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Mismatch between community respiration and the contribution of heterotrophic bacteria in the NE Atlantic open ocean: What causes high respiration in oligotrophic waters?

by Xosé Anxelu G. Morán¹, Valesca Pérez² and Emilio Fernández²

ABSTRACT

Heterotrophic bacteria have long been considered as the major respirers of the open ocean. During a survey in the eastern N Atlantic Subtropical Gyre we measured ³H-leucine heterotrophic bacterial production (BP), ¹⁴C particulate primary production (PPP) and the O₂ community metabolism rates: gross primary production (GPP), net community production (NCP) and community respiration (CR). We estimated heterotrophic bacterial respiration (BR) from BP and literature models of bacterial growth efficiency \[ \text{BGE} = \text{BP}/(\text{BP + BR}) \]. BP was an order of magnitude lower than PPP (integrated means of 17 ± 2 and 207 ± 29 mg C m⁻² d⁻¹, respectively). Although volumetric PPP and GPP were significantly correlated \( r = 0.51; p = 0.009; n = 25 \), CR (1120 ± 122 mg C m⁻² d⁻¹) bore no significant relationship with either primary or heterotrophic bacterial production and exceeded GPP (578 ± 117 mg C m⁻² d⁻¹) at 5 out of 6 sampling stations. Integrated values of CR were only significantly correlated, but negatively, with chlorophyll \( a \) values \( r = -0.95; p < 0.001; n = 6 \). Integrated BR tended to decrease with increasing CR and, with a mean estimated BGE of 6%, it accounted on average for a much lower fraction of CR (33 ± 7%) than currently assumed. By estimating the contributions of other trophic groups a large amount (48 ± 10%) of the measured CR remained unaccounted for. This gap in respiration estimates casts some doubt on the magnitude of the net heterotrophic balance frequently observed in oligotrophic waters from changes of O₂ over 24 h dark in vitro incubations.

1. Introduction

Determination of plankton-mediated biogeochemical fluxes remains a priority task for biological oceanographers in the context of global change, especially in oceanic areas poorly represented in sampling strategies. The balance between biological production and consumption of organic matter in the upper layers of the ocean is an essential component of models aiming at resolving the global cycling of elements. This metabolic balance in the open ocean – summarized by the variable net community production (NCP) as the result of gross primary production (GPP) minus community respiration (CR) – has generated one of

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the most active debates in biological oceanography of the last years (Geider, 1997; del Giorgio et al., 1997; Williams, 1998; Duarte and Agustí, 1998). A focal geographical location for this controversy has been the N Atlantic Subtropical Gyre, characterized by very low values of phytoplankton biomass and production (Marañón et al., 2000; Teira et al., 2005). The paucity of measurements extended over long periods and hence, our inability to determine the scales of coupling between GPP and CR, has apparently resulted in an overestimation of net heterotrophic periods relative to net autotrophic or balanced ones (Karl et al., 2003). However, research in the eastern N. Atlantic has systematically found that CR exceeds GPP for most of the year (Duarte et al., 2001; González et al., 2002; Serret et al., 2001; Robinson et al., 2002a) apparently rendering this region a subsidized system in terms of organic carbon loading.

In spite of the slowly growing dataset of oceanic CR data, considerable uncertainty on its magnitude remains (Hansell et al., 2004), which becomes even larger when evaluating the possible responses to global change (del Giorgio and Duarte, 2002). In autumn 2001 we performed a cruise that crossed the subtropical and tropical provinces of the N. Atlantic Subtropical Gyre (Longhurst, 1998) and the results add to the above-mentioned empirical basis for a negative NCP in this region (Morán et al., 2004). Yet, for NCP to be a good descriptor of the role of biota in biogeochemical carbon cycling we must be confident of its two components. In spite of the well-known uncertainties associated to the radiocarbon uptake technique, GPP can be compared with simultaneous measurements of $^{14}$C-based photosynthesis (Bender et al., 1999; Laws et al., 2000), but CR is more difficult to constrain with other variables. However, an attempt can be made from measured rates of activity of heterotrophic and autotrophic plankton and published growth efficiencies of different trophic groups (Robinson et al., 2002a, b; Robinson and Williams, 2005). This comparative exercise has yielded estimated CR values within the ±50% range of measured rates, hence supporting the validity of the approach despite the uncertainties associated to each respiration estimate.

