

1 **Basin-scale variability of microbial methanol uptake in the**
2 **Atlantic Ocean**

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

30

31

32 **1. Introduction**

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

1 2012). Methanol has been shown to be ubiquitous in waters of the Atlantic Ocean ranging
2 between <27–361 nM (Beale et al., 2013; Williams et al., 2004; Yang et al., 2013; Yang et
3 al., 2014). Our knowledge of the sources and sinks of methanol is limited and often lacks
4 consensus. For example, recent eddy covariance flux estimates demonstrated a consistent flux
5 of atmospheric methanol into the surface waters of a meridional transect of the Atlantic
6 Ocean (Yang et al., 2013). However, along a similar transect, 12 months earlier, Beale et al.
7 (2013) calculated that the Atlantic Ocean represents an overall source of methanol to the
8 atmosphere (3 Tg yr⁻¹), which was largely attributable to an efflux from the North Atlantic
9 gyre; where surface concentrations were as high as 361 nM. Wet deposition from rainwater
10 has also recently been suggested to represent a supply of methanol to the ocean (Felix et al.,
11 2014).

12 Although *in situ* marine photochemical production of methanol has previously been found to
13 be insignificant (Dixon et al., 2013), there is thought to be a substantial unidentified
14 biological source of methanol in seawater (Dixon et al., 2011a). Biological production by
15 phytoplankton and during the breakdown of marine algal cells are possible sources (Heikes et
16 al., 2002; Nightingale, 1991; Sieburth and Keller, 1989). Recent laboratory culture
17 experiments suggest that methanol is produced by a wide variety of phytoplankton including
18 cyanobacteria (*Prochlorococcus marinus*, *Synechococcus* sp. and *Trichodesmium*
19 *erythraeum*) and Eukarya (*Emiliania huxleyi*, *Phaeodactylum tricornutum* and
20 *Nannochloropsis oculata*, *Dunaliella tertiolecta*) (Mincer and Aicher, 2016, Halsey et al.,
21 2017). The mechanisms of *in situ* methanol production and their regulation remains largely
22 unknown, although Halsey et al. (2017) reported light-dependent rates of methanol
23 production in cultures of the marine green flagellate *Dunaliella tertiolecta* (cell size of 10–12
24 μm).

25 Methylotrophic bacteria are capable of utilising one-carbon compounds including methanol
26 as their sole source of energy (methanol dissimilation) and carbon (methanol assimilation).
27 Methylotrophs are widespread in terrestrial and aquatic systems (Kolb, 2009), but research
28 into these bacteria in marine environments is still at an early stage. Traditionally,
29 methylotrophs were thought to utilise methanol dehydrogenase (MDH encoded by *mxaF*,
30 McDonald and Murrell, 1997) to metabolise methanol to formaldehyde, with further
31 oxidation to CO₂ or incorporation of carbon into biomass (Chistoserdova, 2011;
32 Chistoserdova et al., 2009). However, recent progress in this field has resulted in the
33 discovery of the *xoxF* gene, encoding an alternative MDH (Wilson et al., 2008) and

1 seemingly present in all known gram-negative methylotrophs to date (Chistoserdova, 2011;
2 Chistoserdova et al., 2009). The presence of methylotrophs in seawater has been confirmed
3 using a range of molecular approaches including functional gene primers, stable isotope
4 probing and metaproteomics (Dixon et al., 2013; Grob et al., 2015; Neufeld et al., 2008;
5 Neufeld et al., 2007; Taubert et al., 2015). There are also bacterial cells that utilise methanol
6 and other C₁ compounds for the production of energy but not biomass e.g. SAR11 for which
7 Sun et al. (2011) proposed the new term ‘methylovores’, distinct from true methylotrophs
8 which use C₁ compounds as sources of carbon and energy.

9 Limited studies of microbial methanol assimilation in the Atlantic Ocean have previously
10 shown rates up to 0.42 nmol l⁻¹ h⁻¹ in recently upwelled coastal waters of the Mauritanian
11 Upwelling (Dixon et al., 2013). However, open ocean waters of the Atlantic were
12 substantially lower ranging between 0.002–0.028 nmol l⁻¹ h⁻¹ (Dixon et al., 2013). Microbial
13 methanol dissimilation rates are generally up to 1000-fold higher than rates of assimilation;
14 ranging between 0.70-11.2 and <0.001-0.026 nmol l⁻¹ h⁻¹ respectively for coastal waters
15 (Sargeant et al., 2016; Dixon et al., 2011b). Methanol dissimilation rates ranging between
16 0.08–6.1 nmol l⁻¹ h⁻¹ have also been found in open ocean Atlantic waters (Dixon et al.,
17 2011a). However, despite the ubiquity of methanol in seawater, the spatial extent or
18 quantification of microbial methanol utilisation for energy production on a basin scale has not
19 been previously investigated. Therefore, the objective of this research was to simultaneously
20 characterise the spatial variability in microbial methanol dissimilation rates (at depths to 200
21 m) and in microbial community groups throughout contrasting biogeochemical regions of the
22 Atlantic Ocean. This study represents the first basin-wide approach to investigating methanol
23 as a source of reducing power and energy for microbes.

24

25 **2. Materials and Methods**

26 *2.1. Sampling strategy*

27 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
28 Falmouth, UK (50.15° N, 05.07° W) and arrived in Punta Arenas, Chile (53.14° S, 70.92°
29 W). Water samples were collected daily from pre-dawn (97, 33, 14 and 1 %
30 photosynthetically active radiation (PAR) equivalent depths and 200 m) and solar noon (97
31

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 mg m⁻³ gyre regions, >0.15 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.

11

12 2.2. *Microbial methanol uptake*

13 The oxidation of methanol to CO₂ (dissimilation) was determined using ¹⁴C-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 ~10 nM (final concentration) ¹⁴C-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 ¹⁴CO₂, captured in the precipitate as
23 Sr¹⁴CO₃ (nCi ml⁻¹ h⁻¹), were divided by the total ¹⁴CH₃OH added to the sample (nCi ml⁻¹) to
24 calculate the apparent rate constants, *k* (h⁻¹).

