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Rapid carbon cycling in the oligotrophic ocean

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Abstract

The dynamics of organic carbon production, release and bacterial use was examined across a range of communities spanning from highly oligotrophic ones in the Subtropical Atlantic Ocean, mesotrophic ones in the Mediterranean Sea and productive ones in the Northern African upwelling and the Southern Ocean. A comparative analysis of experiments examining total and particulate organic carbon production across a range of time scales (15 min to 24 h) for 20 communities with contrasting phytoplankton cell status, as assessed by cell lysis rates, and the use of a simple inverse model was used to resolve patterns of carbon flow in the microbial food web. Communities in productive ocean waters accumulated organic carbon over hourly time scales, whereas only a small fraction of net primary production accumulated in communities from oligotrophic waters. These communities supported high phytoplankton cell lysis rates leading to a rapid flux of organic carbon to bacteria, which had high affinity for phytoplankton-derived carbon, much of which was rapidly respired. Conventional assessments of primary production in the oligotrophic ocean severely underestimate net phytoplankton production, as carbon flow in microbial communities from oligotrophic ocean waters occurs within short (minutes) time scales. This explains difficulties to reconcile estimates of primary production with independent estimates of carbon use by bacteria in oligotrophic marine ecosystems.

1 Introduction

Reconciling high bacteria carbon use with estimates of primary production and carbon flow from phytoplankton has remained a challenge for over two decades, particularly for the oligotrophic ocean (Williams, 1984; del Giorgio et al., 1997; del Giorgio and Duarte, 2002). Whereas calculated rates of bacterial carbon use are high relative to primary production and, particularly, relative to estimates of dissolved organic carbon production by phytoplankton, this seemingly high carbon flow through bacteria is not

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recovered in a similar increase in biomass or carbon flow through the food web. This observation lead Williams and LeB (1984) to equate carbon flow through bacteria with the emperor's new suit of clothes of the famous short tale by Hans Christian Andersen.

A high flux of phytoplankton carbon through bacteria in oligotrophic waters requires the release of much of phytoplankton production in dissolved form. Reports of high dissolved organic carbon by phytoplankton in the 1980's raised concerns as to the possible role of experimental artifacts in accounting for this observation, as healthy phytoplankton cells, assumed to prevail in natural communities, could not possibly release in dissolved form such high fractions of their primary production (Sharp, 1977). Yet, subsequent reports have provided supporting evidence for high relative release of dissolved organic carbon in the oligotrophic ocean (Karl et al., 1998; Morán et al., 2002), consistent with recent reports of high phytoplankton cell lysis and mortality rates in oligotrophic waters (Agustí et al., 1998, 2001; Agustí, 2004). The release of a high fraction of the phytoplankton primary production as dissolved organic carbon in the oligotrophic ocean is also pivotal in explaining the high bacterial carbon use relative to particulate primary production in oligotrophic waters (del Giorgio et al., 1997), and, more broadly, the tight coupling between the microbial food web and primary producers characteristic of the oligotrophic ocean (Azam et al., 1983, 1988).

Recent evidence shows that chemotactic responses enable marine bacteria to exploit microscale nutrient patches within tens of seconds (Stocker et al., 2008), a prerequisite for these resources to be utilized, since physical processes will dilute them by mixing at longer time scales (Stocker et al., 2008). Coupling between phytoplankton dissolved organic carbon (DOC) release and bacterial use is therefore a process occurring at the microscale and that must be, therefore, effective at the short time scales, from seconds to minutes, characteristic of the persistence of microscale patchess in the sea (Stocker et al., 2008).

Experimental evaluations of dissolved organic carbon production in the oligotrophic ocean are typically conducted over time scales spanning from 2 to 24 h (e.g. Karl et al., 1998; Morán et al., 2002). Yet, experimental assessments of the time scale of

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dissolved organic carbon release by growing phytoplankton and subsequent use by bacteria have shown that organic carbon is released within time scales as short as 1 min following photosynthetic fixation and that these products are used by bacteria over time scales as short as 5 min from their photosynthetic production (Puskaric and Mortain-Bertrand, 2003). Indeed, examinations of dissolved organic carbon production in experiments ranging from minutes to many hours showed a decline in the apparent rate of dissolved organic carbon production with increasing incubation time (Lancelot, 1979; Moran et al., 2001), providing indirect evidence for bacterial use of dissolved organic carbon release at short time scales. The net result of rapid bacterial use of DOC released by phytoplankton would be an underestimation of both dissolved organic carbon production and, more importantly, net phytoplankton primary production, provided that some of the DOC incorporated by bacteria may be respired and missed by measurements of organic carbon production.

