to the NT region (9.9 ± 3.9 pmol l<sup>-1</sup> h<sup>-1</sup>). Pre-dawn (dark) rates of BLI generally exhibited a similar latitudinal pattern to those from solar noon (light), with more variability between light and dark sampling observed in the waters of the productive EQU region. Bacterial rates of leucine incorporation determined from samples collected at solar noon (y) were approximately 20%

less than those determined at pre-dawn ( $y=0.7815x$ ,  $r=0.7288$ ,  $n=22$ ,  $P<0.001$ ), perhaps reflecting a degree of light inhibition of heterotrophic bacterial production.

### 3.2.2 Depth profiles

Rates of bacterial leucine incorporation varied between 0.5–60.2  $\text{pmol l}^{-1} \text{ h}^{-1}$  throughout the top 200 m of the water column. In the sunlit depths (97-1 % PAR) generally BLI rates followed the pattern  $\text{EQY} > \text{NTG} \approx \text{SG} > \text{NT} > \text{NSG}$  (excluding the outliers of 60.2 and 31.3  $\text{pmol l}^{-1} \text{ h}^{-1}$  observed for the NSG at 14 % PAR from two depth profiles in this province). This trend differs slightly from that observed for surface only data due to sub-surface (1-14 % PAR) maxima observed in both the north and south oligotrophic gyres (NSG, NTG, SG). In the NT, NTG and EQU provinces, BLI rates were generally higher in sunlit depths compared to the dark at 200 m (Fig. 3b). However, there were no statistical differences between the provinces for rates of BLI determined at 200 m.

## 3.3. Bacterial community composition

### 3.3.1 Surface

The total number of operational taxonomic units (OTUs) sequenced throughout the Atlantic Ocean varied between 91–207. Overall, the largest contributors to surface bacterial communities were *Prochlorococcus* and SAR11 16S rRNA gene sequences (Fig. 5a); which together accounted for between 21-60 % of all OTUs (21% in the SG and 60% in the NSG). These bacteria typically numerically dominate surface waters of nutrient depleted oceanic regions e.g. Gomez-Pereira et al. (2013). The numbers of *Prochlorococcus*, determined via flow cytometry, for the same surface samples from which 16S rRNA genes were amplified range between  $0.81 \times 10^5$  for the NTG region and  $3.10 \times 10^5$  cells  $\text{ml}^{-1}$  for the EQU region (see Table 2 for summary). *Prochlorococcus* 16S rRNA gene sequences contributed an average of  $28 \pm 12$  % of the community composition of surface samples throughout the surface Atlantic Ocean. Numbers of SAR11 16S rRNA gene sequences contributed a maximum of 24 % to the total 16S rRNA gene sequences for the NSG region, and overall contributed an average of  $11 \pm 3$  % to the bacterial community in surface waters of the Atlantic Ocean. There was a clear shift between surface bacterial communities in the two



northern gyre provinces with *Prochlorococcus* and SAR11 16S rRNA gene sequences decreasing from the NSG to the NTG region (59 and 33 % of total 16S rRNA gene sequences respectively). *Oceanspirillales* and *Flavobacteriales* 16S rRNA gene sequences contributed approximately double the amount (compared to the total 16S rRNA sequences) in the NTG compared to the NSG region (25 and 12 % respectively).

Microbial communities of the surface waters of the NT, NSG and EQU provinces were dominated by *Prochlorococcus*, *Alteromonadales* and SAR11, together representing between 64–72 % of 16S rRNA gene sequences. These orders were less dominant in the more oligotrophic waters of the NTG and SG, accounting for 43 % and 34 % of 16S rRNA gene sequences respectively. In these oligotrophic regions (NTG and SG) microbial communities appear less dominated by a few orders, with a more even spread of bacterial orders contributing to the community composition (Fig. 5a).

### 3.3.2 Depth profiles

The largest contributors to bacterial communities at the 33 % PAR depths were, like surface communities, *Prochlorococcus* and SAR11 16S rRNA gene sequences (Fig. 5b). Together they accounted for between 47-70 % of all OTUs, with the minimum and maximum contributions in the SG and EQU provinces respectively. If the proportion of sequences contributing individually <5% were included then collectively they accounted for between 69-91 % of all 16S rRNA gene sequences. The main differences between the surface and 33% PAR equivalent depth (14-31 m) are the increasing dominance of the cyanobacteria *Prochlorococcus*, and the decrease in relative contribution of *Alteromonadales* at 33% PAR depths, particularly in the NT region.

In the darker 1 % PAR depths (59-127 m) *Prochlorococcus* and SAR11 16S rRNA gene sequences (Fig. 5c) still accounted for between 32-65 % of all OTUs, with the minimum and maximum contributions in the SG and EQU respectively. With the addition of sequences for each Order contributing <5 % to the total 16SrRNA gene sequences, these three categories accounted for 60-81% of all 16S rRNA gene sequences retrieved throughout each of the regions sampled. Two notable differences at this light level in the SG region compared to the other provinces are the 12 % contribution made by the Order III *Incertae Sedis* which belongs to the *Bacteroidetes* class, and the relative reduction in contribution made by *Prochlorococcus* (11 % compared to an Atlantic average of  $27 \pm 15$  % at 1 % PAR). However,

the latter trend is not confirmed in the cell numbers of *Prochlorococcus* determined via flow cytometry (Table 2).

In the permanent dark of 200 m, SAR11 bacteria contributed between 14-29 % in northern regions, which contrasted to only 4-5 % in the EQU and SG provinces. The SAR324 clade contributed 8-11 % in the northern gyre. Both uncultivated bacteria and those that individually comprised <5 % contributed relatively highly to the OTUs (10-36 % and 21-33 % respectively). These two groupings together with the SAR11 and SAR324 make up 83-89 % in northern regions and between 37-56 % in the SG and EQU provinces respectively. For the EQU region the *Alteromonadales* order is also significant at 25 % (which collectively comprise 81 % of all OTUs for EQU), whilst for the SG the cyanobacteria *Prochlorococcus* and *Synechococcus* comprise 52 % (which collectively comprise 89 % of all OTUs for SG).