25

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 ¹⁴CH₃OH added to the sample (nCi ml⁻¹) to calculate the apparent rate
30 constants, *k* (h⁻¹). For both methanol assimilation and dissimilation, the specific activity of
31 ¹⁴C-labelled methanol (57.1 mCi mmol⁻¹) was multiplied by the apparent rate constants to
32 calculate rates of microbial methanol uptake (nmol l⁻¹ h⁻¹) following the approach of Dixon et

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

4

5 *2.3. Bacterial leucine incorporation*

6 Rates of bacterial leucine incorporation were measured using the incorporation of ^3H -leucine
7 into bacterial protein in seawater samples using the method described by Smith and Azam
8 (1992). A final concentration of 25 nM (6.8 μl) of ^3H -leucine (calculated using the specific
9 activity of 161 Ci mmol $^{-1}$, concentrations 1 mCi ml $^{-1}$, American Radiolabelled Chemicals Inc,
10 Saint Louis, MO, USA) was incubated with 1.7 ml seawater samples. Incubations were
11 conducted in triplicate with 'killed' controls (5 % TCA, final concentrations), at *in situ*
12 temperature and in the dark.

13

14 *2.4. Bacterial community composition*

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

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

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

10

11 **3. Results**

12 *3.1. Microbial methanol dissimilation*

13 *3.1.1 Surface*

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

30 *3.1.2 Depth distributions*

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

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

18

19 *3.2. Bacterial leucine incorporation rates*

20 *3.2.1 Surface*

21 Rates of bacterial leucine incorporation (BLI) varied between 2.9–25.2 pmol l⁻¹ h⁻¹ in the pre-
22 dawn surface waters of the Atlantic transect (Fig. 2b). On average, surface rates of BLI were
23 highest in the relatively more productive EQU upwelling region (18.3 ± 4.8 pmol l⁻¹ h⁻¹), and
24 lowest in the northern sub-tropical gyre (NSG, 5.2 ± 2.3 pmol l⁻¹ h⁻¹). Surface rates of BLI
25 averaged 7.8 ± 2.3 pmol l⁻¹ h⁻¹ and 7.7 ± 2.4 pmol l⁻¹ h⁻¹ in the NTG and SG regions
26 respectively. The one measurement of BLI in the ST suggested much higher rates (25.2 pmol
27 l⁻¹ h⁻¹) than previously determined during the transect, even when compared to the NT region
28 (9.9 ± 3.9 pmol l⁻¹ h⁻¹). Pre-dawn (dark) rates of BLI generally exhibited a similar latitudinal
29 pattern to those from solar noon (light), with more variability between light and dark
30 sampling observed in the waters of the productive EQU region. Bacterial rates of leucine
31 incorporation determined from samples collected at solar noon (y) were approximately 20%

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

3

4 *3.2.2 Depth profiles*

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

14

15 *3.3. Bacterial community composition*

16 *3.3.1 Surface*

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

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

6

7 Microbial communities of the surface waters of the NT, NSG and EQU provinces were
8 dominated by *Prochlorococcus*, *Alteromonadales* and SAR11, together representing between
9 64–72 % of 16S rRNA gene sequences. These orders were less dominant in the more
10 oligotrophic waters of the NTG and SG, accounting for 43 % and 34 % of 16S rRNA gene
11 sequences respectively. In these oligotrophic regions (NTG and SG) microbial communities
12 appear less dominated by a few orders, with a more even spread of bacterial orders
13 contributing to the community composition (Fig. 5a).

14

15 3.3.2 Depth profiles

16 The largest contributors to bacterial communities at the 33 % PAR depths were, like surface
17 communities, *Prochlorococcus* and SAR11 16S rRNA gene sequences (Fig. 5b). Together
18 they accounted for between 47-70 % of all OTUs, with the minimum and maximum
19 contributions in the SG and EQU provinces respectively. If the proportion of sequences
20 contributing individually <5% were included then collectively they accounted for between
21 69-91 % of all 16S rRNA gene sequences. The main differences between the surface and
22 33% PAR equivalent depth (14-31 m) are the increasing dominance of the cyanobacteria
23 *Prochlorococcus*, and the decrease in relative contribution of *Alteromonadales* at 33% PAR
24 depths, particularly in the NT region.

25

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

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

3

4 In the permanent dark of 200 m, SAR11 bacteria contributed between 14-29 % in northern
5 regions, which contrasted to only 4-5 % in the EQU and SG provinces. The SAR324 clade
6 contributed 8-11 % in the northern gyre. Both uncultivated bacteria and those that
7 individually comprised <5 % contributed relatively highly to the OTUs (10-36 % and 21-33
8 % respectively). These two groupings together with the SAR11 and SAR324 make up 83-89
9 % in northern regions and between 37-56 % in the SG and EQU provinces respectively. For
10 the EQU region the *Alteromonadales* order is also significant at 25 % (which collectively
11 comprise 81 % of all OTUs for EQU), whilst for the SG the cyanobacteria *Prochlorococcus*
12 and *Synechococcus* comprise 52 % (which collectively comprise 89 % of all OTUs for SG).

13

14 **4. Discussion**

15 *4.1. Basin scale variability in biological methanol uptake*

16 Maximum rates of methanol dissimilation in the Atlantic Ocean were recorded in the NSG
17 province at 33 % PAR light depth (25 m, $1.68 \text{ nmol l}^{-1} \text{ h}^{-1}$, Fig. 2 and Fig. 4a). An overview
18 of the variation in rates of methanol dissimilation to CO_2 throughout the top 200 m of the
19 water column in the Atlantic Ocean is shown in Fig. 4a, which illustrates sub-surface maxima
20 in northerly latitudes. However, no statistically significant differences were found between
21 rates of methanol dissimilation in the euphotic zone (97-1 % PAR) compared to the aphotic
22 zone (samples from 200 m) in the NSG ($t_{\text{NSG}}=2.63$, $t_{20}=2.85$ for $P<0.01$), NTG ($t_{\text{NTG}}=0.02$,
23 $t_{12}=3.05$ for $P<0.01$), EQU ($t_{\text{EQU}}=1.01$, $t_{18}=2.88$ for $P<0.01$) and SG regions ($t_{\text{SG}}=0.88$,
24 $t_{19}=2.88$ for $P<0.01$). This is consistent with a previous study in the north east Atlantic Ocean,
25 which similarly reported no significant variability in methanol dissimilation rates with depth
26 (Dixon and Nightingale, 2012). Nevertheless, greater variability with depth was observed for
27 methanol dissimilation rates from the northern gyre ($F_{\text{NSG}}=3.22$ where $F_{3,17}=3.20$, $P<0.05$
28 and $F_{\text{NTG}}=5.14$ where $F_{2,10}=4.10$, $P<0.05$). Variability in rates from the euphotic zone were
29 found to be significantly higher than those from 200 m in northern ($t_{\text{NT}}=3.17$, $t_{20}=2.85$ for
30 $P<0.01$) and southern temperate regions ($t_{\text{ST}}=5.03$, $t_{10}=3.17$ for $P<0.01$).

