The transformation of leucine incorporation rates to prokaryotic carbon production rates requires the use of either theoretical or empirically determined conversion factors. Empirical leucine-to-carbon conversion factors (eCFs) vary widely across environments, and little is known about their potential controlling factors. We conducted 10 surface seawater manipulation experiments across the world’s oceans, where the growth of the natural prokaryotic assemblages was promoted by filtration (i.e., removal of grazers [F treatment]) or filtration combined with dilution (i.e., also relieving resource competition [FD treatment]). The impact of sunlight exposure was also evaluated in the FD treatments, and we did not find a significant effect on the eCFs. The eCFs varied from 0.09 to 1.47 kg C mol Leu⁻¹ and were significantly lower in the F than in the F samples. Also, changes in bacterial community composition during the incubations, as assessed by automated ribosomal intergenic spacer analysis (ARISA), were more pronounced in the F than in the F treatments, compared to unmanipulated controls. Thus, we discourage the common procedure of diluting samples (in addition to filtration) for eCF determination. The eCFs in the filtered treatment were negatively correlated with the initial chlorophyll a concentration, picocyanobacterial abundance (mostly Prochlorococcus), and the percentage of heterotrophic prokaryotes with high nucleic acid content (%HNA). The latter two variables explained 80% of the eCF variability in the F treatment, supporting the view that both Prochlorococcus and HNA prokaryotes incorporate leucine in substantial amounts, although this results in relatively low carbon production rates in the oligotrophic ocean.
bacterial community composition (11). However, only a few of these studies were conducted in open ocean surface waters (4, 6), which might hamper our ability to derive an empirical model able to predict CFs from environmental variables in vast extensions of the global ocean. Indeed, the wider the gradient of environmental conditions surveyed, the wider the range of CFs found. As an example, CFs varied by two orders of magnitude along a trophic gradient from shelf break upwelling to oligotrophic open-ocean Atlantic waters (4), and leucine-to-carbon CFs were significantly correlated only with bacterial growth efficiency, which is not a routinely measured variable. While the few published leucine-to-carbon CFs in surface oligotrophic oceanic waters are consistently low (4, 6, 17), no studies on the large-scale variability of CFs in relation to environmental factors have been conducted so far in these low-production areas.

The aim of our work was to evaluate the effect of filtration, dilution, and sunlight exposure on leucine-to-carbon CF estimates and to relate the empirically derived CFs with environmental factors in surface oceanic oligotrophic waters of the world’s oceans, during the Malaspina 2010 circumnavigation expedition, designed to cover a longitudinal range of tropical and subtropical waters between 30°N and 30°S.

MATERIALS AND METHODS

Sample collection and experimental setup. Experiments were carried out at 10 stations located in the tropical and subtropical Atlantic, Indian, and Pacific oceans between 14 December 2010 and 14 July 2011 during the Malaspina 2010 circumnavigation expedition on board the R/V Hespérides (Fig. 1). Conductivity-temperature-depth (CTD) casts were carried out at each station with a Sea-Bird Electronics 911 Plus probe attached to a rosette equipped with Niskin bottles. Samples for phosphate and nitrate measurements were frozen, and the concentrations were determined by flow cytometry and leucine incorporation rate measurements. The integrative method was used for the leucine-to-carbon conversion factor calculation (18) using the time intervals where an increase in heterotrophic prokaryotic biomass was observed. The conversion factor was calculated as the HPB (kg C liter⁻¹) produced over the selected time period of the experiment (essentially, the final HPB minus the initial HPB for that time period) divided by the total amount of leucine (mol leucine liter⁻¹) incorporated during that time period. The total amount was measured by integrating the incorporation rates over the selected time period.

Bacterial community composition was assessed at the beginning and at the end of the experiments using the automated ribosomal intergenic spacer analysis (ARISA) fingerprinting technique (see below).