## 4. Discussion

### 4.1. Basin scale variability in biological methanol uptake

Maximum rates of methanol dissimilation in the Atlantic Ocean were recorded in the NSG province at 33 % PAR light depth (25 m,  $1.68 \text{ nmol l}^{-1} \text{ h}^{-1}$ , Fig. 2 and Fig. 4a). An overview of the variation in rates of methanol dissimilation to  $\text{CO}_2$  throughout the top 200 m of the water column in the Atlantic Ocean is shown in Fig. 4a, which illustrates sub-surface maxima in northerly latitudes. However, no statistically significant differences were found between rates of methanol dissimilation in the euphotic zone (97–1 % PAR) compared to the aphotic zone (samples from 200 m) in the NSG ( $t_{\text{NSG}}=2.63$ ,  $t_{20}=2.85$  for  $P<0.01$ ), NTG ( $t_{\text{NTG}}=0.02$ ,  $t_{12}=3.05$  for  $P<0.01$ ), EQU ( $t_{\text{EQU}}=1.01$ ,  $t_{18}=2.88$  for  $P<0.01$ ) and SG regions ( $t_{\text{SG}}=0.88$ ,  $t_{19}=2.88$  for  $P<0.01$ ). This is consistent with a previous study in the north east Atlantic Ocean, which similarly reported no significant variability in methanol dissimilation rates with depth (Dixon and Nightingale, 2012). Nevertheless, greater variability with depth was observed for methanol dissimilation rates from the northern gyre ( $F_{\text{NSG}}=3.22$  where  $F_{3,17}=3.20$ ,  $P\leq 0.05$  and  $F_{\text{NTG}}=5.14$  where  $F_{2,10}=4.10$ ,  $P<0.05$ ). Variability in rates from the euphotic zone were found to be significantly higher than those from 200 m in northern ( $t_{\text{NT}}=3.17$ ,  $t_{20}=2.85$  for  $P<0.01$ ) and southern temperate regions ( $t_{\text{ST}}=5.03$ ,  $t_{10}=3.17$  for  $P<0.01$ ).

Although the highest rates of methanol dissimilation were determined in the NSG, these values were approximately seven times lower than the maxima determined during a seasonal

study of the temperate western English Channel (0.5–11.2 nmol l<sup>-1</sup> h<sup>-1</sup>, Sargeant et al., 2016). Rates determined in the temperate waters of the south Atlantic (0.11–0.45 nmol l<sup>-1</sup> h<sup>-1</sup>) are most comparable to the lowest rates determined during late spring and early summer of ~0.50 nmol l<sup>-1</sup> h<sup>-1</sup> in temperate northern coastal waters (Sargeant et al., 2016). The seasonal study in the western English Channel showed maximum rates of up to 11.2 nmol l<sup>-1</sup> h<sup>-1</sup> during autumn and winter months (Sargeant et al., 2016). The differences in methanol dissimilation rates between the temperate waters of the North (0.83±0.42 nmol l<sup>-1</sup> h<sup>-1</sup>) and South (0.27±0.13 nmol l<sup>-1</sup> h<sup>-1</sup>) Atlantic may therefore reflect seasonal differences between hemispheres i.e. sampling in the NT region occurred during late autumn compared to late spring in the ST region.

Methanol assimilation rates were generally two orders of magnitude lower than dissimilation rates, reaching a maximum of 0.028 nmol l<sup>-1</sup> h<sup>-1</sup> in the top 200m throughout the Atlantic Ocean (Fig. 4b). Rates of methanol assimilation exhibited sub-surface maxima (at 33% PAR equivalent depth) which were particularly evident just north of the Equator (EQU) and in the northern gyre (NSG) of 0.015±0.004 nmol l<sup>-1</sup> h<sup>-1</sup>. These subsurface rates were on average higher than surface values (0.004±0.004 nmol l<sup>-1</sup> h<sup>-1</sup>). Results are similar to findings by Dixon and Nightingale (2012) who also demonstrated sub-surface maxima between 20–30 m in the north east Atlantic. The methanol assimilation rates are shown for direct comparison to dissimilation, but have been previously discussed in more detail in Dixon et al. (2012).

#### 4.2. Bacterial community and productivity

In contrast to microbial methanol dissimilation, rates of bacterial leucine incorporation were lowest in the northern oligotrophic gyre (NSG 5.2 ± 2.3 pmol l<sup>-1</sup> h<sup>-1</sup>, NTG 7.8 ± 2.3 pmol l<sup>-1</sup> h<sup>-1</sup>) reflecting lower microbial activity in these regions of the Atlantic. Surface microbial methanol dissimilation rates exhibited a statistically significant inverse correlation with bacterial leucine incorporation, ( $r = -0.351$ ,  $n = 36$ ,  $P \leq 0.05$ ). This is consistent with findings from a seasonal study in the western English Channel, where surface rates of methanol dissimilation were also inversely correlated to bacterial production (Sargeant et al., 2016). For all the depth data a negative correlation was also found in the NTG, EQU and SG regions ( $r = -0.372$ ,  $n = 52$ ,  $P \leq 0.01$ ), but NT and NSG areas showed methanol dissimilation rates independent of BLI. The productivity of heterotrophic bacteria is generally associated with the concentrations of phytoplankton-derived dissolved organic matter (DOM) e.g.

1 proteins, lipids and carbohydrates which are utilised as sources of energy and carbon (Benner  
2 and Herndl, 2011; Nagata, 2008; Ogawa and Tanoue, 2003). Results from this present study  
3 indicate that in regions of low heterotrophic bacterial production i.e. in the northern Atlantic  
4 Gyre (minimum rate of bacterial leucine incorporation of  $3 \text{ pmol l}^{-1} \text{ h}^{-1}$ ) rates of methanol  
5 dissimilation were relatively higher. In oligotrophic regions, phytoplankton-derived DOM is  
6 scarce, suggesting that those bacteria able to metabolise methanol are using the carbon from  
7 methanol as an alternative source of energy (and to a lesser extent carbon).

8 Although the bacterial community 16S rRNA gene sequence data did not display any clear  
9 patterns with changing biogeochemical province (in contrast to microbial methanol  
10 dissimilation rates), the bacterial community was shown to be depth-stratified throughout the  
11 Atlantic Ocean (Fig. 6a). A non-metric multi-dimensional scale (MDS) plot of a Bray-Curtis  
12 similarity matrix of 16S rRNA gene sequences (Fig. 6a) found bacterial community samples  
13 to cluster into three distinct groupings possibly reflecting light levels: sunlit (97 and 33 %  
14 PAR), minimal light (1 % PAR) and dark (200 m). Bacterial community samples from the  
15 same PAR equivalent depths were found to group together regardless of biogeochemical  
16 province. A larger cluster formed of samples from 97 and 33% PAR is likely to be formed of  
17 bacterial communities originating from the well-mixed surface layer of the water column,  
18 accounting for their similarity in composition. When all environmental parameters were  
19 considered together (including bacterial numbers and BLI) a Euclidean distance matrix non-  
20 metric MDS also demonstrated photic waters (97-1 % PAR) clustered together, and were  
21 significantly different to dark waters from 200m (Fig. 6b). However, no significant  
22 differences were observed between rates of methanol dissimilation determined from the  
23 euphotic zone (samples from 97-1 % PAR equivalent depths) compared to the aphotic zone  
24 (samples from 200 m depth) for gyre and equatorial regions (NSG  $t_{\text{NSG}}=2.63$  ( $t_{20}=2.85$  for  
25  $P<0.01$ ), NTG  $t_{\text{NTG}}=0.02$  ( $t_{12}=3.05$  for  $P<0.01$ ), EQU  $t_{\text{EQU}}=1.01$  ( $t_{18}=2.88$  for  $P<0.01$ ) and SG  
26  $t_{\text{SG}}=0.88$  ( $t_{19}=2.88$  for  $P<0.01$ )) although, clear differences between provinces were evident  
27 (Fig. 6c). This is consistent with results from Dixon and Nightingale (2012) who also found  
28 no significant variation of methanol dissimilation with depth in the north east Atlantic Ocean.  
29 These data suggest that light levels do not have a strong role to play in microbial methanol  
30 dissimilation in waters of the Atlantic, despite the overall bacterial community showing  
31 strong variability with depth (or incident light). Depth-stratification of microbial communities  
32 has been observed previously by Carlson et al. [2004], DeLong et al. [2006] and between  
33 euphotic and aphotic zones in the north western Sargasso Sea (Carlson et al., 2004).