31

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

1 study of the temperate western English Channel (0.5–11.2 nmol l⁻¹ h⁻¹, Sargeant et al., 2016).
2 Rates determined in the temperate waters of the south Atlantic (0.11–0.45 nmol l⁻¹ h⁻¹) are
3 most comparable to the lowest rates determined during late spring and early summer of ~0.50
4 nmol l⁻¹ h⁻¹ in temperate northern coastal waters (Sargeant et al., 2016). The seasonal study
5 in the western English Channel showed maximum rates of up to 11.2 nmol l⁻¹ h⁻¹ during
6 autumn and winter months (Sargeant et al., 2016). The differences in methanol dissimilation
7 rates between the temperate waters of the North (0.83±0.42 nmol l⁻¹ h⁻¹) and South
8 (0.27±0.13 nmol l⁻¹ h⁻¹) Atlantic may therefore reflect seasonal differences between
9 hemispheres i.e. sampling in the NT region occurred during late autumn compared to late
10 spring in the ST region.

11

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

21

22 *4.2. Bacterial community and productivity*

23 In contrast to microbial methanol dissimilation, rates of bacterial leucine incorporation were
24 lowest in the northern oligotrophic gyre (NSG 5.2 ± 2.3 pmol l⁻¹ h⁻¹, NTG 7.8 ± 2.3 pmol l⁻¹
25 h⁻¹) reflecting lower microbial activity in these regions of the Atlantic. Surface microbial
26 methanol dissimilation rates exhibited a statistically significant inverse correlation with
27 bacterial leucine incorporation, ($r = -0.351$, $n = 36$, $P \leq 0.05$). This is consistent with
28 findings from a seasonal study in the western English Channel, where surface rates of
29 methanol dissimilation were also inversely correlated to bacterial production (Sargeant et al.,
30 2016). For all the depth data a negative correlation was also found in the NTG, EQU and SG
31 regions ($r = -0.372$, $n = 52$, $P \leq 0.01$), but NT and NSG areas showed methanol dissimilation
32 rates independent of BLI. The productivity of heterotrophic bacteria is generally associated
33 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 pmol l⁻¹ 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 $t_{NSG}=2.63$ ($t_{20}=2.85$ for
25 $P<0.01$), NTG $t_{NTG}=0.02$ ($t_{12}=3.05$ for $P<0.01$), EQU $t_{EQU}=1.01$ ($t_{18}=2.88$ for $P<0.01$) and SG
26 $t_{SG}=0.88$ ($t_{19}=2.88$ 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).

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

8

9 *4.3. Methanol dissimilation and SAR11*

10 SAR11 cells have been shown to utilise methanol, but only as a source of energy (Sun et al.,
11 2011). The numbers of SAR11 16S rRNA gene sequences exhibited a statistically significant
12 correlation with rates of microbial methanol dissimilation throughout the Atlantic basin ($r =$
13 0.477, $n = 20$, $P < 0.05$), where the number of SAR11 16S rRNA gene sequences explained
14 approximately half of the spatial variability in rates of methanol dissimilation. It should be
15 noted that this correlation has been made with amplicon ratios, relating to the relative success
16 of SAR11 in the community, rather than with SAR11 cell numbers specifically. In culture,
17 SAR11 cells (strain HTCC1062) have previously been shown to utilise methanol as a source
18 of energy at a rate of $\sim 5 \times 10^{-20}$ moles cell $^{-1}$ h $^{-1}$ (Sun et al., 2011), which equates to 2 nmol l $^{-1}$
19 h $^{-1}$ (using a culture cell abundance of 4×10^7 cells mL $^{-1}$, Sun et al., 2011). SAR11 cells
20 dominate ($59 \pm 4\%$) the low nucleic acid (LNA) fraction of bacterioplankton consistently
21 across the Atlantic Ocean, where typically numbers of LNA range between $0.2\text{--}1.0 \times 10^9$ cells
22 l $^{-1}$ (Mary et al., 2006a). Thus estimates of *in situ* SAR11 numbers range between 0.12–0.59 \times
23 10^9 cells l $^{-1}$. This is consistent with estimates from the Sargasso Sea of $\sim 0.1 \times 10^9$ cells l $^{-1}$
24 (where they are reported to contribute $\sim 25\%$ of total prokaryotic abundance of 0.4×10^6 cells
25 mL $^{-1}$, Malmstrom et al., 2004). Thus, we estimate that SAR11 cells of the Atlantic Ocean
26 could be oxidising methanol at rates between 5–29.5 pmol l $^{-1}$ h $^{-1}$, which could account for
27 between 0.3–59 % of the rates of methanol dissimilation in surface Atlantic waters.