Flow cytometry analyses. Samples were fixed and processed with a FACSCalibur flow cytometer (BD Biosciences) with a blue laser emitting at 488 nm. Samples of 1.2 ml of seawater were fixed with a paraformaldehyde-glutaraldehyde mix (1% and 0.05% final concentrations, respectively) and stored at −80°C until analysis in the laboratory within 7 months after the end of the cruise. Samples were stained with SYBR green I, at a final concentration of 1:10,000, for 15 min in the dark at room temperature. The average flow rate used was 12 μl min⁻¹, and acquisition time ranged from 30 to 260 s depending on cell concentration in each sample. Data were inspected in a FL1-versus-light side scatter (SSC, also termed right-angle light scatter [RALS]) plot and analyzed as detailed by Gasol and del Giorgio (19), including the differentiation of the two widespread groups of low-nucleic-acid (LNA) and high-nucleic-acid (HNA) prokaryotes. Latex beads (1 μm; Molecular Probes) were always used as internal standards. The biovolume of prokaryotic cells was estimated using the calibration obtained by Calvo-Díaz and Morán (20) relating relative light side scatter (population SSC divided by bead SSC) to cell diam-
TABLE 1 Environmental conditions at the beginning of the leucine-to-carbon conversion factor experimentsa

<table>
<thead>
<tr>
<th>Exp and ocean</th>
<th>Temp (°C)</th>
<th>Chla concn (μg liter−1)</th>
<th>PO4− concn (μmol liter−1)</th>
<th>NO3− concn (μmol liter−1)</th>
<th>Leucine incorporation (pmol Leu liter−1 h−1)</th>
<th>HPB (μg C liter−1)</th>
<th>%HNA</th>
<th>Picocyanobacterial abundance (10^4 cells ml−1)</th>
<th>C content (fg C cell−1)</th>
<th>Virus abundance (10^6 ml−1)</th>
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<tbody>
<tr>
<td>1; N Atlantic</td>
<td>24.80</td>
<td>0.18</td>
<td>NA</td>
<td>0.509b</td>
<td>30.2</td>
<td>6.39</td>
<td>41</td>
<td>9.68</td>
<td>13.2</td>
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<td>2; Eq Atlantic</td>
<td>27.50</td>
<td>0.15</td>
<td>0.078</td>
<td>NA</td>
<td>168.6</td>
<td>11.14</td>
<td>51</td>
<td>13.30</td>
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</tr>
<tr>
<td>3; S Atlantic</td>
<td>22.50</td>
<td>0.03</td>
<td>0.170</td>
<td>0.361b</td>
<td>34.5</td>
<td>3.84</td>
<td>25</td>
<td>1.37</td>
<td>13.1</td>
<td>NA</td>
</tr>
<tr>
<td>4; Indian</td>
<td>25.90</td>
<td>0.09</td>
<td>0.033</td>
<td>0.262b</td>
<td>22.4</td>
<td>4.36</td>
<td>47</td>
<td>3.31</td>
<td>12.3</td>
<td>1.54</td>
</tr>
<tr>
<td>5; Indian</td>
<td>21.70</td>
<td>0.07</td>
<td>0.019</td>
<td>0.191</td>
<td>6.3</td>
<td>2</td>
<td>38</td>
<td>5.74</td>
<td>9.8</td>
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</tr>
<tr>
<td>6; S Pacific</td>
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<td>0.143</td>
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<td>4.12</td>
<td>53</td>
<td>6.23</td>
<td>12.9</td>
<td>3.10</td>
</tr>
<tr>
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<td>0.319</td>
<td>2.280</td>
<td>2,362.4</td>
<td>14</td>
<td>51</td>
<td>1.51</td>
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<td>4.35</td>
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<tr>
<td>8; N Pacific</td>
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<td>0.083</td>
<td>0.028</td>
<td>6.2</td>
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<tr>
<td>9; S Pacific</td>
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<td>0.229</td>
<td>0.377</td>
<td>68.3</td>
<td>7.21</td>
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<tr>
<td>10; N Atlantic</td>
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<td>0.068</td>
<td>0.340</td>
<td>35.8</td>
<td>6</td>
<td>27</td>
<td>4.67</td>
<td>12.5</td>
<td>4.35</td>
</tr>
</tbody>
</table>