We focus here on the relationship between the respiration of the whole planktonic community and heterotrophic bacterial respiration (BR), as estimated from measured heterotrophic bacterial production (BP) and from computed bacterial growth efficiency \[ \text{BGE} = \frac{\text{BP}}{\text{BP+BR}} \] values based on empirical models. The rationale of this comparison is that heterotrophic bacteria have been frequently considered as the main respirers of open-ocean microbial communities (e.g., Williams, 1981; Pomeroy et al., 1995; Rivkin and Legendre, 2001). The demand for a better knowledge of oceanic CR and the need to enlarge the as yet scarce dataset available (Williams and del Giorgio, 2005) must be accompanied by periodic assessments of the reliability of the techniques used. If, as we show here, BR turns out to be much lower than CR, it can be due to an underestimation of BR, an overestimation of CR or both. Each type of measurement has its own assumptions and possible sources of error, but most importantly, BR and CR were obtained with substantially different incubation periods. We relate our findings with the known effect of long incubations on enclosed microbial plankton communities. Our results show that the
absolute values of CR measured in this area may be difficult to reconcile with independent estimates of the contribution of the various microbial plankton groups.

2. Material and methods

Data of community metabolism, particulate primary production and bacterial activity were collected in successive days at 5 to 7 depths of 6 stations extending from 29 to 10N in autumn 2001 during the cruise CIRCANA-I on board the R/V Hesperides. More information on hydrography, size-fractionated chlorophyll \(a\) and the abundance of microbial plankton can be found in Morán et al. (2004). A short description of the most relevant findings is included in Section 3.

Gross primary production (GPP), net community production (NCP) and dark community respiration (CR) were determined from in vitro changes in dissolved oxygen after light and dark bottle incubations over 24 h. At each depth 4 replicate 120 mL borosilicate bottles were fixed immediately for initial \(O_2\) concentrations, 4 bottles were kept in the dark and 4 bottles were incubated under \textit{in situ}-simulated irradiance at surface temperature in an on deck incubator. After the incubation, dissolved \(O_2\) concentration was measured with an automated Winkler titration system using a potentiometric end point (Pomeroy et al., 1994). Aliquots of fixed samples were delivered with a 50-ml overflow pipette. Production and respiration rates were calculated from the difference between the averaged light and dark replicates and time zero analyses: NCP = measured \(\Delta O_2\) in light bottles (mean 24 h \([O_2] – \text{mean initial } [O_2]\)); CR = measured \(\Delta O_2\) in dark bottles (mean initial \([O_2]\) – mean 24 h \([O_2]\)); GPP = NCP + CR. Since all bottles were flushed with surface seawater, a \(Q_{10}\) of 2 was applied for correcting rates of deep samples, which were incubated at higher values than their \textit{in situ} temperature. Although the choice of photosynthetic (PQ) and respiratory (RQ) quotients for converting oxygen to carbon units may result in different estimates, this is a minor source of error (Williams and del Giorgio, 2005). For comparative reasons we used a PQ of 1.25 and a RQ of 0.89 (Robinson et al., 2002a; Robinson and Williams, 2005). The comparison of CR measurements with estimates of respiration of bacteria and other planktonic trophic groups were made in C units.

The rate of particulate primary production (PPP) was estimated with the \(^{14}\text{C}\) method as the sum of the production in three size-fractions (>20, 2 - 20, <2 \(\mu\)m). Three clear plus one black 70 ml polypropylene bottles were filled with seawater collected from 5 - 6 depths from the surface down to the 1% irradiance level. After addition of 740 KBq of NaH\(^{14}\text{CO}_3\) to each bottle, they were incubated for 5-7 h starting at \(\sim 1100\) local time in the same incubator used for oxygen measurements. After incubation, samples were vacuum-filtered at <50 mm Hg and collected onto polycarbonate filters. Inorganic \(^{14}\text{C}\) was eliminated after exposure to concentrated HCl fumes for 12 h. Filters were placed into vials and 4 ml of scintillation cocktail was added before measurement of radioactivity in a LKB Winspectral 1414 liquid scintillation counter. Disintegrations per minute (dpm) were converted into carbon units by using a constant concentration of dissolved inorganic carbon of 25700 mg C m\(^{-3}\) and an isotope discrimination factor of 1.06. The same temperature correction used for oxygen was
applied to deep, lower temperature samples. Daily values were obtained by multiplying hourly rates by total hours of sunlight.