28 A seasonal investigation in the western English Channel reported bacterial numbers ranging
29 between $2.0\text{--}15.8 \times 10^5$ cells ml $^{-1}$ (Sargeant et al., 2016) which agrees well with data from
30 Mary et al. (2006b, $2.0\text{--}16.0 \times 10^5$ cells ml $^{-1}$). Assuming that SAR11 contribute between 9–
31 20% of total bacterioplankton (Mary et al., 2006b) suggests SAR11 numbers range between
32 $0.18\text{--}3.16 \times 10^5$ cells ml $^{-1}$ at this coastal site. Using the above estimate of $\sim 5 \times 10^{-20}$ moles cell $^{-1}$

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

9

10 Previously methylotrophic bacteria such as *Methylophaga* sp., *Methylococcaceae* sp. and
11 *Hyphomicrobium* sp. have been identified, using *mxAF* functional gene primers (which
12 encode for the classical methanol dehydrogenase), from the same DNA samples analysed for
13 16S rRNA genes in this study, from the upper water column of Atlantic Ocean provinces
14 (Dixon et al., 2013). Although numerically very rare (1-11 16S rRNA gene sequences per
15 sample), 16S rRNA gene sequences identified as *Methylophaga* spp., *Methylophaga* sp.
16 DMS021 (EU001861) and uncultured *Methylophaga* sp. (EU031899), were found in each of
17 the Atlantic Ocean provinces in this study (at 97% PAR or 200m depth), consistent with
18 previous identification of *Methylophaga* spp. in these Atlantic provinces using *mxAF* gene
19 cloning in (Dixon et al., 2013). More recently the *xoxF* gene, which encodes an alternative
20 methanol dehydrogenase, has also been found to be widespread in coastal marine
21 environments (Taubert et al., 2015). SAR11 bacteria are thought to contain an Fe alcohol
22 dehydrogenase, which although not specific for methanol, can oxidise methanol (and other
23 short chain alcohols) to formaldehyde which is then thought to be converted to CO_2 by a
24 tetrahydrofolate-linked C1 transfer pathway to produce energy (Sun et al., 2012). Thus it
25 seems likely that both methylotrophic bacteria possessing *mxAF* and/or *xoxF*, together with
26 microbes such as SAR11 (Sun et al., 2011), are largely responsible for the turnover of
27 methanol in seawater.

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

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

7

8 *4.4. Marine methanol cycling*

9 Data from this study substantially add to the measurements of microbial methanol
10 dissimilation rates in seawater. This extended spatial coverage clearly demonstrates that
11 methanol dissimilation is a widespread microbial process taking place in light and dark
12 environments throughout the Atlantic Ocean. Dissimilation rates are typically two orders of
13 magnitude greater than assimilation rates across most of the Atlantic Basin. These data
14 suggest that methanol is an important source of energy for microbes. This is particularly true
15 in the northern oligotrophic waters of the Atlantic Ocean, where corresponding *in situ*
16 methanol concentrations range between 148-281 nM (Table 1). What is not clear is the source
17 of methanol in open ocean waters, which is suspected to be biological in nature (Dixon et al.,
18 2011a). Although direct flux estimates suggest that the atmosphere could also act as a source
19 to the ocean (Yang et al, 2013), the magnitude of this flux is insufficient to support the
20 observed rates of microbial methanol consumed by bacteria, and hence is suspected to be a
21 minor contribution (Dixon et al., 2011a). Recent culture studies indicate that
22 *Prochlorococcus* sp., *Synechococcus* sp. and *Trichodesmium* sp. could produce methanol
23 (Mincer and Aicher, 2016, Halsey et al., 2017), but *in situ* production mechanisms are
24 unknown. Further work is needed to fully elucidate and quantify the sources of methanol in
25 marine waters.

26

27 **5. Conclusions**

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

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

5

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18

19 **Conflict of Interest Statement**

20 The Authors declare no conflict of interest with this manuscript.

21

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1 **Figure and Table legends.**

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

7
8 **Figure 2.** Variability in a) microbial methanol dissimilation rates (using the specific activity
9 of $^{14}\text{CH}_3\text{OH}$) and b) bacterial leucine incorporation (BLI), in surface waters of the Atlantic
10 Ocean. Rates were determined from pre-dawn (♦ solid line) and solar noon (◊ dashed line)
11 CTD casts. Error bars represent ± 1 s.d. of triplicate samples, dashed vertical lines indicate
12 Atlantic province boundaries.

13
14 **Figure 3.** Average depth profiles in Atlantic provinces for a) microbial methanol
15 dissimilation (using the specific activity of $^{14}\text{CH}_3\text{OH}$) and b) bacterial leucine incorporation
16 (BLI) in pre-dawn waters. Error bars represent ± 1 s.d. of variation within the province,
17 province averages derived from NT (n = 5), NSG (n = 5), NTG (n = 3), EQU (n = 4), SG (n =
18 5) and ST (n = 3), except for BLI where there is no data from the ST.

19
20 **Figure 4.** Microbial methanol (a) dissimilation and (b) assimilation rates ($\text{nmol l}^{-1} \text{ h}^{-1}$) in the
21 top 200 m of an Atlantic Meridional transect (contour plots).

22
23 **Figure 5.** Changes in bacterial community composition (Order, identified using 16S rRNA
24 gene sequencing) for a) 97 % PAR surface 5m, b) 33 % PAR 10-31m, c) 1 % PAR 15-54m
25 and d) 200 m for different provinces (NT, NSG, NTG, EQU and SG) of the Atlantic Ocean.
26 Analysis is based on a rarefied sample of 386 sequences per sample. Bacterial Orders
27 individually contributing to less than 5% of the total sample sequences were pooled together
28 into 'Others (<5%)' for clarity. Where ■ *Prochlorococcus*, ■ *Alteromonadales*, □
29 SAR11 clade, ■ *Oceanospirillales*, ■ *Rhodospirillales*, ■ *Flavobacteriales*, ■
30 *Rhodobacterales*, ■ *Sphingomonadales*, ■ *Synechococcus*, ■ *Acidimicrobiales*, ■
31 Order III *Incertae Sedis*, ■ SAR324 clade (Marine group B), ■ uncultivated bacterium, ■
32 other bacteria individually comprising <5%.

1 **Figure 6.** Non-metric multi-dimensional scale (MDS) plots of (a) a Bray-Curtis similarity
2 matrix of the 16S rRNA gene sequences of the bacterial community, (b) a Euclidean distance
3 matrix of environmental parameters (salinity, temperature, chl. a, primary productivity,
4 inorganic nutrients, flow cytometry cell numbers, BLI) and (c) a Euclidean distance matrix of
5 rates of methanol dissimilation. Dashed lines highlight significant sample grouping. Plots
6 generated using PRIMER-E (www.primer-e.com). For (a) and (b) ■ represents samples from
7 200 m i.e. 0 % PAR.

