

Interactive comment on “Rapid biological oxidation of methanol in the tropical Atlantic: significance as a microbial carbon source” by J. L. Dixon et al.

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Anonymous Referee #2

This study follows a previous work published in the ISME journal (“Microbial methanol uptake in northeast Atlantic waters”; Dixon et al., 2010), about microbial uptake of methanol into particulate biomass, oxidation rates to CO₂ and biological turnover time of methanol in temperate North Atlantic waters. In both studies they apply the same methodology to compare uptake and oxidation rates and turnover times of methanol in tropical and temperate North Atlantic waters.

There was no data from tropical/oligotrophic stations in Dixon et al., 2011 which fo-
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cussed on the uptake and use of methanol as a carbon source for growth only in coastal, shelf and European waters.

In fact, they include the same 3 stations from the Dixon’s et al. 2011 study to compare uptake rates into particles. Additionally, they measure bacterial leucine uptake rates and estimate the methanol contribution to bacterial carbon demand

The main conclusions of the study are: 1. Measured concentrations of methanol in subtropical North Atlantic waters are up to 300nM (more than 3 times higher than in temperate NA waters), with uptake rates up to 146nM d⁻¹ (about 5 times higher than in temperate NA waters) and turnover time as low as 1 day -extremely low turnover time compared with the lowest turnover time (11 days) estimated for shelf waters in the temperate NA. 2. Methanol contributes on average 13% to BCD in the Central NA Gyre (!) – with a maximum of 54% 3. Based on air to sea gas exchanges estimations, they conclude that the atmosphere is not a major source for methanol and suggest an “in situ” (as yet unidentified) methanol source.

(12) The paper is well written and concise, although sometimes is difficult (at least for me) to follow the origin of data (i.e. which data correspond to actual measurements and which have been obtained from the literature or averaged from other studies).

The only data which was not determined on concurrent studies was in situ methanol concentrations for stations 1-6 which is clearly explained in Table 2 ‘f’ superscript. We have used literature values for empirical carbon conversion factors which is also clearly explained in Table 1 superscript ‘a’.

(13) My main concern with this work is the great degree of assumptions used to derive their conclusions. To publish this paper, I think the authors should constrain better the uncertainty in their estimates (although then the conclusions might change).

The assumptions used in this paper are clearly indicated and have precedent in other peer reviewed papers, as indicated in the manuscript. The cumulative uncertainty has

been well constrained i.e. upper and lower limits on the % methanol contribution to BCD as a function of chl a and is clearly shown and explained by the dotted lines in Figure 3. The overall conclusions and hypothesis do not change.

Major comments: (14) . Methanol concentrations, and estimates of methanol oxidation to CO₂ (E) and uptake rates into particles (G). Methanol concentrations in seawater were not measured at stations where ¹⁴C labelled methanol uptake/oxidation experiments were performed (stations 1, 2 and 3, close to eutrophic-mesotrophic NW Africa coastal transition zone). Since E and G are derived from the product of “k” (apparent rate constant) multiplied by the in situ concentration of methanol, it is necessary to know the latter to have a precise estimate of the rates. Moreover, calculations of turnover time, the ratio E:G and the %Carbon from methanol contributing to BCD depend also on the in situ methanol concentration (Table 2).

Stations 1 and 2 were not influenced by the NW African upwelling and were oligotrophic when we sampled. Station 3 was located within the local influence of the Cape Verde islands as discussed in section 3.1.1. However the nutrient concentrations have now been added to Table 1 for information and clarity on the tropic status of the stations. The turnover times and E:G ratio are completely independent of the in situ methanol concentrations and are derived purely from the radiochemical experiments i.e. the turnover time is ‘k-1’ as explained in Table 2 superscript ‘c’. ‘k’ is explained in methods section 2.2. (3903 lines 11-14). E:G is the ratio of k(oxidation divided by k (uptake into particles). Methanol oxidation rates in nmol L⁻¹ h⁻¹ only require the in situ methanol concentrations which have been estimated using the best available data, especially considering that there are only 2 papers published containing any seawater methanol concentrations.

(15) The authors use (for their calculations) a range of values of methanol concentrations derived from in situ measurements in the North Atlantic during the AMT-19 cruise (across the centre of the oligotrophic NA subtropical Gyre). Given the large variability observed in methanol concentrations between regions (Dixon et al 2010, Williams et

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al., 2004), I understand that the authors cannot extrapolate the open ocean values of the AMT cruise to the near shelf stations of this study. At best they should include a wider range of concentrations, including eutrophic regions too, but in that case I am not sure how transcendent would be the conclusions.