A possible underestimation of gross primary production (GPP) in the ocean by the conventional particulate ^{14}C production method has been suggested to be likely involved in an apparent major imbalance between community respiration and gross primary production in unproductive phytoplankton communities (del Giorgio and Duarte, 2002; del Giorgio and Williams, 2005). However, even approaches that strive to account for phytoplankton DOC production (Karl et al., 1998; Moran et al., 2002) may severely underestimate GPP in the ocean if bacterial use of released products occur at time scales shorter than incubation times (typically of the order of 3 h).

Here we test the postulated rapid dynamics of DOC production and use in an attempt to elucidate gross rates of primary production and its short-term fate in oceanic plankton communities. We examined these dynamics across a range of communities spanning from highly oligotrophic ones in the Subtropical Atlantic Ocean, mesotrophic ones in the Mediterranean Sea and productive ones in the Northern African upwelling and the Southern Ocean. The approach used involved the comparative analysis of experiments examining total and particulate organic carbon production across a range of time scales (15 min to 24 h) across 20 different plankton communities, and the use

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of a simple inverse model to resolve patterns of carbon flow in the microbial food web consistent with the experimental results. Lastly, we test the role of phytoplankton cell lysis as the process conducive to high release of DOC, modulating the relationship between particulate and total primary production.

2 Methods

The experiments were carried out during four oceanographic cruises: (1) the COCA-2 cruise (19 May–14 June 2003) on board R/V *Hespérides*, occupying the transition zone between the northeast coastal upwelling of Africa and the oceanic waters of the North Atlantic at 26° W, where a total of 8 experiments were conducted across the transects, spanning from highly oligotrophic waters in the Subtropical Atlantic Gyre to highly productive ones at the Northern African coastal upwelling; (2) the BADE I cruise (September–October 2003) on board R/V *Pelagia*, examining a gyre in the Central NW Mediterranean, South of Mallorca Island where four experiments were conducted in mesotrophic waters; (3) the BADE II cruise (September–October 2004) on board R/V *Pelagia*, in the same area in the subtropical Northeast Atlantic Ocean as the COCA-2 cruise, where four experiments were conducted along a range from highly oligotrophic to productive environments; and (4) the ICEPOS 2005 cruise conducted on board R/V *Hespérides* (2 to 21 February 2006), where four experiments were conducted encompassing moderately to highly productive waters along the Antarctic Peninsula.

The experiments were conducted using surface (5 m depth) communities, sampled using 12 l Niskin bottles attached to a Rosette sampling system. Time-course experiments examining the incorporation of carbon into the dissolved and particulate fractions were conducted using ^{14}C additions (Steeman-Nielsen, 1952). Two dark and two light 125 ml polycarbonate bottles were filled for each time step used in the experiments, and were inoculated with different amounts of $NaH^{14}CO_3$ (range 10 to 80 µCi) depending on the incubation time and productivity of the waters. The bottles were incubated on deck at in situ temperature in a controlled-temperature bath and using neutral screens

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to adjust for in situ irradiance. In order to resolve the time course of the incorporation of ^{14}C in different pools (particulated and dissolved), incubation bottles (2 dark and 2 light bottles) were retrieved at 15 min, 30 min, 1 h, 3 h, 6 h; and, in three of the experiments, at 12 h and 24 h.