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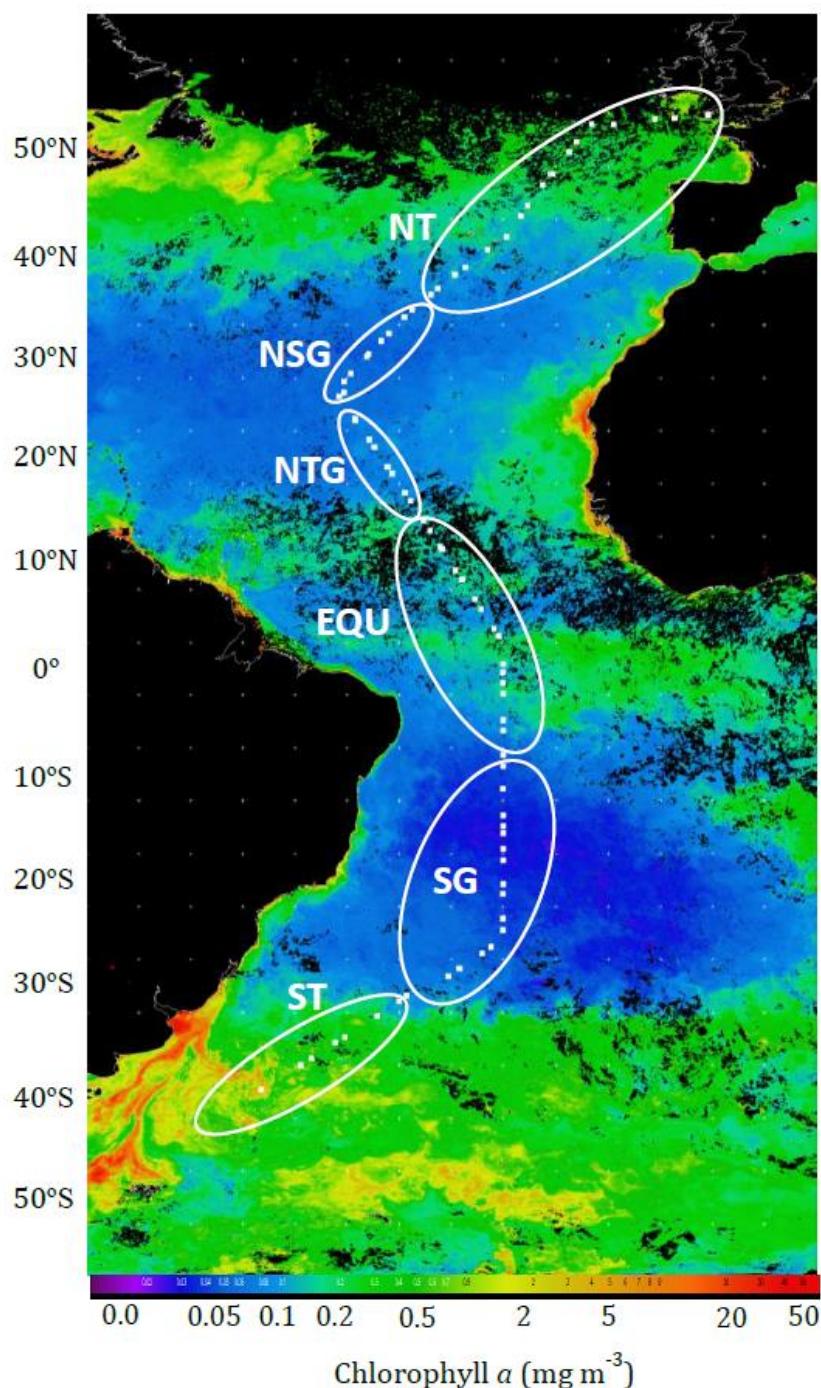
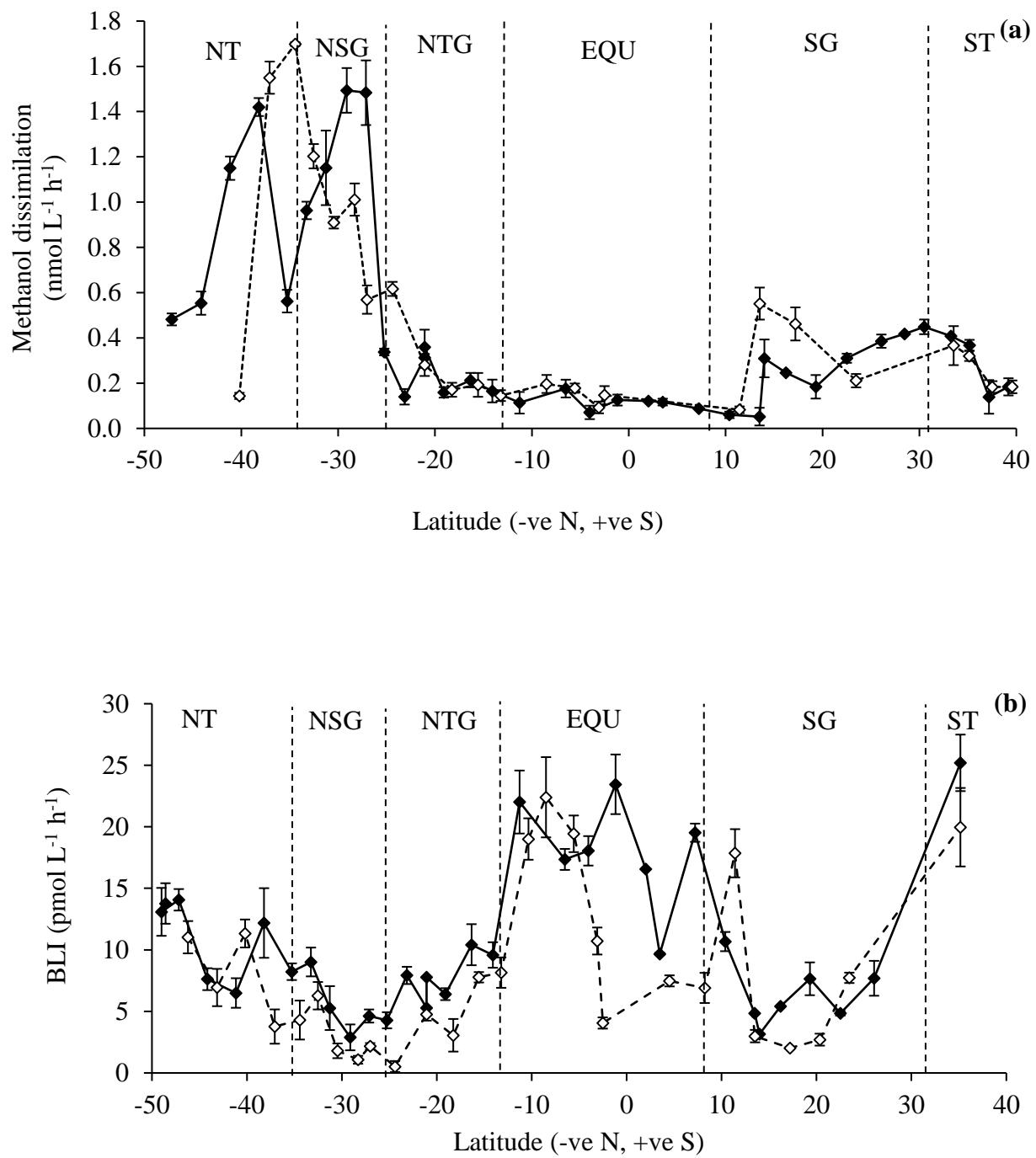
Figure 1.