aData, Temperature, chlorophyll a (Chla), phosphate concentration, nitrate concentration, and virus abundance correspond to unmanipulated seawater. Leucine incorporation rates, heterotrophic prokaryotic biomass (HPB), percent high-nucleic-acid-content prokaryotes (%HNA), picocyanobacterial abundance, and prokaryotic cell carbon content correspond to 0.8-μm-filtered seawater. Eq, equatorial; NA, not available.

aCounts of Prochlorococcus picocyanobacteria, which tended to overlap partially with the HNA prokaryote cluster, were subtracted from total heterotrophic prokaryotic counts by independent assessment in nonstained aliquots. Synechococcus and Prochlorococcus cyanobacteria were in turn identified in plots of SSC versus red fluorescence (FL3; >650 nm) and of orange fluorescence (FL2; 585 nm) versus FL3. Picocyanobacterial biomass was calculated by using the following volume-to-carbon conversion factors: 230 fg C μm−3 for Synechococcus and 240 fg C μm−3 for Prochlorococcus (22).

RESULTS

Initial conditions. A considerable range of initial seawater conditions was observed in the experiments, both for environmental variables (Table 1) and for bacterial community composition (Fig. 2). Chlorophyll a concentrations in the unfiltered seawater ranged from 0.03 μg liter−1 in the South Atlantic (experiment 3) to 0.21 μg liter−1 in the western tropical Pacific (experiment 9) (Table 1).
Phosphate concentration also varied about 10-fold, from 0.02 μM in the Indian Ocean to 0.32 μM in the equatorial Pacific (Table 1). In contrast, nitrate concentration varied by two orders of magnitude, from 0.03 μM in the North Pacific to 2.28 μM in the equatorial Pacific (Table 1). Leucine incorporation rates in the 0.8-μm-filtered seawater varied by three orders of magnitude, whereas planktrophic heterotrophic biomass (PHB) and picocyanobacterial abundance (PCA) varied by one order of magnitude (Table 1). Approximately 50% of the picocyanobacteria in the original sample were present in the <0.8-μm fraction (data not shown). Prochlorococcus represented >90% of the picocyanobacteria in all the experiments except in experiment 9 (eastern tropical North Pacific), which was dominated by Synechococcus (details not shown). Heterotrophic prokaryotes were generally dominated by LNA cells, with the proportion of HNA cells ranging from 27 to 53%. The initial bacterial community composition as assessed by ARISA showed significant differences among sampling sites (Fig. 2), although there were no significant differences in the bacterial assemblages among experiments 1, 2, 8, and 10 (samples from Atlantic and North Pacific oceans), between experiments 6 and 7 (samples from equatorial and South Pacific oceans), and between experiments 4 and 5 (samples from the Indian Ocean). The highest bacterial community composition similarity (68%) was observed between the experiments conducted in the Indian Ocean. Bacterial community composition in the eastern tropical North Pacific Ocean (experiment 9) differed most from those in other locations (<35% similarity).

**eCFs.** Empirical leucine-to-carbon conversion factors (eCFs) were determined by comparison of leucine incorporation rates with the increase in bacterial biomass during the experimental incubations (Fig. 3). The resulting eCFs ranged from 0.09 ± 0.01 to 1.47 ± 0.08 kg C mol Leu⁻¹, showing values close to or higher than 1 kg C mol Leu⁻¹ in the filtered treatments in 4 out of 10 experiments. Overall, eCFs were higher in the filtered than the filtered and diluted treatments (Fig. 4). The eCFs in the LF, LFD, and DFD treatments followed similar variability patterns (Fig. 4), although significant differences were found among treatments (RMANOVA; \( P = 0.018 \)). Pairwise comparisons showed that eCFs were significantly higher in the LF than in both LFD and DFD treatments (Bonferroni test; \( P < 0.042 \)). No significant differences between LFD and DFD treatments were found (Bonferroni test; \( P > 0.05 \)), although eCFs were lower in the light than in the dark treatments in experiments 8 and 10.