The methanol concentrations assumed for station 6 (L4 coastal) were based on the average concentrations determined at this station during the same season i.e. May/June but in a different year which is the best match we can do. The assumed methanol concentrations used for stations 4 and 5 (more seasonally eutrophic in nature) were based on concentrations determined at the same latitude, and very close longitude, but during a different time on an AMT cruise (N=6). They do take full account of the variability seen in this region i.e. 48-80 nM which was based on actual measurements. This data was also used in the ISME paper of Dixon et al., 2011. So we do not agree with the reviewer, as the concentrations we have used have been closely matched already to take into account and reflect the tropic conditions of different regions in the Atlantic ocean. This has been clearly explained in the footnotes of Table 2.

(16a). Bacterial production (BP), bacterial respiration (BR), bacteria growth efficiency (BGE) and carbon demand (BCD) BP was calculated using a carbon to leucine conversion factor (CF) of 0.73 kgC mol leu⁻¹. The authors claim that this value represents an average value used in other studies close to their sampling locations. However, the fact is that “the sampling locations” in the tropical NA spans a transition zone from eutrophic to oligotrophic waters.

These stations were oligotrophic when we sampled them – which is now evident by the inclusion of nutrient data (amended Table 1). Furthermore, we have spent a lot of time comparing our sampling locations, seasons and depth (surface only) with other literature data to choose the most appropriate CF, and stand by our claim that this value used does represent an average value used in the literature for similar sampling conditions. When we sampled these locations they were simply not in a transition zone, as they are not in areas regularly influenced by the Mauritanian upwelling, we think that

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the reviewer is mistaken. We have subsequently in 2009 undertaken a further cruise specifically designed to be in transitional waters that are heterogeneously affected by the Mauritanian upwelling. Stations 1 and 2 of this publication were from SOLAS INSPIRE cruise and were specifically chosen, using satellite data, for their oligotrophic nature. This has been further shown by the extremely low nM nutrient concentrations.

(16b). Eutrophic mesotrophic stations closer to the upwelling (i.e. 1, 2 and 3, used to calculate de E:G ratio) would presumably have a CF >1.5 (Alonso-Saez et al., 2007; see also discussion in del Giorgio et al. 2011; L&O 56, 1-16), whereas the most oligotrophic stations would presumably have a CF closer to 0.2 (Alonso-Saez et al., 2007). Moreover, empirical CFs in temperate waters may vary from <0.5 to >2. This variability –consistently found in coastal-open ocean gradients- should be considered, unless you estimate the CFs for your study taking into account leucine respiration during your experiments (Alonso-Saez et al 2007).

Please see response above. Stations 1 and 2 were totally oligotrophic during our sampling time, so comments about eutrophic-mesotrophic- eutrophic gradients affecting our stations 1, 2 and 7-12a are not relevant. Yes, of course there are occasions in the literature e.g. Alonso-Saez et al., 2007, Martinez-Garcia et al., 2010 when CF for oligotrophic stations have been reported to be lower. To reflect this, and show the variability this makes to our calculations and results/conclusions we have already allowed for this and used conversion factors ranging between 0.17 and 1.55 kg C mol Leu-1 to construct the upper and lower limits in Figure 3. This has all been clearly explained in the legend of Figure 3. Perhaps the reviewer missed this?

(16c). BR –that is used together with BP to calculate BCD- was derived from the general equation of Robinson (2008), relating BP and BR: $BR=3.69 BP^{0.58}$. This equation explains only 52% of the variance of BR (at a global scale!); hence this must be considered in the final calculations of BCD. Perhaps would be better to derive an equation from published concomitant values of BR and BP from the regions of study, or use a range of BR values published from the same region.

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Bacterial carbon demand in this study was calculated as BP (which was measured in all cases) divided by BGE. The BGE used was taken as the average of 2 independently determined values in order to allow for this variance and to try and analyse how accurate our calculations of BR derived from BP as above actually were. i.e. we also derived BGE using in situ determined chlorophyll a concentrations. This is all in Table 1. The resulting BGE we used in our calculations was an average of the 2. We think that this is an acceptably robust way to determine BGE. For the oligotrophic stations the relationship between BGE (chl a) and BGE (BP & BR) was actually $BGE (chl a) = 0.6 (BGE (BP \& BR) + 0.027$ ($r=0.658$, $P \leq 0.05$, $n=14$).