The net production of heterotrophic prokaryotes, commonly termed bacterial production (BP) although the method does not distinguish between Bacteria and Archaea, was estimated from °H-leucine incorporation rates as described in Smith and Azam (1992). Four replicates ± 2 immediately killed controls of 1 mL samples were inoculated with 50 nmol °H-leucine and incubated for 1 - 2 h in the dark at surface temperature. We performed three extra experiments at station 6 comparing light and dark incubations and found that incubation in the light resulted in slightly higher leucine incorporation rates in two out of three experiments, with an overall mean value of 15% ± 13%. Leucine incorporation rates were corrected for differences of temperature following Zubkov et al. (2000). To convert Leu to C units, we used a value of 0.73 kg C mol Leu⁻¹, which was the average of three empirical determinations carried out in surface waters on two different cruises crossing the region (see Morán et al. (2004) for details). The use of a single factor for all data is justified since the three original conversion factors were rather similar (0.80, 0.95 and 0.46 kg C mol Leu⁻¹) and clearly well below the theoretical ones (1.55 or 3.1 kg C mol Leu⁻¹). Two additional determinations during those cruises carried out at 150 and 250 m, 0.50 and 0.73 kg C mol Leu⁻¹, respectively, give further support to the use of a common factor for all depths. Since bacterial heterotrophic respiration (BR) was not measured in the cruise, we calculated it as BP/BGE – BP. There are several models available in the literature that predict bacterial growth efficiency (BGE) from other physical or biological variables (Table 1). When applied to our data, individual BGE values obtained with these models ranged from 2.1 to 23.8%. The specific choice for carbon budget calculations is discussed below.
Table 1 also includes the calculations used to estimate the contribution of other planktonic trophic groups to total community respiration. Although incubations with the $^{14}$C method for estimating PPP yields values somewhere between gross and net primary production, there is experimental evidence relating PPP and GPP measurements with corresponding phytoplankton respiration. Phytoplankton (including *Prochlorococcus* and *Synechococcus* cyanobacteria) was thus assumed to respire 40% (2 x 20%) of daylight PPP when exposed to a 12:12 h light:dark period (Marra and Barber, 2004), or 35% of GPP (Duarte and Cebrián, 1996). For bacterian grazers we assumed that they consume all the daily BP (Zubkov *et al.*, 2000), that 20% of the assimilated carbon is egested (Nagata, 2000; Robinson *et al.*, 2002a) and that they use the remaining fraction with a gross growth efficiency of 25% (Straile, 1997). The contribution of herbivores was made on the observation that they take up on average 80% of daily PPP in the subtropical Atlantic (Quevedo and Anadón, 2001) assuming the same egested fraction and growth efficiency as for the bacterivores.

Stratification is a distinct property of the eastern N. Atlantic upper ocean, so this ecosystem might be better split into two self-contained layers, the mixed layer and that below the thermocline. However, our analysis would be seriously compromised by restricting it to the upper mixed layer, with only two to three depths sampled within it. Integrated values are, therefore, given for the photic layer (1% of surface irradiance), which ranged from 60 to 120 m during this survey. This also allows a direct comparison with most previous reports for the region (Duarte *et al.*, 2001; Serret *et al.*, 2001; Agustí *et al.*, 2001; González *et al.*, 2002).

3. Results and discussion

In a strongly stratified water-column, with a 4.7 to 15.1°C difference between the surface and 100 m depth, both integrated chlorophyll $a$ and particulate primary production (PPP) were consistently low, with respective ranges of 16-28 mg m$^{-2}$ and 135-321 mg C m$^{-2}$ d$^{-1}$. PPP measurements for the region usually do not exceed 300 mg C m$^{-2}$ d$^{-1}$ except in winter (Marañón *et al.*, 2000; Teira *et al.*, 2005). Picoplanktonic organisms were the dominant contributors to total phytoplankton biomass (70%) but not production (38%, cf. 43% nanoplanckton, Morán *et al.*, 2004). Differential picophytoplanktonic contributions to biomass and production are a persistent feature of these waters (Fernández *et al.*, 2003). The abundance of heterotrophic bacteria in the photic layer averaged 9.4 ± 1.3 $10^5$ cells mL$^{-1}$ with most of the cells belonging to the low nucleic acid (LNA) group (55 ± 2%), suggesting a low overall activity of the community (Gasol *et al.*, 1999). Indeed, integrated heterotrophic bacterial production (BP) was on average one order of magnitude lower than PPP (15 ± 2 mg C m$^{-2}$ d$^{-1}$). BP values were somewhat lower than those found in previous surveys conducted farther north (González *et al.*, 2001; Teira *et al.*, 2003) or in the Atlantic Meridional Transects (AMT) 3 and 4 (Zubkov *et al.*, 2000), but similar to the AMT-11 (Pérez *et al.*, 2005). The large difference between BP and PPP, in agreement with previous
work (Hoppe et al., 2002; Teira et al., 2003), was also reflected in the estimated biomass of both planktonic compartments with a phytoplankton:heterotrophic bacteria biomass ratio of 1.32 ± 0.16, using the C to chl a empirical model of Marañón et al. (2000). Our CR values (104.9 ± 11.4 mmol O$_2$ m$^{-2}$ d$^{-1}$) were in good agreement with previous measurements (González et al. 2001, 2002; Robinson et al., 2002a; Serret et al., 2001, 2002), confirming a relative constancy at ~ 100-150 mmol O$_2$ m$^{-2}$ d$^{-1}$. CR greatly exceeded GPP at all but one station, with a mean negative NCP of -43.6 ± 14.9 mmol O$_2$ m$^{-2}$ d$^{-1}$. CR was also less variable (CV=27%) than GPP (CV=50%) or NCP (CV=83%), as found by other studies (Duarte and Agustí, 1998; Serret et al., 2001; Arístegui and Harrison, 2002; Robinson and Williams, 2005). Therefore, our community metabolism data support the overall net heterotrophic nature of open-ocean, oligotrophic waters of the N. Atlantic noted by these and other researchers (Agustí et al., 2001).