After the incubation period, sample bottles were placed in dark plastic bags until filtration. Two aliquots of 5 ml were sampled and placed in 20 ml scintillation vials for determination of total labelled organic carbon (TOC). Liquid samples (TOC) were acidified with 100 µl of HCl 10 % and were shaken overnight until addition of 20 ml of Packard Ultima Gold XR scintillation cocktail. The remaining volume was filtered through 0.22 µm membrane filters for determination of the total labelled particulate carbon (POC>0.22 µm) retained in the filters. Ideally, this fraction is expected to include all photosynthetically-produced particulate organic carbon contained in algal biomass plus the bacterial POC resulting from bacterial uptake of DOC released by algae as well as any bacterial biomass ingested by micrograzers. Filters were fumed with concentrated HCl (37 %) to remove inorganic carbon for a minimum of 12 h before addition of 10 ml of Packard Ultima Gold XR scintillation cocktail. Radioactivity was measured after 24 h on board in a EG&G/WALLAC 1414-001 WINESPECTRAL liquid scintillation counter.

Samples of 200 ml were filtered through Whatmann GF/F filters to estimate total chlorophyll-*a* concentration (chl-*a*). Chl-*a* was measured fluorometrically unsing Turner Designs fluorometer in 90 % acetone extracts of filters preserved frozen following Parsons et al. (1984). Phytoplankton cell lysis rates were estimated from the quantification of the dissolved esterase activity (EA), measured in triplicate using the spectrofluorometric technique described by van Boekel et al. (1993) as modified by Agustí et al. (1998) and Agustí and Duarte (2000). In short, 5 ml water samples were filtered through a 0.45 µm Millipore Millex filter, and 50 µl of ethylene diaminetetraacetic acid (EDTA) and 50 µl of fluorescein diacetate (FDA, Sigma Chemical) were added to the samples (to a final concentration of 0.02 and 0.2 mM, respectively) and mixed in a vortex mixer. After incubating the samples for 1 h at 20 °C, the fluorescence emission

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was measured immediately in a Shimadzu RF-5000 spectrofluorometer at 451 and 510 nm excitation and emission (10 nm bandwidth) wavelengths, respectively. Fluorescein production (nmol fluorescein l^{-1} h^{-1}) was calculated using a standard calibration curve obtained by measuring the fluorescence of a range of fluorescein (Sigma Chemical) solutions (3–2000 nmol fluorescein l^{-1} of filtered seawater). The initial particulate esterase activity (PEA_0) was calculated from the measured chl-*a* concentration using a ratio of PEA to chl-*a* derived from phytoplankton cultures (Agustí et al., 1998). The phytoplankton cell lysis rate (h^{-1}) was calculated from the decrease in PEA with time (t) due to the production of dissolved EA during cell lysis by:

$$\mu l(t^{-1}) = \frac{\ln\left(\frac{PEA_t}{PEA_0}\right)}{t} \quad (1)$$

where PEA_0, represents the initial particulate esterase activity, estimated as described above, and PEA_t, is the particulate esterase activity expected after a time interval t (i.e. PEA, minus the production of dissolved EA calculated over the time interval t).

The production of dissolved EA was derived from a combination of the measured dissolved EA and estimates of the rate of loss of the activity of the enzyme calculated by experiments conducted in parallel to sampling. Due to technical reasons, lysis rates and chlorophyll a concentration could not be measured in the BADE cruises.

3 Results

The communities investigated ranged broadly in biomass, as indicated by chl-*a* (0.04 to 4.26 µg chl-*a* l^{-1}, Table 1) and primary production, with POC production rates (measured over >6 h) ranging from (0.62 to 32.6 µg C L^{-1} h^{-1}, Table 1). Calculated cell lysis rates ranged greatly among communities (0.003 to 1.09 day^{-1}, Table 1) and decreased as community biomass increased ($R^2_{\text{Lysis vs. chl}-a}$ = 0.75, $P < 0.0001$). POC production (measured over >6 h) declined strongly with increasing lysis rates (Fig. 1). The percent extracellular organic carbon release (PER) in the experiments ranged broadly across

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communities (0.3 % to 85.7 %, Table 1) and increased with increasing cell lysis rates ($R^2 = 0.75$, $P < 0.0001$), to reach an asymptotic PER of about 70 % with lysis rates in excess of 0.5 day^{-1} (Fig. 1).