Heywood et al. (2006) suggested that the physical separation of low nutrient surface waters in gyre regions from mixing with more nutrient rich waters below a defined pycnocline, in combination with differing levels of light availability, could partially explain changes in bacterial community composition throughout the water column. Therefore, these results could indicate that methanol dissimilation is limited to specific microbial groups that are present relatively uniformly between the surface and 200m, although more depth variability is shown north of 25°N where rates of methanol dissimilation are the highest and most variable.

#### 4.3. Methanol dissimilation and SAR11

SAR11 cells have been shown to utilise methanol, but only as a source of energy (Sun et al., 2011). The numbers of SAR11 16S rRNA gene sequences exhibited a statistically significant correlation with rates of microbial methanol dissimilation throughout the Atlantic basin ( $r = 0.477$ ,  $n = 20$ ,  $P < 0.05$ ), where the number of SAR11 16S rRNA gene sequences explained approximately half of the spatial variability in rates of methanol dissimilation. It should be noted that this correlation has been made with amplicon ratios, relating to the relative success of SAR11 in the community, rather than with SAR11 cell numbers specifically. In culture, SAR11 cells (strain HTCC1062) have previously been shown to utilise methanol as a source of energy at a rate of  $\sim 5 \times 10^{-20}$  moles cell<sup>-1</sup> h<sup>-1</sup> (Sun et al., 2011), which equates to 2 nmol l<sup>-1</sup> h<sup>-1</sup> (using a culture cell abundance of  $4 \times 10^7$  cells mL<sup>-1</sup>, Sun et al., 2011). SAR11 cells dominate ( $59 \pm 4\%$ ) the low nucleic acid (LNA) fraction of bacterioplankton consistently across the Atlantic Ocean, where typically numbers of LNA range between  $0.2$ - $1.0 \times 10^9$  cells l<sup>-1</sup> (Mary et al., 2006a). Thus estimates of *in situ* SAR11 numbers range between  $0.12$ - $0.59 \times 10^9$  cells l<sup>-1</sup>. This is consistent with estimates from the Sargasso Sea of  $\sim 0.1 \times 10^9$  cells l<sup>-1</sup> (where they are reported to contribute  $\sim 25\%$  of total prokaryotic abundance of  $0.4 \times 10^6$  cells mL<sup>-1</sup>, Malmstrom et al., 2004). Thus, we estimate that SAR11 cells of the Atlantic Ocean could be oxidising methanol at rates between  $5$ - $29.5$  pmol l<sup>-1</sup> h<sup>-1</sup>, which could account for between  $0.3$ - $59\%$  of the rates of methanol dissimilation in surface Atlantic waters.

A seasonal investigation in the western English Channel reported bacterial numbers ranging between  $2.0$ - $15.8 \times 10^5$  cells mL<sup>-1</sup> (Sargeant et al., 2016) which agrees well with data from Mary et al. (2006b,  $2.0$ - $16.0 \times 10^5$  cells mL<sup>-1</sup>). Assuming that SAR11 contribute between  $9$ - $20\%$  of total bacterioplankton (Mary et al., 2006b) suggests SAR11 numbers range between  $0.18$ - $3.16 \times 10^5$  cells mL<sup>-1</sup> at this coastal site. Using the above estimate of  $\sim 5 \times 10^{-20}$  moles cell<sup>-1</sup>

<sup>1</sup> h<sup>-1</sup> for rates of methanol dissimilation in cultured SAR11 cells suggests that SAR11 could oxidise methanol at rates ranging between 0.9-15.8 pmol l<sup>-1</sup> h<sup>-1</sup> in temperate coastal regions. This equates to <0.01-2.3% of microbial community methanol dissimilation rates (0.7-11.2 nmol l<sup>-1</sup> h<sup>-1</sup>, Sargeant et al., 2016). Therefore, we suggest that cells of the SAR11 clade are more likely to make a larger contribution to marine microbial methanol dissimilation in open ocean environments, where alternative sources of carbon are more limited relative to temperate coastal waters. More work is required to add clarity and understanding to the role that SAR11 cells play in marine community methanol dissimilation.

Previously methylotrophic bacteria such as *Methylophaga* sp., *Methylococcaceae* sp. and *Hyphomicrobium* sp. have been identified, using *mxoF* functional gene primers (which encode for the classical methanol dehydrogenase), from the same DNA samples analysed for 16S rRNA genes in this study, from the upper water column of Atlantic Ocean provinces (Dixon et al., 2013). Although numerically very rare (1-11 16S rRNA gene sequences per sample), 16S rRNA gene sequences identified as *Methylophaga* spp., *Methylophaga* sp. DMS021 (EU001861) and uncultured *Methylophaga* sp. (EU031899), were found in each of the Atlantic Ocean provinces in this study (at 97% PAR or 200m depth), consistent with previous identification of *Methlophaga* spp. in these Atlantic provinces using *mxoF* gene cloning in (Dixon et al., 2013). More recently the *xoxF* gene, which encodes an alternative methanol dehydrogenase, has also been found to be widespread in coastal marine environments (Taubert et al., 2015). SAR11 bacteria are thought to contain an Fe alcohol dehydrogenase, which although not specific for methanol, can oxidise methanol (and other short chain alcohols) to formaldehyde which is then thought to be converted to CO<sub>2</sub> by a tetrahydrofolate-linked C1 transfer pathway to produce energy (Sun et al., 2012). Thus it seems likely that both methylotrophic bacteria possessing *mxoF* and/or *xoxF*, together with microbes such as SAR11 (Sun et al., 2011), are largely responsible for the turnover of methanol in seawater.

Members of *Betaproteobacteria*, OM43, have been shown to be potentially important obligate methylotrophs, with cultivated cells of strain HTCC2181 dissimilating 3.5 times more methanol than was assimilated (Halsey et al., 2012). OM43 were not successfully identified in the 16S rRNA sequences in this study, which could be an artefact of the relatively low sequence coverage (386 sequences per sample) leading to this taxon not being

detectable. During a previous coastal study, also analysing 16S rRNA pyrosequence data, in the western English Channel (Sargeant et al., 2016) only a single sequence of the OM43 clade, HTCC2181, was identified. This is a limitation of this type of environmental sequencing effort and should be a consideration in planning any future projects aiming to understand microbial function through process measurements alongside the generation of metagenomic datasets.”