Figure 2.



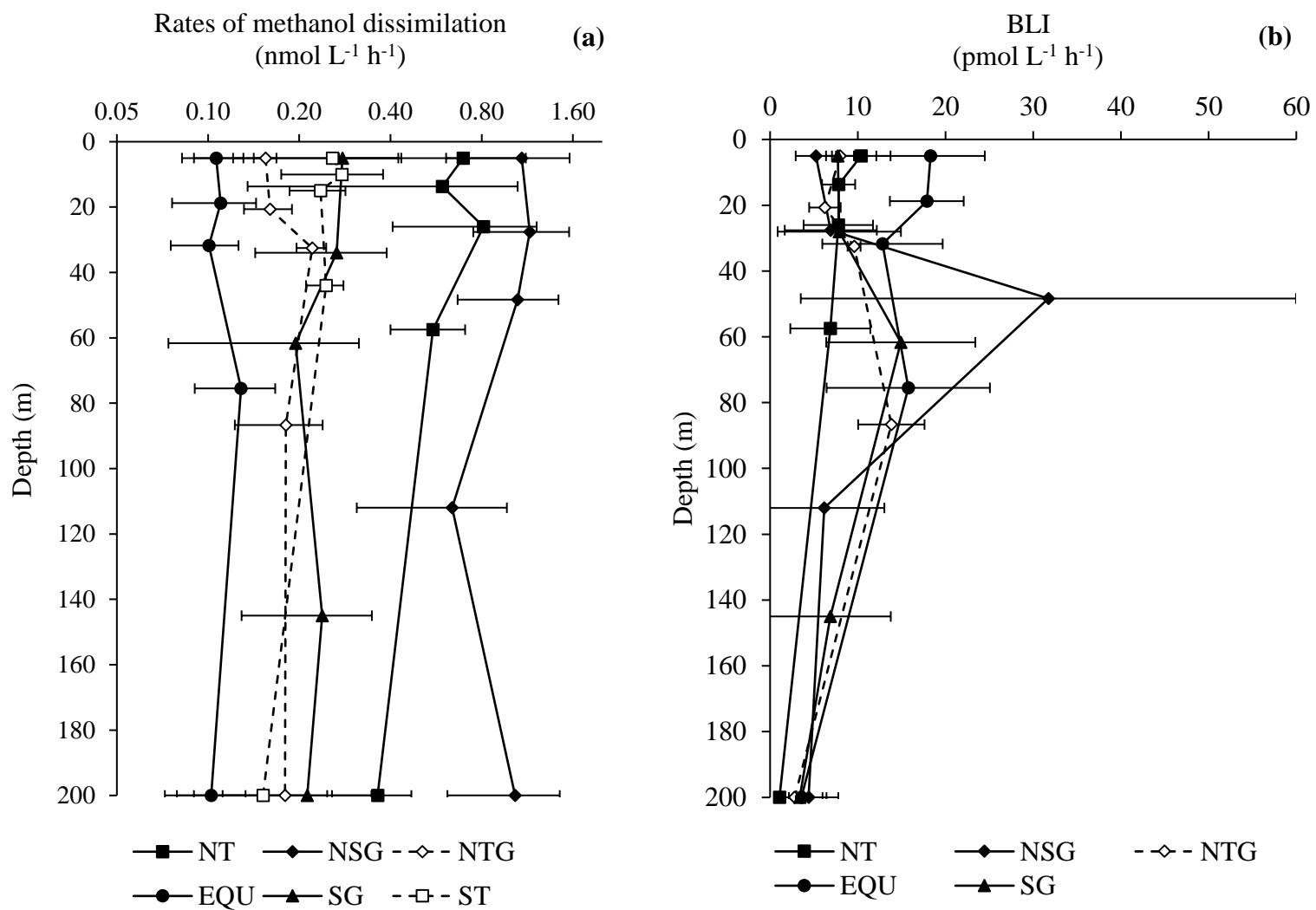


Figure 3.

Figure 4.