The time course of cumulative TOC and POC produced showed a contrasting behavior among experiments (Fig. 2). The accumulated TOC declined sharply from high values at the shortest time interval used (15 min) to low values at longer sampling times, and increased slowly, in parallel to POC, thereafter in the oligotrophic communities examined at the NE Subtropical Atlantic Gyre. In contrast, the accumulated TOC and POC increased linearly and in parallel with incubation time in productive waters, including those in the NW African upwelling and the Southern Ocean and the most productive community studied in the NW Mediterranean (Fig. 2). The parallel increase in accumulated TOC and POC over time indicates that the apparent percent extracellular release (PER) declines over time, as the slope of POC vs. time is expected to be shallower than that for PER, at $\frac{\text{Slope}_{\text{TOC}} \times \text{PER}}{100}$, under constant PER.

The linear slopes of cumulative TOC and POC produced versus time ranged broadly from negative (−2.54 and −0.007 $\mu g\,C\,l^{-1}\,h^{-1}$, respectively) to highly positive (27.48 and 23.06 $\mu g\,C\,l^{-1}\,h^{-1}$, respectively), and were closely related among experiments ($r = 0.98$, $P < 0.0001$, Table 3). Closer examination showed that the slopes of cumulative TOC and POC versus time differed greatly at low POC production rates (Table 3). A total of fourteen of the 20 experiments conducted exhibited a significant linear increase in the total TOC produced over time, while five others showed a high initial TOC production followed by a sharp decline in total TOC produced (e.g. Fig. 2, Table 3). A linear increase in the total TOC produced over time was observed in productive, high-biomass communities. The significance of the expected linear increase in accumulated TOC produced over time (as the probability that there be no increase in TOC over time; H_0: slope TOC over time = 0) increased with increasing chl-*a* concentration and average POC production rates (Fig. 3, Table 3), indicating that the linear increase in TOC over time assumed in the ^{14}C method to assess primary production only applied to relatively productive communities. The accumulated TOC produced tended to increase

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significantly over time when chl-*a* concentrations and POC production rates increased above 1 µg chl-*a* l^{-1} and 1 µg C l^{-1} h^{-1}, respectively (Fig. 3). In contrast, the short-term TOC production rate (i.e. calculated after 15 min incubations) was positive in all cases, and total POC production increased over time along the experiment in all but one experiment (Table 3). Short-term rates of TOC production, measured throughout the first 15 min of incubation, were independent of lysis rates ($P > 0.05$, Fig. 4), but short-term DOC production rates, measured over 15 min, increased strongly with increasing lysis rates ($R^2 = 0.75$, $P < 0.01$, Fig. 4).

4 Discussion

The results presented indicate that the expected linear accumulation of total organic carbon production over time scales of hours assumed by conventional application of the ^{14}C method is only observed in relatively productive communities. Unproductive communities in oligotrophic waters present a high short-term TOC production rate and a high DOC production rate, followed by a loss of accumulated TOC produced which must derive from respiratory losses mediated by bacteria. As a consequence rates of TOC, POC and DOC production are relatively independent of incubation time for productive communities, but vary greatly over time, by more than two orders of magnitude in some cases, for unproductive ones.

These results indicate that the fate of photosynthetic carbon differs greatly among unproductive and productive communities. Communities in productive regions accumulate TOC over time, largely in particulate form, consistent with results from comparable time-series ^{14}C addition experiments conducted in productive North Atlantic waters in the past (Lancelot, 1979). However, communities in oligotrophic waters show a high initial accumulation of TOC followed by a sharp decline and a very low rate of POC accumulation compared to short-term TOC production. The high average DOC production rates in unproductive communities (>50 % of TOC production) cannot be explained by excretion by healthy cells, as argued in the past (Sharp, 1977). Sharp (1977) showed that healthy cells do not excrete significant amounts of DOC,

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consistent with very low (<10 %), extracellular release of organic carbon in the more productive communities investigated, but concluded, following Duursma (1965), that dead or decaying phytoplankton cells must be responsible for high DOC release rates in the oligotrophic ocean. Yet, their conclusions could not be tested, since techniques to assess cell viability and lysis rates were not then available.