A comparison of pooled volumetric data of community metabolism and particulate primary production expressed in carbon units is shown in Figure 1. GPP was higher than CR at only 3 out of 26 determinations (Fig. 1A). Although in situ primary production rate has been claimed as one of the main drivers of CR (del Giorgio et al., 1997; del Giorgio and Duarte, 2000) no significant correlation was found between PPP or GPP and CR, either for volumetric (Fig. 1A) or integrated units (data not shown) in our dataset. The uncoupling between primary production and respiration has also been reported by Arístegui and Harrison (2002) for a region farther north. Being a related variable, NCP was as expected significantly correlated with GPP (Fig. 1B, \( r = 0.60; p = 0.001; n = 25 \)). Likewise, volumetric GPP was also significantly and positively correlated with independent measurements of PPP (Fig. 1C, \( r = 0.51; p = 0.009; n = 25 \)), although it exceeded it 3-fold on average, similar to the findings of Pérez et al. (2005) for the equatorial Atlantic. Both methods of estimating primary productivity often yield similar differences (Bender et al., 1999; Laws et al., 2000; Robinson et al., 2002a). With a PQ of 1.25, the mean molar ratio of PPP to GPP was 0.57 ± 0.10, slightly higher to that obtained in other open ocean studies using a PQ of 1.2 (Bender et al., 1999; Laws et al., 2000; Pérez et al., 2005). Among the causes explaining this discrepancy are the high rates of DOC production in oligotrophic waters (Karl et al., 1998), which may make up to 60% of total photosynthetic carbon fixation. Dissolved primary production would be measured as GPP but obviously not in PPP. In addition, phytoplankton respiration is included in GPP but not in PPP determinations. Although the fraction of primary production directly usable by bacteria was not measured, PPP was still significantly correlated with BP (see Fig. 8A in Morán et al., 2004), as expected from a direct dependence of heterotrophic bacterial growth on recently fixed photosynthate.

Individual heterotrophic bacterial production rates ranged from 0.002 to 0.69 mg C m$^{-3}$ d$^{-1}$, with less variable integrated values (10.4-22.5 mg C m$^{-2}$ d$^{-1}$). Using López-Urrutia and Morán (2007) model of BGE (Table 1), mean integrated carbon demand (BP+BR) was 303 ± 28 mg C m$^{-2}$ d$^{-1}$, more than the estimated supply of recent photosynthate (Morán et al., 2004). Most of the carbon taken up by heterotrophic bacteria was respired, with the
corresponding BR ranging more than two-fold from 189 to 453 mg C m\(^{-2}\) d\(^{-1}\) (Table 2). The relationship between BR and CR was just the opposite of that expected (Fig. 2A), i.e., BR decreased with CR, although the correlation was not significant due to the small number of samples \((r = -0.64; p = 0.17; n = 6)\). Mean ± SE contribution of heterotrophic bacterioplankton to total respiration was 33 ± 7% (Fig. 2B, Table 2). One could argue that the choice of BGE seriously influenced our analysis. In fact, there is appreciable disagreement between different BGE models (Table 1). Using the model of del Giorgio and