#### 4.4. Marine methanol cycling

Data from this study substantially add to the measurements of microbial methanol dissimilation rates in seawater. This extended spatial coverage clearly demonstrates that methanol dissimilation is a widespread microbial process taking place in light and dark environments throughout the Atlantic Ocean. Dissimilation rates are typically two orders of magnitude greater than assimilation rates across most of the Atlantic Basin. These data suggest that methanol is an important source of energy for microbes. This is particularly true in the northern oligotrophic waters of the Atlantic Ocean, where corresponding *in situ* methanol concentrations range between 148-281 nM (Table 1). What is not clear is the source of methanol in open ocean waters, which is suspected to be biological in nature (Dixon et al., 2011a). Although direct flux estimates suggest that the atmosphere could also act as a source to the ocean (Yang et al, 2013), the magnitude of this flux is insufficient to support the observed rates of microbial methanol consumed by bacteria, and hence is suspected to be a minor contribution (Dixon et al., 2011a). Recent culture studies indicate that *Prochlorococcus* sp., *Synechococcus* sp. and *Trichodesmium* sp. could produce methanol (Mincer and Aicher, 2016, Halsey et al., 2017), but *in situ* production mechanisms are unknown. Further work is needed to fully elucidate and quantify the sources of methanol in marine waters.

## 5. Conclusions

This study reports the first basin-wide understanding of microbial methanol dissimilation rates in seawater. Radiochemical assays have demonstrated active metabolism throughout the top 200 m of the water column, with rates being substantially higher in the northern subtropical Atlantic gyre. Microbial methanol dissimilation rates showed a positive

correlation with the numbers of SAR11 16S rRNA gene sequences, and an inverse relationship with bacterial leucine incorporation. Future work should determine marine methanol sources and understand the relative contribution of various microbial orders to methanol loss processes.

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## Conflict of Interest Statement

The Authors declare no conflict of interest with this manuscript.

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## Figure and Table legends.

**Figure 1.** Remotely sensed MODIS-Aqua chlorophyll *a* composite image of the Atlantic Ocean from November 2009 (image courtesy of NEODAAS). White squares represent sampling points and ovals indicate samples within different oceanic provinces, labelled with province names NT (northern temperate), NSG (northern subtropical gyre), NTG (northern tropical gyre), EQU (equatorial upwelling), SG (southern gyre), ST (southern temperate).

**Figure 2.** Variability in a) microbial methanol dissimilation rates (using the specific activity of  $^{14}\text{CH}_3\text{OH}$ ) and b) bacterial leucine incorporation (BLI), in surface waters of the Atlantic Ocean. Rates were determined from pre-dawn ( $\blacklozenge$  solid line) and solar noon ( $\blacklozenge$  dashed line) CTD casts. Error bars represent  $\pm 1$  s.d. of triplicate samples, dashed vertical lines indicate Atlantic province boundaries.

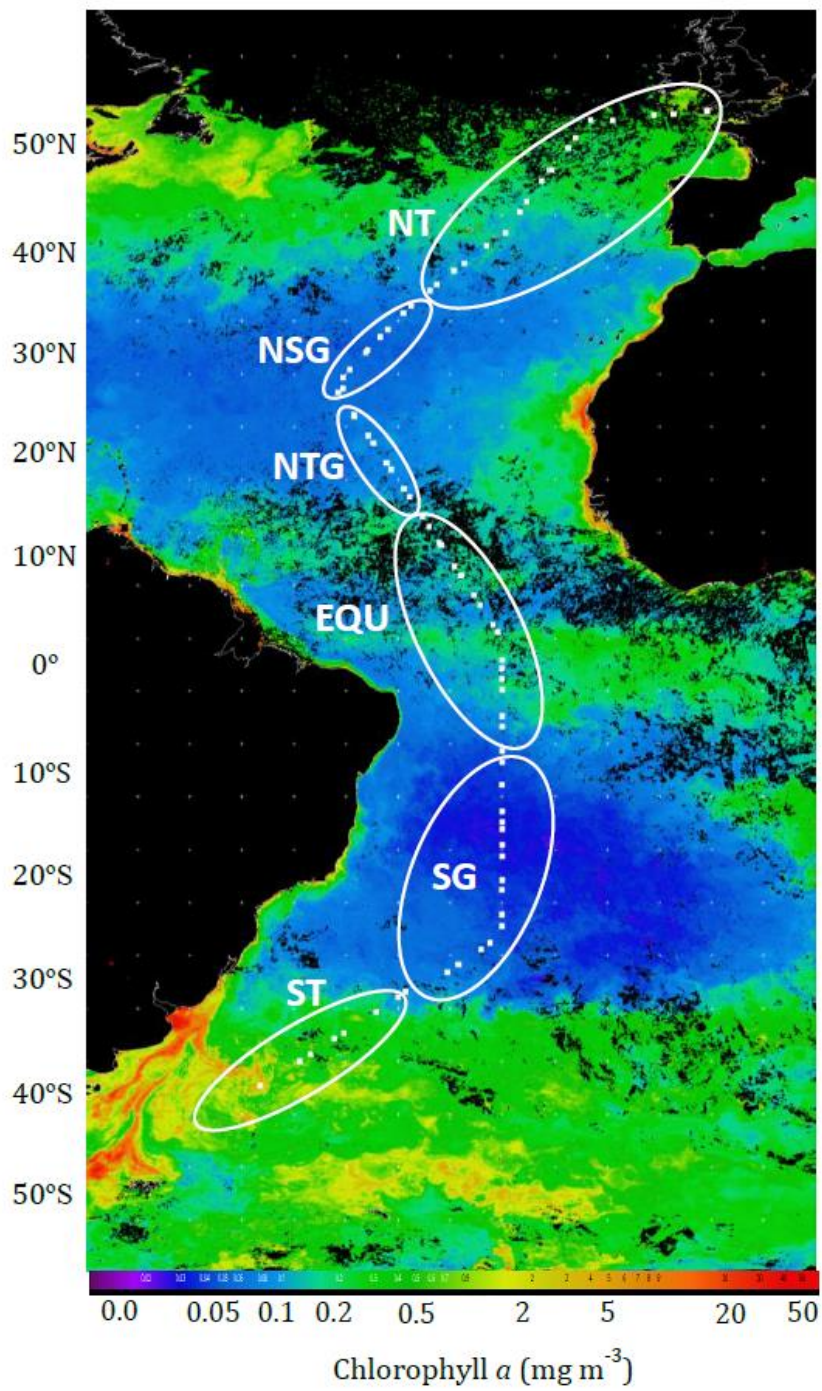
**Figure 3.** Average depth profiles in Atlantic provinces for a) microbial methanol dissimilation (using the specific activity of  $^{14}\text{CH}_3\text{OH}$ ) and b) bacterial leucine incorporation (BLI) in pre-dawn waters. Error bars represent  $\pm 1$  s.d. of variation within the province, province averages derived from NT ( $n = 5$ ), NSG ( $n = 5$ ), NTG ( $n = 3$ ), EQU ( $n = 4$ ), SG ( $n = 5$ ) and ST ( $n = 3$ ), except for BLI where there is no data from the ST.