Assessments of cell status in oligotrophic waters has provided evidence that a large proportion of phytoplankton cells are dead or compromised (Agustí, 2004), suggesting that cell lysis is a significant vector for DOC production in oligotrophic waters (Agustí et al., 1998, 2001), driving a significant flux of DOC to the microbial food web (Agustí et al., 2001). The experiments presented here confirm that healthy cells do not release substantial amounts of DOC and that the high release rate of DOC in oligrotrophic environments is attributable to high phytoplankton cell lysis. Indeed, the average percent DOC production rates and the initial DOC release rate were closely correlated with independent estimates of cell lysis rates measured for the communities prior to incubation, providing strong evidence that the high flux of DOC in unproductive communities, about 70 % of TOC production, is driven by cell lysis.

The decline in total accumulated TOC at time scales >15 min in unproductive communities provides evidence for rapid loss of organic carbon through bacterial respiration, as DOC taken up by bacteria should be recovered as labeled POC unless respired. These results indicate that the pathway of carbon through the microbial food web, from the uptake of inorganic carbon by phytoplankton, to their release by lysed cells to their incorporation and subsequent respiration by bacteria (Fig. 5) can be rather fast, involving time scales of 15 to 30 min, so that longer incubation terms result in very low apparent rates that approach net community production rates rather than the net rates of phytoplankton production. Laboratory experiments have indeed demonstrated the process from photosynthetic carbon incorporation by autotrophs to release of organic carbon and the subsequent incorporation and respiration by bacteria to be rather fast, on the order of 5 min (Puskaric and Mortain-Bertrand, 2003).

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The inference that the pattern of time course of organic carbon accumulation by unproductive communities observed here (e.g. Fig. 2a–c) can be explained by high phytoplankton cell lysis rates combined with rapid bacterial uptake and subsequent respiration was examined using a simple inverse model. More specifically, the inverse model was used to assess what processes are consistent with two puzzling features of the time-series experiments (Fig. 2): (a) the parallel increase in total TOC and POC produced at incubation times >1 h, suggesting a decline in PER over time; and (b) the initial rise and subsequent decline in labeled TOC, but not POC, in unproductive communities (Fig. 2a–c), compared to the sustained increase in TOC and POC produced in productive ones (Fig. 3d–f). The model included, in a parsimonious manner, the various process involved in the flow of inorganic carbon to phytoplankton, bacteria and back to CO_2 (Fig. 5, Table 2), including net primary production (NPP = gross primary production – phytoplankton respiration), as indicated by the initial rate of TOC production, measured after 15 min incubation time, the percent extracellular carbon released by phytoplankton (PER), bacteria uptake of phytoplankton-derived dissolved organic carbon (BCU, $\mu g\,C\,l^{-1}\,h^{-1}$), modeled from the accumulated phytoplankton-derived dissolved organic carbon using Michaelis-Menten kinetics, and bacterial production and respiration (BP and BR, respectively, both with units $\mu g\,C\,l^{-1}\,h^{-1}$), modeled using bacterial growth efficiency (BGE = range 1 to 10 % for open ocean communities, del Giorgio and Cole, 1998).

Most combinations of parameters resulted in a linear increase in the total TOC and POC produced through time, with different slopes (slope POC$=\frac{\text{Slope}_{\text{TOC}}\times\text{PER}}{100}$; Fig. 6a), as assumed by conventional applications of the ^{14}C technique. In contrast, the total TOC and POC produced through time tended to increase in parallel, i.e. with a similar slope, in the experiments (Fig. 2, Table 2). The model could reproduce this behavior, but yield a lower rate of increase in TOC and POC over time, if a high V_{max} and relatively low K_m for phytoplankton-derived DOC were used (Fig. 6b, Table 2). Lastly, the fast initial increase in TOC, but not POC, and subsequent decline to follow a parallel increase over time to that of POC, as observed in the unproductive communities

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studied (Fig. 2a–c), could only be reproduced by the model if, in addition to high V_{max} and relatively low K_m for phytoplankton-derived DOC, a PER in excess of 40 % and a time lag of about 15 min for bacterial use of phytoplankton-derived DOC were introduced (Fig. 6c, Table 2). The initial increment in TOC increased with increasing time lag and PER. An increase in BGE over the 2 % to 10 % range increased the slopes of TOC and POC accumulation over time but did not otherwise change the shape of their time courses.