In order to relate the observed variability in eCFs with environmental factors, we conducted a correlation analysis (Table 2). The conversion factors in the FD treatment (under either light or dark conditions) did not significantly correlate with any of the considered variables. In contrast, the eCFs in the LF treatment showed significant and strong negative correlations to chlorophyll a concentration, picocyanobacterial abundance, and the percentage of HNA prokaryotes (\( r \) ranging from \(-0.67\) to \(-0.80\); \( P < 0.05 \)) (Table 2). Significant semilogarithmic or linear relationships were found between the LF eCFs and picocyanobacterial abundance (Fig. 5A) or the %HNA (Fig. 5B), explaining 64% and 56% of the observed variability, respectively. A multiple linear regression model including LF eCFs as a dependent variable and both %HNA and log picocyanobacterial abundance as independent variables explained 80% of the variability in the LF eCFs (eCFs = 4.98 ±1.04 - 0.73 ±0.25 \times \log\text{picocyanobacterial abundance} - 0.021 [±0.09] \times \%\text{HNA}; \( r^2 = 0.80 \); adjusted \( r^2 = 0.75 \); \( P = 0.005 \); \( n = 10 \)) (standard errors are in brackets). Log picocyanobacterial abundance had a greater effect on F eCFs (beta coefficient = \(-0.57\); \( P = 0.022 \)) than %HNA (beta coefficient = \(-0.46\); \( P = 0.049 \)).

**FIG 2** Dendrogram based on automated ribosomal intergenic spacer analysis (ARISA) profile similarities of unmanipulated seawater at the beginning of the experiments. Dashed lines represent statistically significant clusters (\( P < 0.05 \)) according to the SIMPROF test. Eq, equatorial; Atl, Atlantic; Ind, Indian; Pac, Pacific.
Bacterial community composition in the unmanipulated control treatment showed, on average, 35% similarity to the initial bacterial community composition after 3 days of incubation (data not shown). The manipulation of nutrient availability (by dilution), nutrient availability and grazing pressure (filtration and dilution), and light caused changes in bacterial community composition (Fig. 6). However, the resulting community was mostly determined by the initial bacterial assemblage, since the samples clustered primarily by experiment. Some experiments showing similar eCFs also showed similar bacterial communities, such as experiments 1 and 2 or experiments 8 and 10 (Fig. 4 and 6). The effect of solar radiation on the bacterial community composition was negligible in most of the diluted treatments, as no significant differences were observed between LFD and DFD samples (SIMPROF test; \(P < 0.05\)), except in experiments 4, 8, and 10. The average similarity between LFD and DFD samples was 63%. On the other hand, when comparing the unmanipulated control samples (LC) with LF and LFD samples, we observed that filtration alone (LF) had fewer effects on bacterial community composition (average similarity of 60%) than the combination of filtration and dilution (LFD) (average similarity of 46%). Indeed, bacterial community composition in LF samples was not significantly different from that in LC samples in 4 out of 8 experiments (SIMPROF test; \(P < 0.05\)) (Fig. 6). In contrast, bacterial community composition in LFD samples did not significantly differ from that in LC samples in only 1 out of 8 experiments (SIMPROF test; \(P < 0.05\)) (Fig. 6).