**Figure 4.** Microbial methanol (a) dissimilation and (b) assimilation rates ( $\text{nmol l}^{-1} \text{h}^{-1}$ ) in the top 200 m of an Atlantic Meridional transect (contour plots).

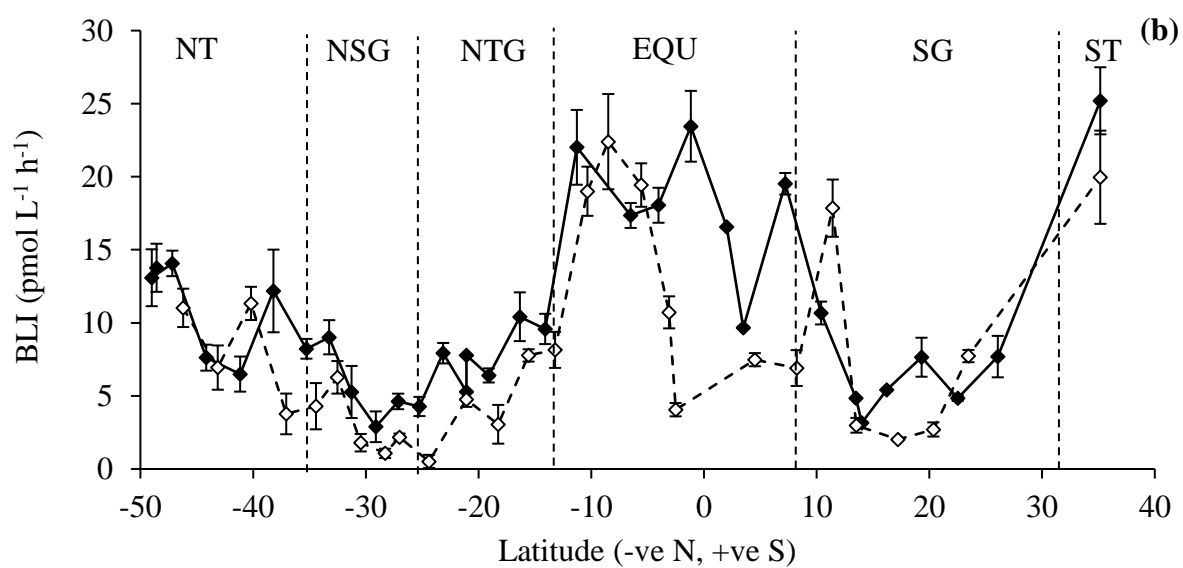
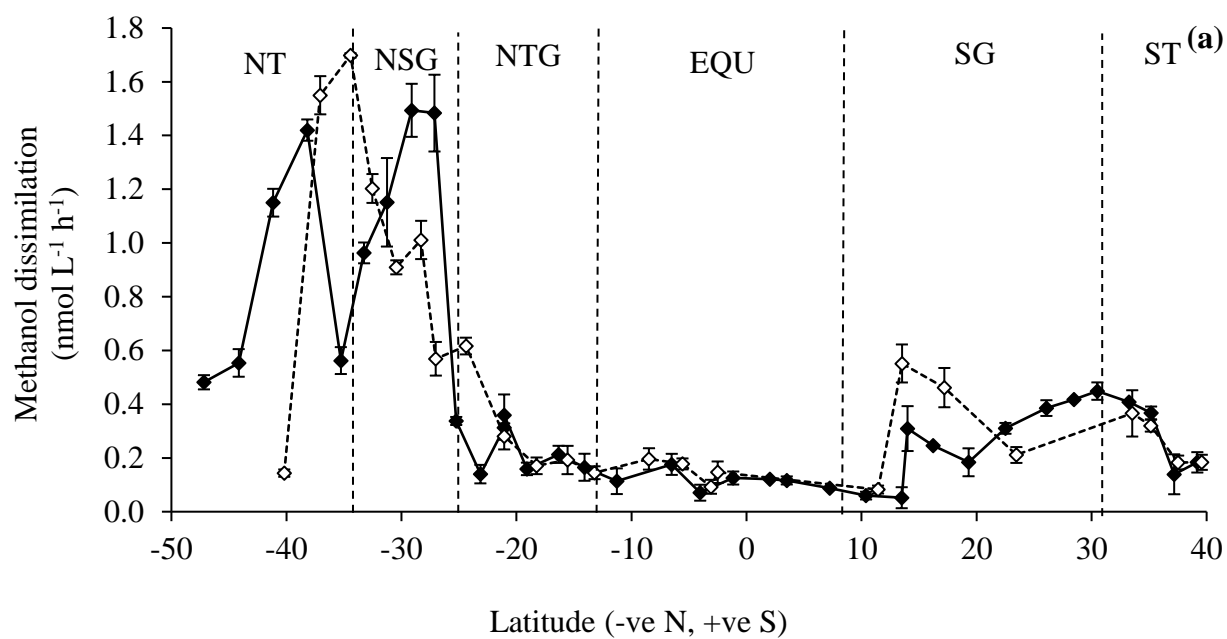
**Figure 5.** Changes in bacterial community composition (Order, identified using 16S rRNA gene sequencing) for a) 97 % PAR surface 5m, b) 33 % PAR 10-31m, c) 1 % PAR 15-54m and d) 200 m for different provinces (NT, NSG, NTG, EQU and SG) of the Atlantic Ocean. Analysis is based on a rarefied sample of 386 sequences per sample. Bacterial Orders individually contributing to less than 5% of the total sample sequences were pooled together into 'Others (<5%)' for clarity. Where  $\blacksquare$  *Prochlorococcus*,  $\blacksquare$  *Alteromonadales*,  $\square$  SAR11 clade,  $\blacksquare$  *Oceanospirillales*,  $\blacksquare$  *Rhodospirillales*,  $\blacksquare$  *Flavobacteriales*,  $\blacksquare$  *Rhodobacterales*,  $\blacksquare$  *Sphingomonadales*,  $\blacksquare$  *Synechococcus*,  $\blacksquare$  *Acidimicrobiales*,  $\blacksquare$  Order III *Incertae Sedis*,  $\blacksquare$  SAR324 clade (Marine group B),  $\blacksquare$  uncultivated bacterium,  $\blacksquare$  other bacteria individually comprising <5%.

**Figure 6.** Non-metric multi-dimensional scale (MDS) plots of (a) a Bray-Curtis similarity matrix of the 16S rRNA gene sequences of the bacterial community, (b) a Euclidean distance matrix of environmental parameters (salinity, temperature, chl. a, primary productivity, inorganic nutrients, flow cytometry cell numbers, BLI) and (c) a Euclidean distance matrix of rates of methanol dissimilation. Dashed lines highlight significant sample grouping. Plots generated using PRIMER-E ([www.primers-e.com](http://www.primers-e.com)). For (a) and (b) ■ represents samples from 200 m i.e. 0 % PAR.