A high V_{max} and low K_m , necessary to yield the time parallel course of TOC and POC accumulation observed, implies a high affinity of bacteria for phytoplankton-derived DOC, as the affinity, A, is defined as $A = \frac{V_{max}}{K_m}$ (Button, 1991). This is consistent with the expectation, derived from theoretical principles and experimental studies on bacteria isolated from the open ocean, that "*good oligotrophs have small Michaelis constants*", K_m, and high affinity and V_{max} (Button, 1991). High V_{max} and low K_m , characteristic of bacteria growing under oligotrophic conditions, yield a parallel accumulation of TOC and POC, with the apparent PER decreasing through time. The apparent PER after 1 h in the cases shown corresponded to 17 % (Fig. 7b) and 12.5 % (Fig. 7c), despite the actual PER generating these data was prescribed in the model as 90 %, constant over time. A decrease in PER with increasing incubation time had been noticed consistently in the past (Sharp, 1977; Lancelot, 1979; Moran et al., 2001) although often interpreted as real rather than apparent change. Yet, Lancelot (1979) already concluded, using times-series ^{14}C addition experiments comparable to those examined here on productive communities, that the decline is due to heterotrophic respiration of DOC released. The model results presented here suggest that high affinity for phytoplankton-derived DOC by open ocean bacteria can yield low apparent PER, decreasing with increasing incubation time, in the presence of a uniformly high PER.

The rapid initial accumulation of TOC, but not POC, followed by a decline and subsequent gradual, parallel increases in accumulated TOC and POC, observed in oligotrophic waters (Fig. 3d–f) requires (1) high PER, (2) high bacterial affinity for phytoplankton DOC, and (3) an initial time lag of up to 15 min between DOC release and

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bacterial use (Fig. 7c). This initial time lag was already noticed by Lancelot (1979), who explained it as the time required for internal molecules involved in PER to reach an internal equilibrium. In addition to an internal time lag, the time lag detected can also involve an external component, dependent on the time required for a steady-state on the phytoplankton PER-bacteria respiration process to be reached, as ^{14}C-labelled organic carbon will only be lost from the community once respired by bacteria. ^{14}C-labelled DOC released by phytoplankton will initially accumulate as POC incorporated by high-affinity bacteria, to be subsequently depleted due to bacteria respiration. Indeed, the initial rate of accumulation of TOC follows a rate close to NPP. Hence, the model results suggest that the cycling of carbon in the microbial food web occurs at a characteristic time scale of 10–15 min.

The results from the simple inverse model presented indicate that the time-course ^{14}C addition experiments conducted should be considered as a perturbation experiment, where addition of labeled C allows GPP to be detected before the steady-state equilibrium between phytoplankton DOC release and its uptake and subsequent respiration by bacteria is reestablished on the newly added ^{14}C pool.

The experimental and modeling results presented provide evidence for a rapid release of DOC and subsequent uptake and respiration by bacteria in the oligotrophic ocean. Recent results that show rapid aggregation of bacteria around patches of DOC, such as those expected from lysing cells (Stocker et al., 2008) provide evidence for the existence of the postulated phycosphere allowing for significant carbon flux between phytoplankton and bacteria to occur over time scales of minutes and spatial scales of millimeters (cf. Azam and Malfatti, 2007). Azam and Malfatti (2007) called for an approach to microbial oceanography that involves the assessment of biogeochemical processes at such small spatial and temporal scales to be subsequently scaled-up. Our results concur with this assessment, as we show that ^{14}C uptake experiments conducted over hours, as are conventional in oceanography, grossly underestimates net phytoplankton primary production in oligotrophic waters to approach the net production of the phytoplankton-bacteria assemblage. The decline in ^{14}C-based primary

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production rates with incubation time observed here has been know for almost 50 yr (Barnett and Hirota, 1967), but was believed to result from a time lag between uptake and DOC excretion, so that rates measured at short time intervals would approach net primary production whereas those at larger time-scales would underestimate it (Barnett and Hirota, 1967).