Figure 1. Relationship between \(^{14}\text{C}-\) and \(\text{O}_2\)-based volumetric measurements of primary production and respiration at the 6 stations, using a PQ of 1.25 and a RQ of 0.89. Notice the different scale of the Y-axis in the 3 plots. Continuous lines represent 1:1 values and dashed lines represent significant correlations.
A weighted BGE value of 2.4% was obtained with the model of Rivkin and Legendre (2001). The model we used (López-Urrutia and Morán, 2007) lay in between these two widespread models (Fig. 2B), with a weighted BGE value of 5.8%. Broad empirical relationships may not be representative of small-scale studies such as this one, but even with the range of possible BGE values shown in Figure 2B the differences between BR and CR persist. BGE values rarely exceed 20% in open ocean oligotrophic waters (del Giorgio and Cole, 1998). In a recent survey in the NE Atlantic, Alonso-Sáez et al. (2007) found an average BGE for offshore stations of 9%, very similar to the mean they obtained in a review for oligotrophic open ocean waters (12%, range 1-43%). Therefore, we feel that the range of BGE values used (4.4-7.2%) was reasonable for constraining the upper limit of BR in the study region. Any higher BGE would obviously further decrease the share of heterotrophic bacteria to CR shown in Figure 2B. Hence, contrary to the analysis of Robinson et al. (2002a), who estimated a four-fold greater BP from bacterial abundances and subsequent application of the del Giorgio and Cole (1998) model, our results strongly indicate that the bulk CR in the region was not readily attributable to BR. In order to arrive at an average BR:CR ratio of ~0.90, a rough average of the estimation given by Robinson et al. (2002a) for these waters, the mean BGE should have been as low as 1.3%. Yet, it seems difficult to accept that heterotrophic bacteria respire 99% of the assimilated organic carbon for long periods of time.

The mismatch between BR and CR lead us to look for other possible contributions to the measured respiration rates as shown in Table 2. We decided to estimate phytoplankton respiration with the independently measured PPP rather than a variable that results from the same variable of interest (GPP=NCP+CR). Respiration by autotrophs thus amounted to a mean 8% of CR (Table 2), and a maximum of 17%. Based on GPP measurements, mean ± SE phytoplankton contribution to CR (data not shown) would have been 20 ± 5%. Besides the lack of relationship with GPP or PPP, CR was highly negatively correlated

Table 2. Estimated respiration rates and relative contribution to total community respiration (among parentheses, %) of different plankton trophic groups at the 6 stations. Details and assumptions of the calculations are given in Table 1. Mean ± SE are also given. ?: unaccounted for.

<table>
<thead>
<tr>
<th>Station</th>
<th>Community respiration</th>
<th>Bacteria</th>
<th>Phytoplankton</th>
<th>Bacterivores</th>
<th>Herbivores</th>
<th>?</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(mg C m⁻² d⁻¹)</td>
<td>(mg C m⁻² d⁻¹)</td>
<td>(mg C m⁻² d⁻¹)</td>
<td>(mg C m⁻² d⁻¹)</td>
<td>(mg C m⁻² d⁻¹)</td>
<td>(mg C m⁻² d⁻¹)</td>
</tr>
<tr>
<td>1</td>
<td>1407</td>
<td>268 (19.0)</td>
<td>54 (3.8)</td>
<td>6 (0.4)</td>
<td>65 (4.6)</td>
<td>1014 (72.1)</td>
</tr>
<tr>
<td>2</td>
<td>762</td>
<td>453 (59.4)</td>
<td>57 (7.5)</td>
<td>9 (1.1)</td>
<td>68 (9.0)</td>
<td>175 (23.0)</td>
</tr>
<tr>
<td>3</td>
<td>762</td>
<td>370 (48.5)</td>
<td>128 (16.8)</td>
<td>13 (1.7)</td>
<td>154 (20.2)</td>
<td>97 (12.7)</td>
</tr>
<tr>
<td>4</td>
<td>1415</td>
<td>357 (25.2)</td>
<td>87 (6.1)</td>
<td>10 (0.7)</td>
<td>104 (7.4)</td>
<td>858 (60.6)</td>
</tr>
<tr>
<td>5</td>
<td>1251</td>
<td>189 (15.1)</td>
<td>99 (7.9)</td>
<td>8 (0.6)</td>
<td>118 (9.5)</td>
<td>838 (66.9)</td>
</tr>
<tr>
<td>6</td>
<td>1126</td>
<td>346 (30.7)</td>
<td>73 (6.5)</td>
<td>10 (0.9)</td>
<td>87 (7.7)</td>
<td>610 (54.1)</td>
</tr>
<tr>
<td>Mean ± SE</td>
<td>1120 ± 122</td>
<td>330 ± 37</td>
<td>83 ± 11</td>
<td>9 ± 1</td>
<td>99 ± 47</td>
<td>599 ± 156</td>
</tr>
<tr>
<td></td>
<td>(100)</td>
<td>(33.0 ± 7.1)</td>
<td>(8.1 ± 1.8)</td>
<td>(0.9 ± 0.2)</td>
<td>(9.7 ± 2.2)</td>
<td>(48.2 ± 10.0)</td>
</tr>
</tbody>
</table>