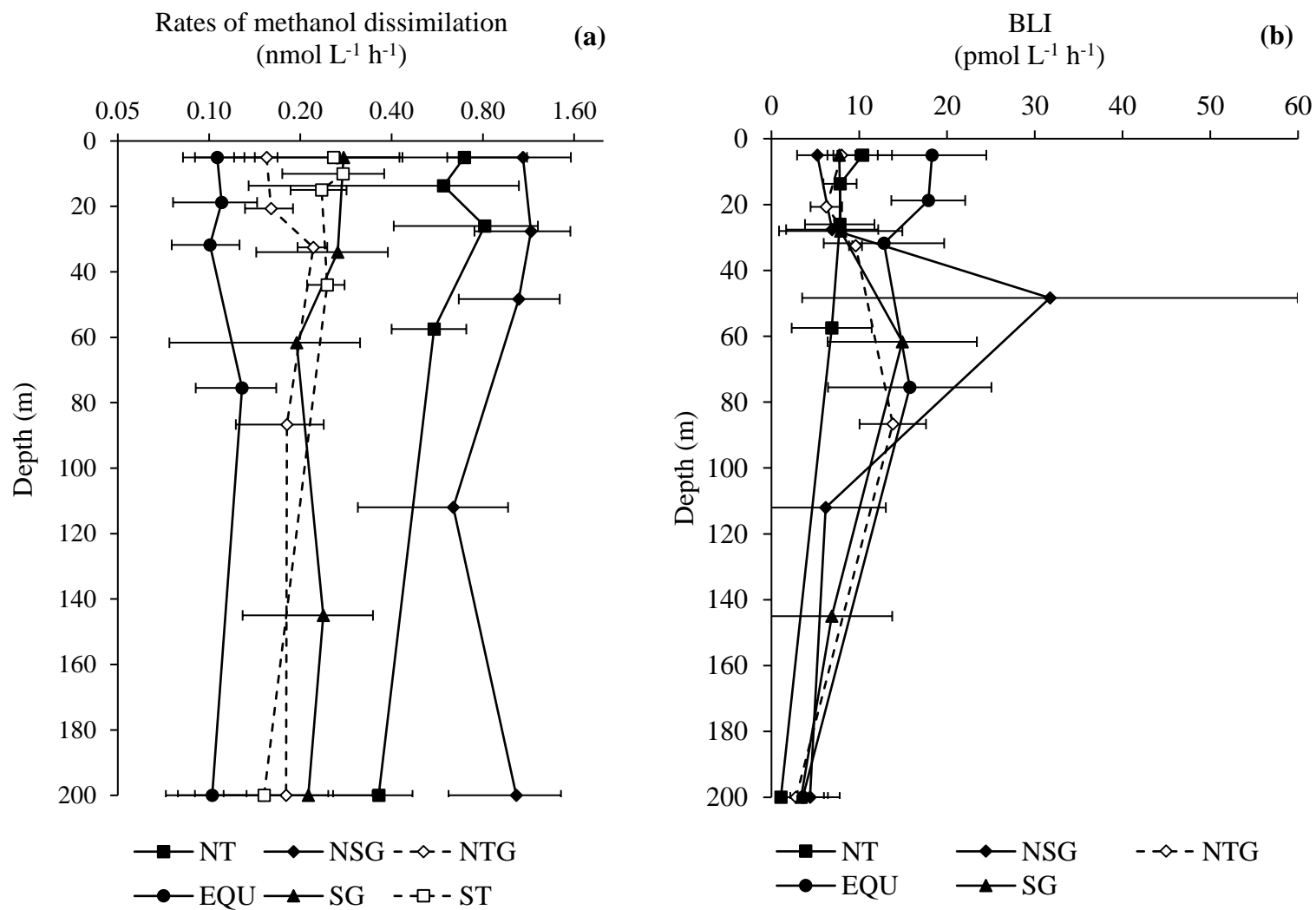
**Table 1.** Summary of rates of methanol uptake (dissimilation and assimilation), methanol concentrations, bacterial leucine incorporation (BLI) and production (BP), numbers of heterotrophic bacteria (BN), *Prochlorococcus* (Pros) and *Synechococcus* (Syns). “Values given are average  $\pm$  standard deviation (range). NA denotes that data is not available.”

**Figure 1.**

**Figure 2.**







**Figure 3.**

Figure 4.