The results presented here show that net phytoplankton production in the oligotrophic ocean represents the emperor's new suite of clothes of carbon flux studies in the sea, since oceanographers assessing primary production at hourly time scales cannot observe it, because much of NPP has already been respired by bacteria. However, the magnitude of net phytoplankton production can be constrained from independent estimates of carbon use by bacteria, which indeed reflect a much higher rate of organic carbon supply in the ecosystem than that derived from conventional (incubation times of hours) ^{14}C-based estimates of primary production. The results presented here explain difficulties to reconcile conventional ^{14}C-based estimates of primary production with independent estimates of bacterial carbon use (Williams, 1984; del Giorgio et al., 1997), which were interpreted as evidence of heterotrophy in the past (del Giorgio et al., 1997).

However, high DOC release, exceeding 40 % of net primary production, is essential to support the rapid and high flux of photosynthetic carbon through bacteria. Such high rates are, again, very unlikely for healthy phytoplankton cells (Sharp, 1977). The strong relationship between independently assessed phytoplankton cell lysis rates and short term DOC flux and mean hourly PER demonstrated here provides, thirty years later, the answer to the rhetoric question "Do healthy cells do it?" (Sharp, 1977). The results presented show that the high DOC release rates by phytoplankton in the oligotrophic ocean are not supported by healthy cells but, rather, but dying ones, as suspected long ago (Duursma, 1965). Evidence for high lysis rates (Agustí et al., 1998, 2001) and a high percentage of dying or compromised cells (Agustí, 2004; Alonso-Laita and Agustí, 2006; Lasternas et al., 2010) as characteristic properties of phytoplankton communities in the oligotrophic ocean is accumulating in parallel to an increased understanding of

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the multiple cellular and molecular mechanisms (Bidle et al., 2004) and environmental drivers (Llabrés and Agustí, 2006; Echeveste et al., 2010) responsible for these high mortality rates.

In summary, the results presented here show that communities in productive ocean waters are able to accumulate organic carbon over hourly time scales, whereas only a small fraction of net primary production accumulated in communities from oligotrophic waters. These communities support high phytoplankton cell lysis rates leading to a rapid flux of organic carbon to bacteria, much of which was respired within 15 min of being produced. The assessments of carbon flow in microbial communities from oligotrophic ocean waters must be reconsidered to include analyses at the short time scales at which these processes operate and to better integrate estimates of cell status and death by phytoplankton and bacterial carbon use to derive a consistent understanding of the high, but hitherto largely invisible, flux of carbon in the oligotrophic ocean, the largest ecosystem in the biosphere.

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Table 1. Mean ±SE and range of Chla, cell lysis rates (day^{-1}), particulate organic carbon production rate (incubation time >6 h), percent extracellular carbon release (PER, %, incubation time >6 h) in the communities tested in the various cruises conducted.

Cruise	Chl-*a* (μg chl-*a* l^{-1})	Lysis d^{-1}
BADE-1	–	–
BADE-2	–	–
COCA-2	1.01 ± 0.56 (0.04–4.27)	0.37 ± 0.13 (0.00–1.09)
ICEPOS	2.24 ± 0.19 (1.88–2.78)	0.06 ± 0.01 (0.04–0.09)
	PER (%)	POC production (μg C L^{-1} h^{-1})
BADE-1	34.18 ± 10.50 (6.68–54.28)	4.77 ± 0.50 (3.54–5.99)
BADE-2	15.30 ± 5.18 (8.86–30.77)	3.45 ± 0.61 (2.14–5.06)
COCA-2	47.50 ± 11.22 (0.27–85.77)	7.97 ± 4.24 (0.62–32.31)
ICEPOS	15.80 ± 6.06 (3.79–32.11)	6.54 ± 1.34 (4.58–10.44)

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Table 2. Values for the parameters of the simulation model yielding the results displayed in the three panels (cases a, b and c) in Fig. 6. The parameters involved (cf. Fig. 5) are net primary production (NEP = GPP – phytoplankton respiration), as indicated by the initial rate of TOC production, measured after 15 min incubation time, the percent extracellular carbon released by phytoplankton (PER), bacteria uptake of phytoplankton-derived dissolved organic carbon, modeled from the Michaelis-Menten kinetics using maximum uptake rates (V_{max}) and half-saturation constants (K_m) and, the fate, bacterial production or respiration, or the assimilated DOC modeled using bacterial growth efficiency (BGE).