(16d). BGE- The derived BGE, after several assumptions in BP and BR (see above), are very low (2-4%). I doubt they represent realistic BGE for the whole region of study. From the 3 papers cited to compare with the results of this study (Alonso-Saez et al, 2007, Moran et al 2007 and Robinson 2008), only in the first one BGE is estimated from direct measurements of BP and BR. In the Alonso –Saez et al study (spanning a zonal gradient of productivity across the same sampling region of this study) BGE ranges from 1% to 56% (average >10%), and correlates well with CF. In summary, I feel the conclusions of this study would be very different, taking into account the uncertainty and variability in the estimates. I believe you cannot apply the same average values of methanol concentration, CF and BR (which were not measured during the cruise!) to all the stations, due to the trophic variability across your sampling regions.

Please see comment above regarding also determining BGE via in situ chlorophyll a concentrations. We think that actually the BGE determined for the oligotrophic stations are very reflective of this area. BGE varies from 1-56% across a gradient of productivity which we simply do not have in these stations. Stations 5 and 6 at the shelf break and in coastal areas actually show BGE (chl a) of 9 and 16% which are higher, reflecting the more mesotrophic conditions. So we feel that we have taken into account all reasonable uncertainty and this is clearly shown in Figure 3. Figure 3 represents a new working hypothesis that has never been shown before which can, and am sure will, be

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tested in the future. We feel that it is also important to bear in mind that this paper is about reporting some of the first measurements of methanol concentrations and microbial methanol loss fluxes in the literature, and makes the first attempts (which are clearly defined) at comparing microbial methanol use with microbial carbon demand. The calculation of BCD and BGE is a highly debatable, and sometimes controversial, area of research within its own right and this paper is not intended for that purpose. Moreover, it uses the best available concurrent datasets in comparison with best available and comparable literature data to propose a new hypothesis. Interestingly, Sun et al (from Giovannoni research group) has just published a paper identifying that SAR11 Alphaproteobacteria, the most abundant heterotrophs in the oceans, have genome encoded pathways for the oxidation of C1 compounds, such as methanol to produce energy only and conclude that C1 oxidation might be a significant conduit by which dissolved organic carbon is recycled to CO₂ in the upper ocean. So we now have the molecular identification of the pathway in the most abundant heterotroph in the oceans which totally reinforces our microbial methanol flux findings (Sun et al., 2011, PLoS ONE, Volume 6, e23973).

Minor comments 1. P 3901, L 28: “: : :microbial methanol turnover times of 12-24 days: : :” Shouldn't it be 11-33 days instead 12-24 (Dixon et al 2011)?

No, 11-33 days would include the data from the off shelf station in Table 2 of Dixon et al., 2011. 12-24 days just covers the coastal and shelf stations as stated in the text 3901 (lines 28-29)

2. Page 3902, Line 1: “: : :nutrient limited tropical waters: : :”Were the waters at stations 1,2 and 3 also nutrient limited?

Yes. Nutrient data added to Table 1 and see response to (14) above.

3. P 3902, Section 2.1. Please, include dates for the cruise

We do not think this is necessary because the sampling dates are clearly shown in

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Tables 1 and 2.

4. P 3904, Section 2.4. How do C14 uptake experiments during 6 hours (stations 1-3) compare with experiments from dawn to dusk (stations 7-12)? Did you check for DOC14 excretion?

We presume you are referring here to the measurements of primary productivity using C14 bicarbonate. These experiments are included just to give the reader an indication of the production status of the water, and are not meant to be a focus. The comparison of PP between 6 hours and close to 12 hours incubation is not known. However, the important consideration is that neither strategy included dark respiration periods. Assuming that C14 bicarbonate uptake is linear between 6-12 hours then the data should be comparable. ¹⁴C bicarbonate excretion i.e. DOC production was not undertaken for these samples..

5. P 3906, 2nd parag. With a single day-night cycle it is difficult to see whether the pattern is reproducible at each station. For instance, it is not evident a rise before dawn at stations 1 and 2. (Notice that the label for station 3 is lacking in the legend)

Yes, however time constraints and other scientist's requirements for the cruise precluded longer diel cycles being logistically possible.

Label for station 3 added.

6. P 3907, section 3.1.2: “Iberian peninsula”?... It seems to me that the stations are far from the Iberia peninsula.

This is the closest land mass. Title changed to ‘West of the Iberian ...’

7. P 3907, L 17-18: “: : :away from the influence of upwelling or continental inputs e.g. dust”. Dust storms cross the Atlantic Ocean and reach the Florida (US) coast. Stations 7-12 are thus potentially under the influence of dust deposition.

Yes, but they were not influenced by ‘dust’ when we sampled them as evidenced by the

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supporting data in Tables 1 and 2 (now including nutrient data). Text changed to reflect this.

8. Table 2. Why station 4 has a range in longitude (16-18W)?

Because it is the average of 3 days i.e. 1st, 4th and 6th July stations as stated in the sampling description in Tables 1 and 2 which were on the same latitude but slightly varying longitude.

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