**DISCUSSION**

Considering the widely demonstrated variability in eCFs, the use of constant theoretical CFs may produce erroneous estimates of prokaryotic heterotrophic production (PHP) by relying only on estimates of substrate incorporation rates rather than also on the fate of the incorporated compounds (i.e., fraction of substrate that is not assimilated into biomass) (6, 28). Several studies have...
shown that the use of theoretical leucine-to-carbon CFs may overestimate both temporal and spatial variability in prokaryotic biomass production (4, 5). However, as CF experiments are time-consuming and labor-intensive, unraveling which environmental factors drive changes in eCFs and deriving empirical models for predicting eCFs from basic environmental variables represent a present challenge in aquatic microbial ecology. The broad range of environmental conditions sampled in our survey (Table 2) allowed us to find an empirical model that could be useful for deriving eCFs in surface oligotrophic oceanic waters from data obtained by flow cytometry, the usual method for estimating microbial plankton abundance.

**Filtration versus filtration and dilution in eCF experiments.** Although there are several extensive studies reporting leucine-to-carbon eCFs across a variety of environmental conditions, a meta-analysis is not easy to conduct due to the great variability in the prokaryotic community pretreatments. Early work by Coveney and Wetzel (9) evaluating the effects of different pretreatments (filtration, dilution, and nutrient addition) on thymidine eCFs consistently found lower eCFs associated with the addition of phosphorus. In the case of leucine, only the study by Alonso-Sáez et al. (11) systematically tested the effect of nutrient addition on the eCFs in prefiltered and 20×-diluted samples from a coastal station along an annual cycle in the Mediterranean Sea. Significantly lower eCFs were found in inorganic-nitrogen-enriched than in unamended seawater samples, and substantial changes in bacterial community composition were observed in association with nutrient additions. Those authors hypothesized that addition of inorganic nutrients could lead to a situation of C limitation, where leucine would be utilized to obtain energy, leading to low net biomass production regardless of high leucine incorporation rates. Interestingly, the addition of carbon compounds such as glucose (10) or glucose and acetate (29) did not seem to affect leucine eCFs in studies performed in temperate coastal and Antartic waters, respectively. Kirchman (30) also found that thymidine and leucine eCFs were not affected by addition of organic compounds in the subarctic Pacific.

Although we did not directly test the effect of nutrient enrichment, we did evaluate the effect of dilution of the sample with 0.2-μm-prefiltered seawater, which can also increase nutrient availability. Most of the published leucine-to-carbon eCFs derive
from incubation of filtered and diluted seawater, with dilution factors ranging from $1 \times 10$ to $20 \times 11$; however, the potential influence of dilution on the leucine-to-carbon eCF estimations had not been tested so far. The significantly lower eCFs obtained in the filtered and diluted (LFD and DFD) compared to the filtered (LF) treatments are in agreement with the aforementioned negative effect of nutrient enrichment on eCF estimates. Thus, our results suggest that dilution treatments may not be appropriate in CF experiments in oligotrophic areas. The effect of dilution was variable among the experiments (Fig. 4), which may be related to the degree of nutrient limitation in each seawater incubation. According to the hypothesis of C limitation proposed by Alonso-Sáez et al. (11) and del Giorgio et al. (6), the effect of filtration and dilution on eCFs is expected to be higher when C is the primary limiting element. Under these conditions, the filtration and dilution increase mineral nutrient availability, which in turn may exacerbate C limitation, as primary production is drastically reduced after filtration, leading to an uncoupling of leucine incorporation and bacterial biomass production (i.e., low eCFs). The greatest difference between eCFs obtained with LFD and eCF obtained with LF was observed in experiment 3, where the lowest chlorophyll a concentration was measured (Table 1), suggesting that strong carbon limitation was the primary cause of the discrepancy.