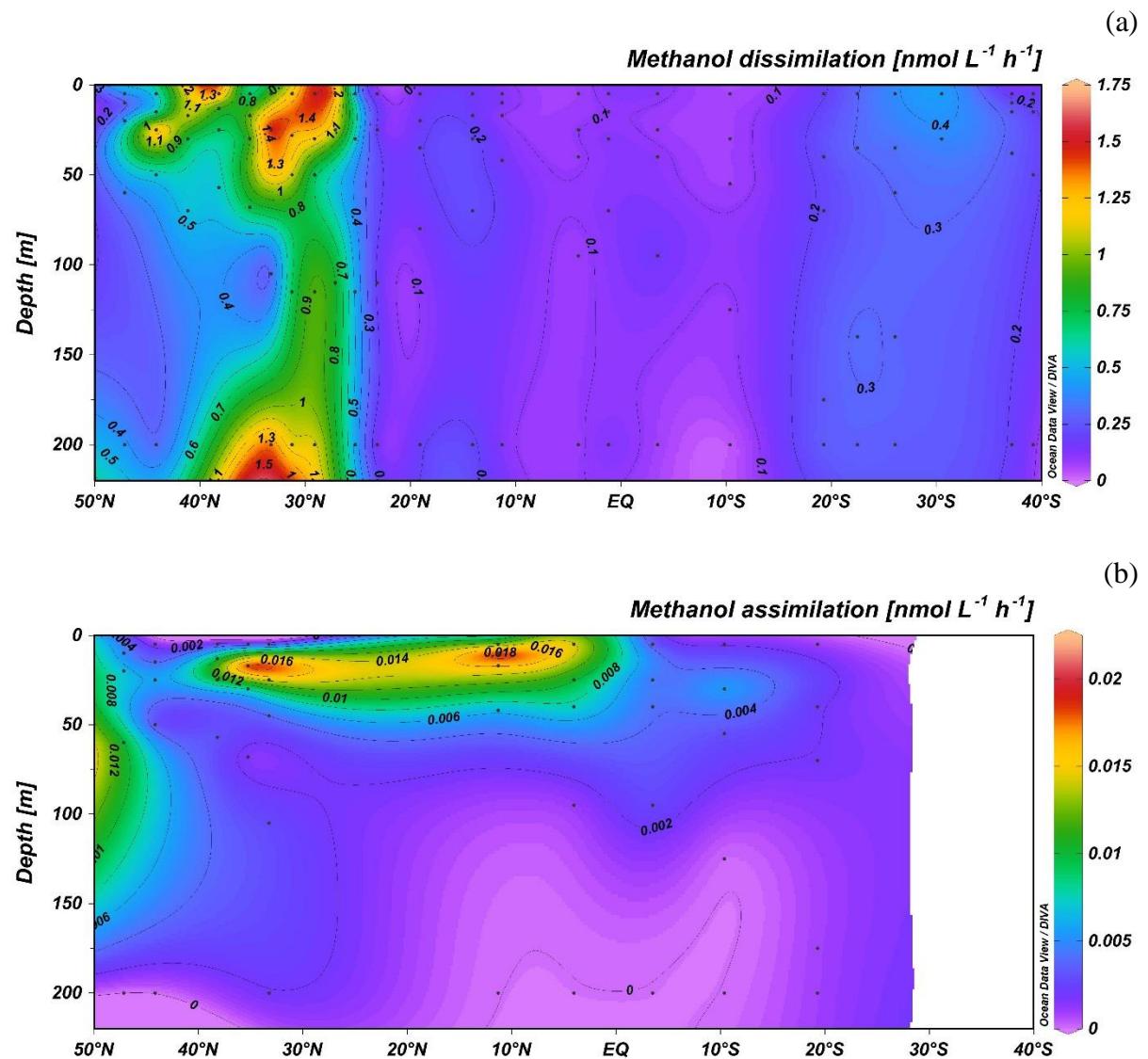


Figure 5.