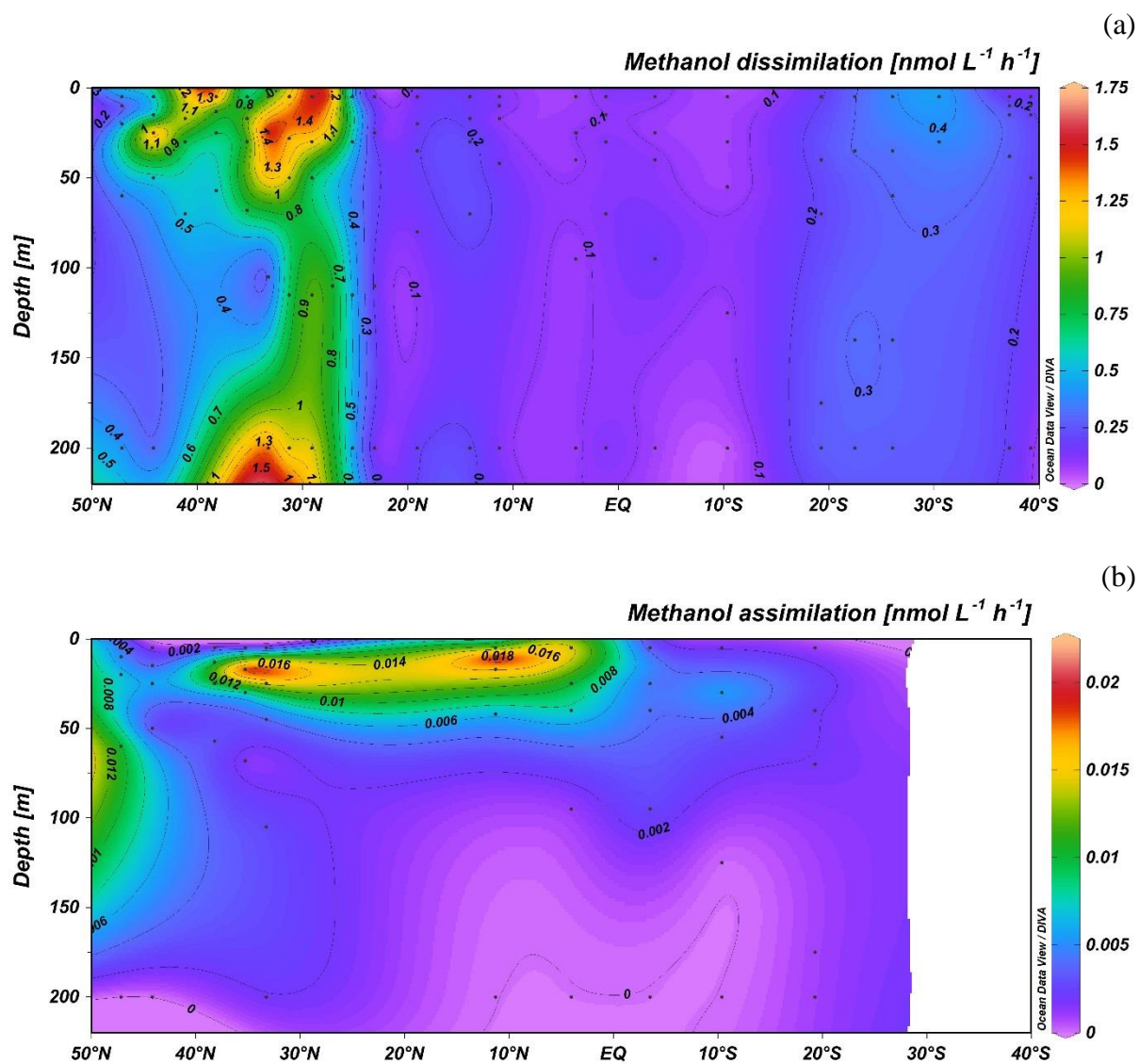
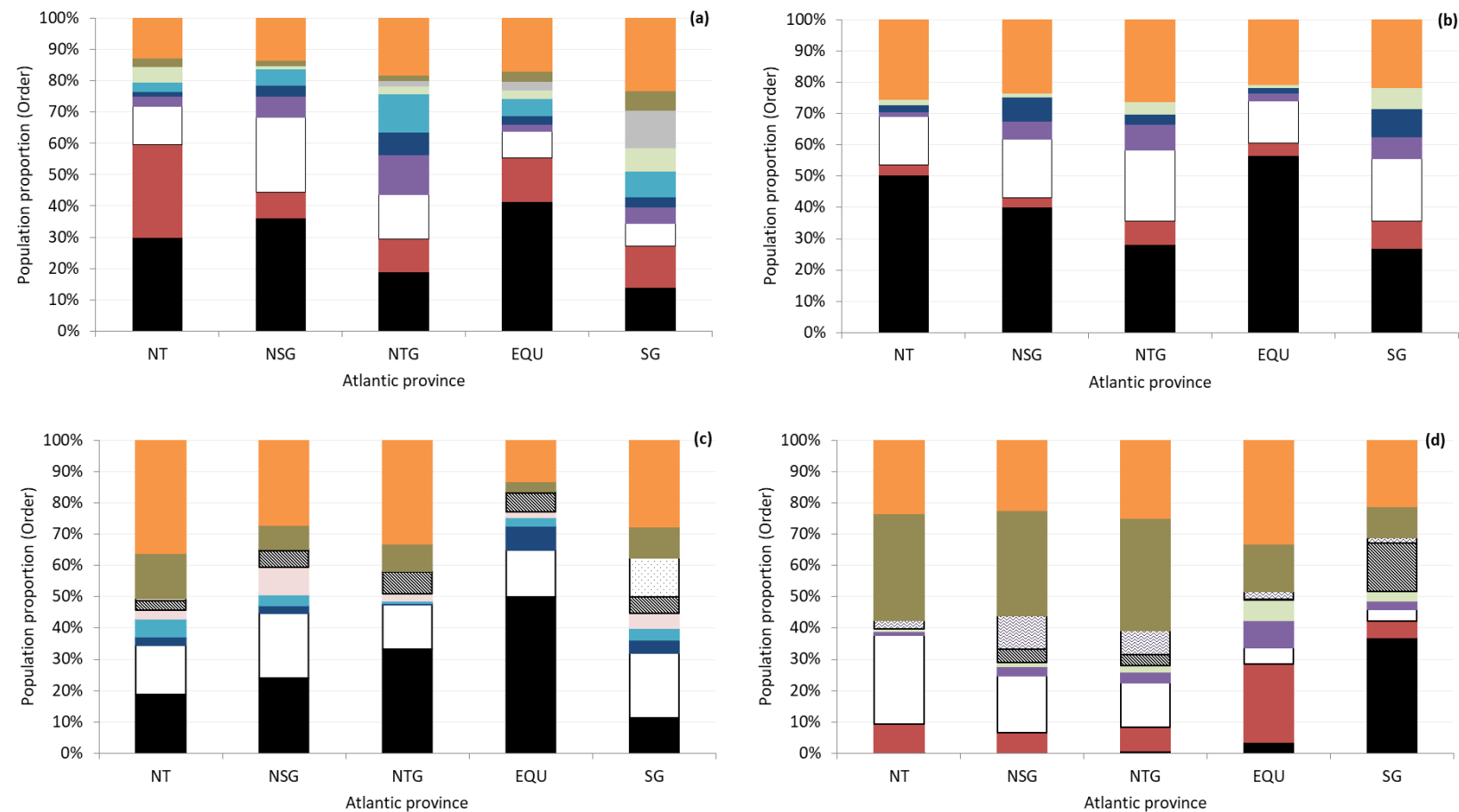
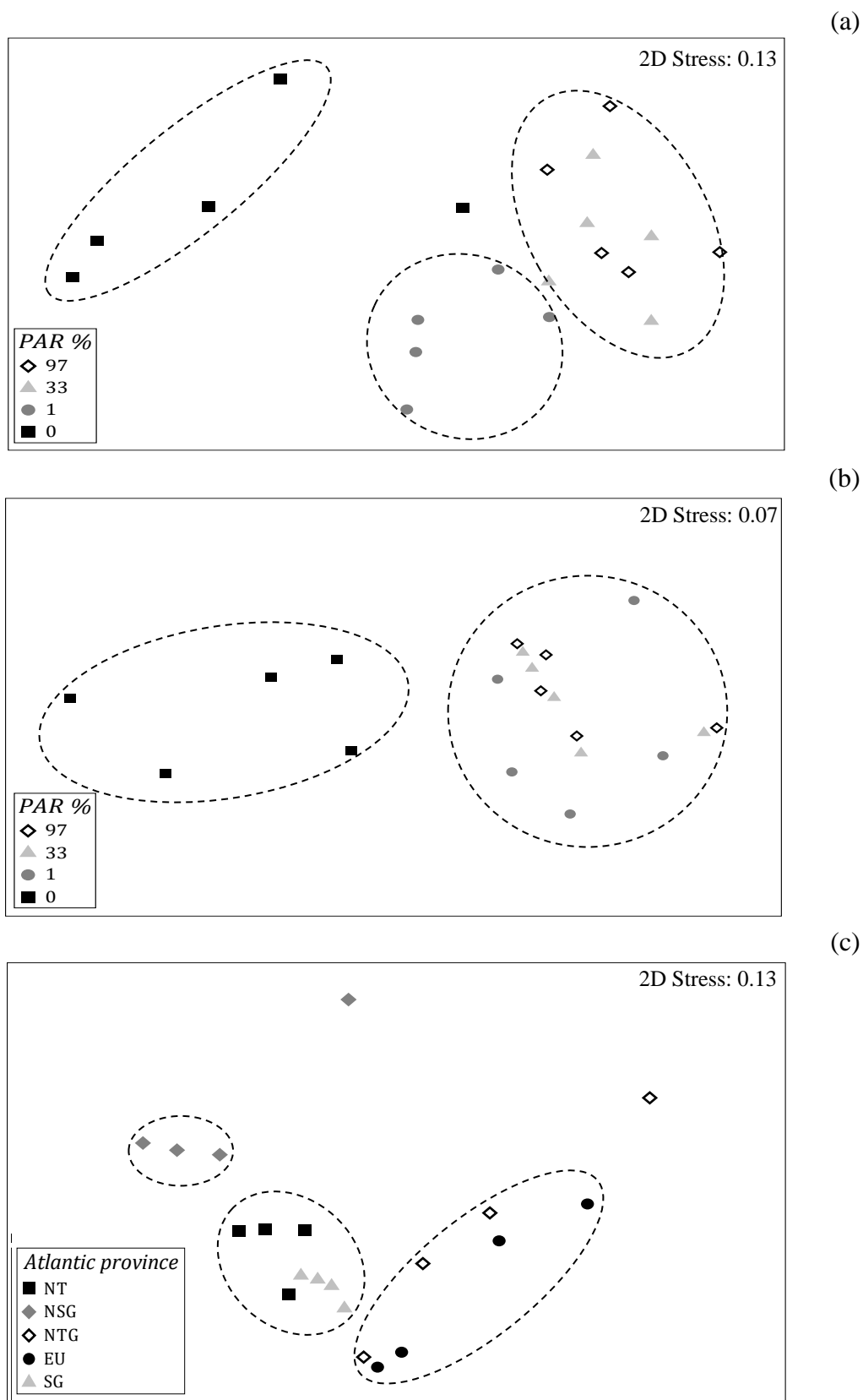