A further argument that might discourage the dilution pre-treatment in CF experiments in oligotrophic areas is the fact that the bacterial community composition developed in LFD treatments was significantly different than that in unmanipulated (LC) seawater, while LF samples were much more similar to the unmanipulated controls (Fig. 6). Although both dilution and nutrient enrichment appear to affect leucine-to-carbon eCFs, we found only one study, conducted in Antarctic waters, where eCFs were estimated in filtered but nondiluted seawater incubations (29). The eCFs derived by those authors were very similar to those obtained by Teira et al. (31) in the same sampling region using the dilution approach. Summertime Southern Ocean waters are already nutrient rich (29, 31), and an increase in nutrient availability through dilution may have little effect on eCFs, supporting the role of trophic status in the effect of the different experimental manipulations for estimating eCFs.

Only the variability in LF eCFs could be explained by any of the environmental factors measured in our study (Table 2). We are aware that other nonincluded ecological factors might also have influenced the eCFs, such as dissolved organic carbon (DOC) concentration, leucine catabolism, or bacterial growth efficiency (4, 6, 15). However, we believe that the lack of a coherent explanation for the variability in eCFs in the diluted treatments may be at least partially related to the inadequacy of the experimental design. Considering the high variability that the methodological approach may introduce in eCF estimates, and in view of the large changes in bacterial communities and the lower leucine yields associated with dilution, we strongly recommend avoiding dilution in CF experiments, at least in oligotrophic waters.

**Relationship between eCFs and environmental factors.** Regardless of the potential methodological problems, some potential explanations for the leucine-to-carbon eCFs variability have been postulated to date. Overall, eCFs higher than the theoretical (1.55 kg C mol Leu$^{-1}$, assuming no isotopic dilution) can be explained by the isotopic dilution of the radiotracer. If the radiotracer is not added at a saturating concentration, then the measured leucine incorporation rates will be lower than the actual rates, leading to artificially high eCFs. This problem may typically occur in coastal eutrophic waters, where the ambient leucine concentration may be higher than the commonly used concentrations of added radiotracer (20 to 40 nmol liter$^{-1}$). In fact, leucine-to-carbon eCFs higher than the theoretical one have been repeatedly found at coastal sites (6, 11, 32–34).

In open ocean oligotrophic waters, the leucine-to-carbon eCFs are consistently lower than the theoretical one (4, 6, 17), which implies an unbalanced bacterial growth in which net bacterial biomass production is low regardless of relatively high leucine incorporation rates. We also measured eCFs lower than the theoretical one in all of the experiments (Fig. 4). An unbalanced bacterial growth under limiting conditions has been described, where protein synthesis is maintained in order to maximize survival rather than growth and reproduction (3, 15, 35), resulting in high turnover rates of intracellular protein (36). However, Alonso-Sáez et al. (4) measured relatively low protein turnover rates in oligotrophic waters of the subtropical Atlantic Ocean and concluded that the low eCFs found in the area were related to leucine catabolism by energy-limited bacterial cells. According to this hypothesis, leucine would be incorporated into the cell but a high portion would be respired before being used for protein synthesis; this would translate into low eCFs if the tritium signals of the incorporated and respired leucine were recovered by cold trichloroacetate acid. High percentages of leucine respiration (40 to 80%) associated to low eCFs have been found by Alonso-Sáez et al. (4) and del Giorgio et al. (6), revealing a significant negative correlation between the percentage of leucine respired and the eCFs ($r = -0.46; P < 0.001; n = 25$) (unpublished data).

Low leucine-to-carbon eCFs have been also related to a faster synthesis of transport proteins relative to cell duplication when substrate availability is low (5). Calvo-Díaz and Morán (5) obtained a significant empirical model to predict eCFs from leucine incorporation rates and cellular carbon contents. However, samples were not prefiltered in their study, and thus grazing, although reduced by dilution, could have influenced their results. In our data set, we did not find any significant correlation between eCFs and either leucine incorporation rates or bacterial cellular carbon content (Table 2). Moreover, by contrast with previous studies (11), we found a negative correlation between LF eCFs and chlorophyll a, indicating that higher eCFs occurred under limiting conditions. However, it is important to note that although we sampled a relatively wide range of environmental conditions, the trophic gradient was rather limited (chlorophyll a $< 0.21$ mg m$^{-2}$), and thus, energy limitation supposedly occurred at all sampling sites.