Cole (1998), a weighted BGE value of 2.4% was obtained. A higher value (12.3%) was obtained with the model of Rivkin and Legendre (2001). The model we used (López-Urrutia and Morán, 2007) lay in between these two widespread models (Fig. 2B), with a weighted BGE value of 5.8%. Broad empirical relationships may not be representative of small-scale studies such as this one, but even with the range of possible BGE values shown in Figure 2B the differences between BR and CR persist. BGE values rarely exceed 20% in open ocean oligotrophic waters (del Giorgio and Cole, 1998). In a recent survey in the NE Atlantic, Alonso-Sáez et al. (2007) found an average BGE for offshore stations of 9%, very similar to the mean they obtained in a review for oligotrophic open ocean waters (12%, range 1-43%). Therefore, we feel that the range of BGE values used (4.4-7.2%) was reasonable for constraining the upper limit of BR in the study region. Any higher BGE would obviously further decrease the share of heterotrophic bacteria to CR shown in Figure 2B. Hence, contrary to the analysis of Robinson et al. (2002a), who estimated a four-fold greater BP from bacterial abundances and subsequent application of the del Giorgio and Cole (1998) model, our results strongly indicate that the bulk CR in the region was not readily attributable to BR. In order to arrive at an average BR:CR ratio of ~0.90, a rough average of the estimation given by Robinson et al. (2002a) for these waters, the mean BGE should have been as low as 1.3%. Yet, it seems difficult to accept that heterotrophic bacteria respire 99% of the assimilated organic carbon for long periods of time.

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with chlorophyll a (Fig. 3, $r = -0.95; p < 0.001; n = 6$), contrary to the findings of Robinson et al. (2002a, b), strongly suggesting a negligible contribution of phytoplankton to total CR in our samples. Autotrophic contribution is only a major portion of CR during phytoplankton blooms (Robinson et al., 2002a, b; Robinson and Williams, 2005) or net autotrophic periods, where CR has been shown to covary with chl a (Navarro et al., 2004). Following Robinson et al. (2002a), the remaining contribution of heterotrophs other than bacteria was split into two groups, bacterivores and herbivores, with micro- and mesozooplankton included in the latter group. We used a common conservative growth efficiency value of 25% (Straile, 1997) with the aim of estimating the maximum possible contribution of grazers to CR. The contribution of bacterivores would range from 0.4 to 1.7% of CR, with as low a mean as 0.9% (Table 2). Although higher that that of bacterivores, mean contribution of herbivores would not exceed 10% (range 5-20%, Table 2). The sum of the

Figure 2. (A) Relationship between integrated values of heterotrophic bacterial respiration and community respiration using the bacterial growth efficiency (BGE $\sim 6\%$) model of López-Urrutia and Morán (2007). Continuous line represents the 1:1 relationship. Error bars represent standard errors. (B) Relationship between the percent contribution of heterotrophic bacteria to community respiration (BR:CR) for the data pairs shown in (A) (filled squares) and for del Giorgio and Cole (1998, BGE $\sim 2\%$, open circles) and Rivkin and Legendre (2001, BGE $\sim 12\%$, open squares) models of bacterial growth efficiency, and community respiration. Only Y-standard errors of filled squares are plotted. Exponential fits to data are only shown for visual reference.

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different contributions would not reach the measured CR at any station, leaving a mean
0.6 g C m⁻² d⁻¹ of respired carbon unaccounted for. These values imply that on average
48% (range 13–72%) of total community respiration could not be explained by the
constituent microbial plankton trophic groups (Table 2).