Figure 5.



**Figure 6.**



**Table 1.** Summary of rates of methanol uptake (dissimilation and assimilation), methanol concentrations, bacterial leucine incorporation (BLI) and production (BP), numbers of heterotrophic bacteria (BN), *Prochlorococcus* (Pros) and *Synechococcus* (SyNs). Values given are average  $\pm$  standard deviation (range). NA denotes that data is not available.

	Overall	Atlantic province					
		NT	NSG	NTG	EQU	SG	ST
Methanol dissimilation (nmol L <sup>-1</sup> h <sup>-1</sup> )	0.45 $\pm$ 0.42 (0.01–1.68)	0.69 $\pm$ 0.35 (0.22–1.50)	0.99 $\pm$ 0.41 (0.15–1.68)	0.18 $\pm$ 0.04 (0.10–0.25)	0.11 $\pm$ 0.03 (0.07–0.17)	0.24 $\pm$ 0.12 (0.01–0.45)	0.20 $\pm$ 0.05 (0.11–0.27)
Methanol assimilation (x 10 <sup>-2</sup> ) (nmol L <sup>-1</sup> h <sup>-1</sup> )	0.51 $\pm$ 0.54 (0.00–2.24)	0.54 $\pm$ 0.53 (0.00–2.23)	0.53 $\pm$ 0.56 (0.17–1.51)	NA	0.67 $\pm$ 0.66 (0.00–2.24)	0.19 $\pm$ 0.16 (0.00–0.57)	NA
BLI (pmol L <sup>-1</sup> h <sup>-1</sup> )	9.4 $\pm$ 8.9 (0.5–60.2)	7.7 $\pm$ 4.0 (0.9–14.2)	9.7 $\pm$ 14.2 (1.0–60.2)	8.0 $\pm$ 4.3 (2.0–17.0)	13.7 $\pm$ 7.9 (0.6–26.4)	8.2 $\pm$ 9.5 (0.5–41.5)	NA
<sup>a</sup> BP(TCF) (ng C L <sup>-1</sup> h <sup>-1</sup> )	14.6 $\pm$ 13.8 (0.8–96.1)	11.9 $\pm$ 6.1 (1.5–22.0)	15.0 $\pm$ 21.9 (1.5–96.1)	12.4 $\pm$ 6.6 (3.2–26.3)	21.2 $\pm$ 12.2 (1.0–41.0)	12.7 $\pm$ 14.8 (0.8–64.3)	NA
<sup>b</sup> BP (ECF) (ng C L <sup>-1</sup> h <sup>-1</sup> )	4.8 $\pm$ 4.6 (0.3–31.6)	3.9 $\pm$ 2.0 (0.5–7.2)	4.9 $\pm$ 7.2 (0.5–31.6)	4.1 $\pm$ 2.2 (1.0–8.7)	7.0 $\pm$ 4.0 (0.3–13.5)	4.2 $\pm$ 4.9 (0.3–21.1)	NA
Numbers of heterotrophic bacteria (x10 <sup>5</sup> ) (cells mL <sup>-1</sup> )	6.5 $\pm$ 6.3 (1.4–82.6)	NA	NA	5.8 $\pm$ 2.0 (1.6–9.8)	8.8 $\pm$ 10.3 (1.4–82.6)	5.4 $\pm$ 4.4 (1.5–35.8)	NA
Numbers of <i>Prochlorococcus</i> sp. (x10 <sup>5</sup> cells mL <sup>-1</sup> )	1.12 $\pm$ 4.62 (0.0–4.62)	0.91 $\pm$ 0.07 (0.0–2.56)	0.89 $\pm$ 0.71 (0.0–4.21)	1.52 $\pm$ 1.23 (0.0–4.19)	1.67 $\pm$ 0.2 (0.0–4.62)	1.20 $\pm$ 0.01 (0.0–2.45)	0.35 $\pm$ 0.22 (0.0–2.33)
Numbers of <i>Synechococcus</i> sp. (x10 <sup>4</sup> cells mL <sup>-1</sup> )	1.64 $\pm$ 31.4 (0.0–31.4)	1.96 $\pm$ 3.61 (0.0–12.7)	0.18 $\pm$ 0.21 (0.0–0.93)	0.15 $\pm$ 0.17 (0.0–0.73)	1.34 $\pm$ 2.69 (0.0–12.8)	0.14 $\pm$ 0.13 (0.0–0.79)	8.30 $\pm$ 10.3 (0.02–31.4)
<sup>c</sup> Methanol (nM)	143 $\pm$ 82 (38–420)	110 $\pm$ 126 (38–420)	203 $\pm$ 38 (154–281)	193 $\pm$ 46 (148–278)	148 $\pm$ 37 (117–241)	110 $\pm$ 33 (58–176)	132

<sup>a</sup>Theoretical conversion factor (TCF) 1.55 kg C mol leu<sup>-1</sup>, <sup>b</sup>empirical conversion factor (ECF) 0.51 kg C mol leu<sup>-1</sup>, <sup>c</sup>From Beale et al., (2013)