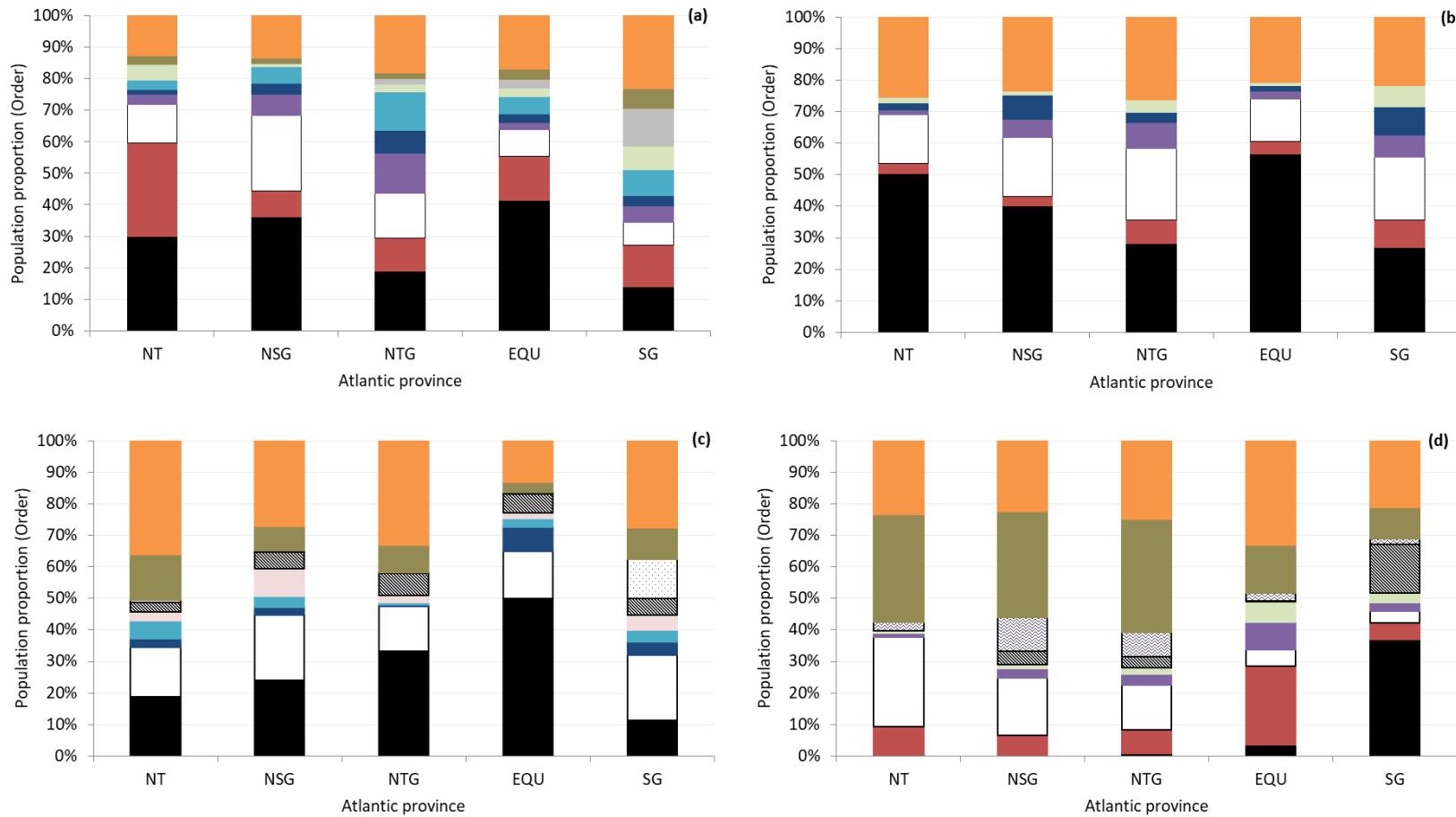


Figure 6.

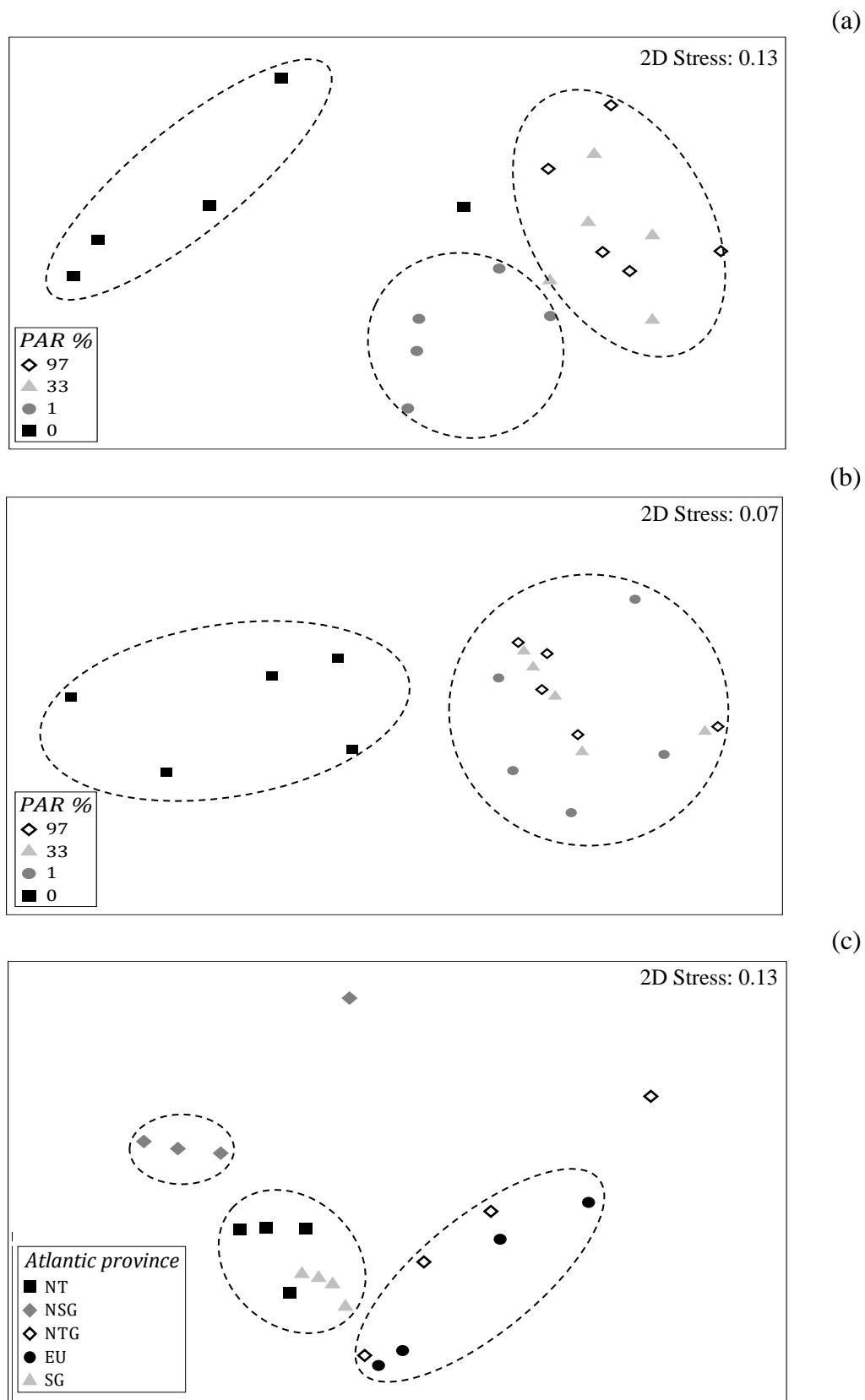


Table 1. Summary of rates of methanol uptake (dissimilation and assimilation), methanol concentrations, bacterial leucine incorporation (BLI) and production (BP), numbers of heterotrophic bacteria (BN), *Prochlorococcus* (Pros) and *Synechococcus* (Syns). Values given are average \pm standard deviation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

| | Overall | NT | NSG | NTG | EQU | SG | ST |
|----------------------------------------------------------------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
| Methanol dissimilation (nmol L ⁻¹ h ⁻¹) | 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 ⁻² nmol L ⁻¹ h ⁻¹) | 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 ⁻¹ h ⁻¹) | 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 |
| ^a BP(TCF) (ng C L ⁻¹ h ⁻¹) | 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 |
| ^b BP (ECF) (ng C L ⁻¹ h ⁻¹) | 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 ⁵) (cells mL ⁻¹) | 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 ⁵ cells mL ⁻¹) | 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 ⁴ cells mL ⁻¹) | 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) |
| ^c 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 |

^aTheoretical conversion factor (TCF) 1.55 kg C mol leu⁻¹, ^bempirical conversion factor (ECF) 0.51 kg C mol leu⁻¹, ^cFrom Beale et al., (2013)