It is obvious that all these calculations rely on the accuracy of the original measurements
of PPP and BP. As far as PPP estimates are concerned, our results are well within the range
of published values (Jochem and Zeitzschel, 1993; Marañón et al., 2000, 2001; Robinson
et al., 2002a; Teira et al., 2005). In contrast, BP lay at the lower end within the range for
open ocean systems (Ducklow, 2000). There is generally more uncertainty when compar-
ing BP estimates because of the need for converting leucine incorporation into carbon
production. Low leucine uptake rates (<100 pmol Leu L⁻¹ h⁻¹), however, are typically
found in oligotrophic, subtropical waters of the Atlantic (Carlson et al., 1996; Zubkov et
al., 2000; H. G. Hoppe, pers. comm.) and the Pacific (Sherr et al., 2001) oceans. Values
similar to ours have also been found in other open ocean environments (JGOFS Arabian
Sea and Equatorial Pacific cruises data bases had mean ± SD values of 25.1 ± 32.3 and
20.4 ± 13.7 pmol Leu L⁻¹ h⁻¹, respectively). Since leucine incorporation rates were
determined during the cruise (mean 11.2 ± 8.0 pmol Leu L⁻¹ h⁻¹), the other possible major
source of error was circumvented by the use of an empirically-determined conversion
factor rather than a published one. Although many studies have used the theoretical
conversion factor (3.1 kg C mol Leu⁻¹, Simon and Azam (1989) or 1.55 kg C mol Leu⁻¹ if
we assume no isotope dilution), there is growing evidence that this value is far from
realistic in oligotrophic environments. The CF we used (0.73 kg C mol Leu⁻¹, see also
Section 2) was, as expected, markedly lower than the theoretical one and similar to other
values obtained in the NE Atlantic: 0.58 by Agustí et al. (2001) and 0.02-1.26 by
Alonso-Sáez et al. (2007).
Heterotrophic bacteria have long been regarded as the major contributors to community respiration in the open-ocean (Williams, 1981; Biddanda et al., 1994; Pomeroy et al., 1995; Rivkin and Legendre, 2001). They were indeed more important respirers than phytoplankton, heterotrophic protists and metazoa according to the calculations shown in Table 2. Interestingly, heterotrophic bacteria would account for a mean 63 ± 4% of the summed contributions of the four microbial groups considered here, a value much closer to those reported by the above mentioned authors. Heterotrophic bacterial contribution to measured CR would only be greater than 50% if the effective BGE values were lower than 2%. Although some authors have revised upwards the contribution of microzooplankton to total respiration (Calbet and Landry, 2004), even their highest estimate of ~60% of PPP being respired by micrograzers would leave a mean 50% of CR unaccounted for with the examined trophic groups. It is, therefore, hard to envision how the estimated contributions can be revised so as to meet 100% of measured CR. It is necessary to note that the errors associated to the sum of estimated respirations of individual plankton groups might reach the 48% difference between measured and estimated respiration (Table 2), but the apparent uncoupling between BR and CR (Fig. 2A) was not reported in any previous studies using this approach (Robinson et al. 2002a, b; Robinson and Williams, 2005). A large discrepancy between both estimates, such that bacteria fail to account for more than 30% of total O₂ consumption, was recently confirmed in an extensive review of the planktonic carbon budget in the subtropical NE Atlantic (Marañón et al., 2007). The point can be made that it is the respiration of heterotrophic bacteria and other components that was severely underestimated. However, as argued before, neither higher Leu to C conversion factors nor lower BGEs are justified in our region in order to raise the estimate of total respiration attributable to heterotrophic bacteria. Dark incubations might have a role in the low BP values measured in open ocean waters if the results of Church et al. (2004) in the NE Pacific are general. However, significantly lower leucine incorporation rates in the light in oligotrophic environments have also been reported (Morán et al., 2002). Furthermore, our results of three experiments comparing light and dark uptake were not supportive of significant enhancement of aminoacid incorporation in the light. With a mean underestimation of 13% ± 12% at station 6, the possible underestimation of BP in the rest of the stations should have been much lower than the 32%–48% obtained by Church et al. (2004).

In an attempt to reconcile this discrepancy of our budget calculation it might be useful to discuss the old problem of long in vitro incubations. Enclosure in bottles, especially at oligotrophic sites, may artificially increase heterotrophic bacterial abundance (Pomeroy et al., 1994; Gattuso et al., 2002) and activity (Sherr et al., 1999). Important losses of primary producers (>50%) have been frequently observed either as chlorophyll a or individual group abundance (Fernández et al., 2003) with in situ planktonic populations remaining stable over long periods (Marañón et al., 2000; Zubkov et al., 2000). Furthermore, nonlinear increases of CR or BR in dark bottles (Pomeroy et al., 1994; Briand et al., 2004) have been documented, although linearity responses are also frequent (Robinson et al., 2002a). We hypothesize that extending the incubation time from 1 - 2 h (as in most BP
measurements) to 24 h (as in most CR experiments) might exacerbate this opposite behavior of autotrophs and heterotrophs confined in bottles. It stems from the available evidence that we could not discard an underestimation of PPP in experiments lasting \( \sim 6 \text{ h} \), usually the minimum period for \( ^{14}\text{C} \) incubations in oligotrophic waters.