The lower LF eCFs associated with high picocyanobacterial abundance, dominated by *Prochlorococcus*, could be related to the ability of these autotrophic bacteria to incorporate leucine (37–40). As both picoautotrophs and heterotrophs contribute to leucine assimilation, but only the biomass production of heterotrophs is taken into account in the calculation of eCFs, a high abundance of picocyanobacteria during the experiments may result in high bulk leucine incorporation rates irrespective of low heterotrophic prokaryotic biomass production. Mean picocyanobacterial biomass during the incubations ($0.86 \pm 0.18 \mu$g C liter$^{-1}$) was lower than heterotrophic prokaryotic biomass ($6.8 \pm 1.1 \mu$g C liter$^{-1}$). Even if picocyanobacteria represented only 2 to 20% of total prokaryotic abundance, their contribution to leucine

---

**Table 1**

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Nutrient Availability</th>
<th>Leucine Incorporation</th>
<th>Bacterial Biomass</th>
<th>ECF</th>
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<tr>
<td>1</td>
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<td>2</td>
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<tr>
<td>3</td>
<td>Moderate</td>
<td>Moderate</td>
<td>Moderate</td>
<td>Moderate</td>
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</tbody>
</table>

**Figure 6**

- Increase in nutrient availability
- Leucine incorporation
- Bacterial biomass
- ECFs

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**Figure 4**

- Leucine incorporation
- Bacterial biomass
- ECFs

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**Figure 3**

- Leucine incorporation
- Bacterial biomass
- ECFs

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incorporation could be considerable, as Prochlorococcus cells may display higher cell-specific incorporation rates than heterotrophic bacteria because of their larger volume (39). The few existing estimates indicate that Prochlorococcus may contribute 24 to 63% of the total microbial planktonic leucine incorporation into proteins (38, 39).

We also found a significantly negative relationship between LF eCFs and the percentage of HNA prokaryotes (Table 2; Fig. 5B). As HNA prokaryotes appear to have higher cell-specific leucine incorporation rates than LNA prokaryotes (39, 41) and tend to be more susceptible to viral infection than LNA prokaryotes (42–44), the low LF eCFs could also be related to high cell-specific leucine incorporation rates along with a low net biomass increase of this bacterial functional group during the incubations. As grazing was minimized by prefiltration, we hypothesize that other factors, such as viral lysis or apoptosis, could be responsible for low net biomass accumulation during our incubations. The fact that picocyanobacterial abundance and the relative abundance of HNA cells explained 80% of the variability observed in LF eCFs in these oligotrophic waters suggests that prokaryotic community composition, and particularly these two prokaryotic groups, have a strong influence on eCF estimates.

In conclusion, we have shown that whereas light exposure does not have a clear effect on the leucine-to-carbon eCFs, the dilution pretreatment tends to reduce the carbon-to-leucine yield and promotes important changes in bacterial community composition (assessed with ARISA fingerprinting) compared to unmanipulated seawater samples. Filtration alone, on the other hand, allowed bacterial biomass increase and did not imply important changes in bacterial community composition, thus appearing to be an adequate experimental approach for deriving empirical conversion factors, as the environmental characteristics would be closer to those found under in situ conditions compared to the filtered and diluted treatments. We also provide a new perspective to explain low eCFs in oceanic oligotrophic waters, in addition to the previously proposed hypotheses of high protein turnover and leucine catabolism. We hypothesize that eCF variability patterns could be driven, in part, by low net biomass accumulation of highly active prokaryotes during incubation. As prokaryote biomass loss cannot be totally avoided during conversion factor experiments, the prokaryotic production rates derived from the application of eCFs may not yield gross biomass production rates, which should be taken into account when microbial carbon budgets are constructed.

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