In an experiment aimed at evaluating changes in picoplankton populations in 125 mL \( \text{O}_2 \) bottles over 24 h we found that autotrophic biomass decreased by 68\% in the dark bottles compared with a \( \sim 3500 \text{ L} \) mesocosm in a temperate, mesotrophic ecosystem (Ría de Vigo, NW Spain) while it remained virtually unchanged in the light ones (12\% decrease; Calvo-Díaz, Marañón and Morán, unpublished data). In this case, the biomass of heterotrophic bacteria was comparable in the mesocosm and in both bottle types (\( \pm 20\% \)). These results indicate that the combination of bottle confinement plus 24 h darkness can cause serious changes in community composition at the end of long incubations with respect to initial values. Evidence for dramatic changes in the picoplankton size-fraction after 2 h bottle enclosure were obtained in the same cruise (Fernández et al., 2003), and serious disruptions of \textit{Prochlorococcus} cell-cycles due to continuous dark confinement have been reported (Jacquet et al., 2001). As a consequence, metabolic rates from dark bottle incubations may not be fully representative of natural oxygen and carbon fluxes. We can only speculate on this, but a decrease in algal biomass could be accompanied by an artificial increase in POC and DOC consumption and hence greater heterotrophic bacterial respiration, which was not taken into account in our estimates of BR based on \( \sim 2 \text{ h} \) BP incubations.

According to Takahashi et al. (2002) the subtropics are a weak sink of CO\(_2\), hence contradicting the net heterotrophy indicated by the current method of measuring changes in dissolved \( \text{O}_2 \) in bottles along 24 h. In a more recent paper, by evaluating all organic carbon inputs and outputs, Hansell et al. (2004) concluded that the net heterotrophy in the NE Atlantic was much lower than previously found from incubation procedures, and that the system could indeed be considered as metabolically balanced. Although part of the consistently high negative values obtained from \( \text{O}_2 \) incubations in the NE Atlantic may be attributable to temporal (González et al., 2002; Karl et al., 2003) or regional variations (Serret et al., 2002), the evidence presented here of a serious mismatch between CR and the relative contribution of the different trophic groups casts some doubt on the actual magnitude of the net heterotrophy of the region. Interestingly, by comparing the estimated rates of community respiration (Table 2) with those of gross primary production, the metabolic balance would still be net heterotrophic on average (CR:GPP ratio of 1.48 \( \pm 0.53 \)), but of a considerable lower magnitude compared with measured CR and GPP rates (3.74 \( \pm 1.41 \)). It must be noted that not only CR, but also GPP and PPP (to a variable extent depending on the incubation length) would be affected by the observed decreases in phytoplankton biomass (Fernández et al., 2003), stressing the need for checking possible changes in standing stocks and metabolic rates within incubation bottles.
4. Concluding remarks

In conclusion, the expected positive linear relationship between integrated O$_2$ consumption and the estimated contribution of bacteria was not found, with heterotrophic bacterial respiration accounting on average for one third of community respiration given a mean, low bacterial growth efficiency of 6%. In addition, community respiration was strongly and negatively correlated with phytoplankton biomass. The heterotrophic metabolic balance in the oligotrophic NE Atlantic could not be totally explained by the summed contributions of individual trophic groups, leaving a mean 48% of community respiration unaccounted for. The cause for the apparent uncoupling between autotrophic and heterotrophic activity and respiration remains to be explained. From these considerations, we can safely conclude that either measurements of heterotrophic bacterial production, community respiration, or both, are in some error in oligotrophic environments. Unless there is a missing major respirer, a possible hypothesis is that 24 h dark in vitro incubations, by means of opposite changes in the biomass and activity of autotrophs and heterotrophs, may contribute to enhance the apparent negative net metabolic balance of oligotrophic, open ocean waters. Long in vitro confinements should be systematically controlled for community changes over the incubation period in order to determine the true coupling between autotrophic and heterotrophic processes and ultimately better constrain carbon fluxes in the upper ocean.

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