Parameter	Units	Panel a	Panel b	Panel c
Net Primary Production	$\mu g\,C\,l^{-1}\,h^{-1}$	50	50	50
Percent extracellular release	%	90	90	90
RDOC	$\mu g\,C\,l^{-1}\,h^{-1}$	40	40	40
Lag DOC release-Bacterial Use	min	0	0	15
Bacteria Growth Efficiency	%	2	2	2
K_{max}	$\mu g\,C\,l^{-1}$	50	20	20
V_{max}	$\mu g\,C\,l^{-1}$	10	500	500

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Table 3. Slopes ($\mu g\,C\,l^{-1}\,h^{-1}$), and corresponding p-values (t-test) of linear regression analysis of total organic carbon (TOC) and particulate organic carbon production (POC) over time, and the short term (measured over 15 min intervals) TOC and POC production rates for the various time course experiments performed.

Cruise	Experiment	Slope TOC ($\mu g\,C\,l^{-1}\,h^{-1}$)	p	Slope POC ($\mu g\,C\,l^{-1}\,h^{-1}$)
COCA-2	1	2.32	0	0.29
COCA-2	2	1.04	0	2.08
COCA-2	3	−1.38	1	−0.007
COCA-2	4	−0.43	1	0.02
COCA-2	5	0.62	1	0.76
COCA-2	6	−2.54	1	0.23
COCA-2	7	19.37	0	19.62
COCA-2	8	27.48	0	23.06
BADE-1	1	4.73	0	4.88
BADE-1	2	3.49	0	3.68
BADE-1	3	4.6	0	4.81
BADE-1	4	2.95	0	3.16
BADE-2	1	4.99	0	5.05
BADE-2	2	1.25	0	1.23
BADE-2	3	2.14	0	2.69
BADE-2	4	2.83	0	2.45
ICEPOS	1	3.26	0	2.99
ICEPOS	2	5.87	0	7.11
ICEPOS	3	4.66	0	4.35
ICEPOS	4	5.73	0	5.87

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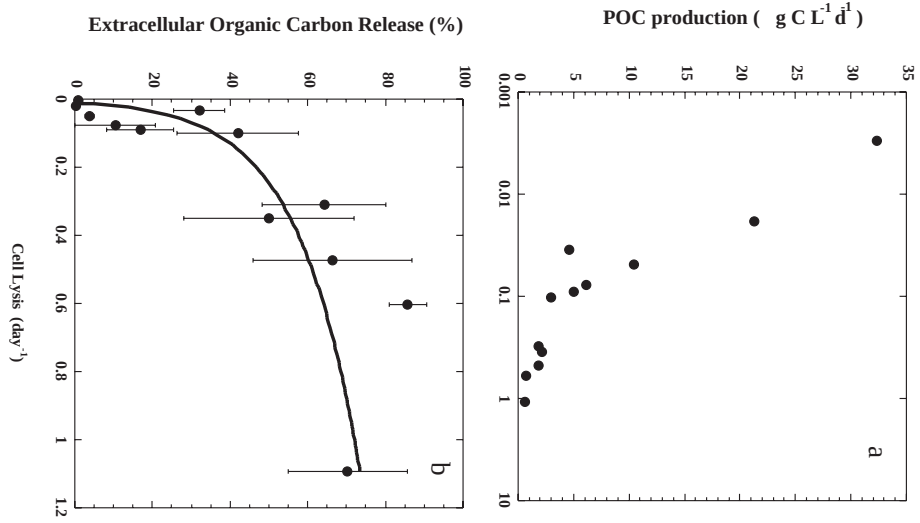

Fig. 1. The relationship between particulate organic carbon production (POC, **a**), and the mean (±SE) percent extracellular organic carbon release (PER, **b**) and cell lysis rates in the communities investigated. The solid line in **(b)** represents the fitted regression equation, PER (%) = 72.0 + 36.2 log (Lysis rate, day^{-1}), $R^2 = 0.74$, $P < 0.05$.

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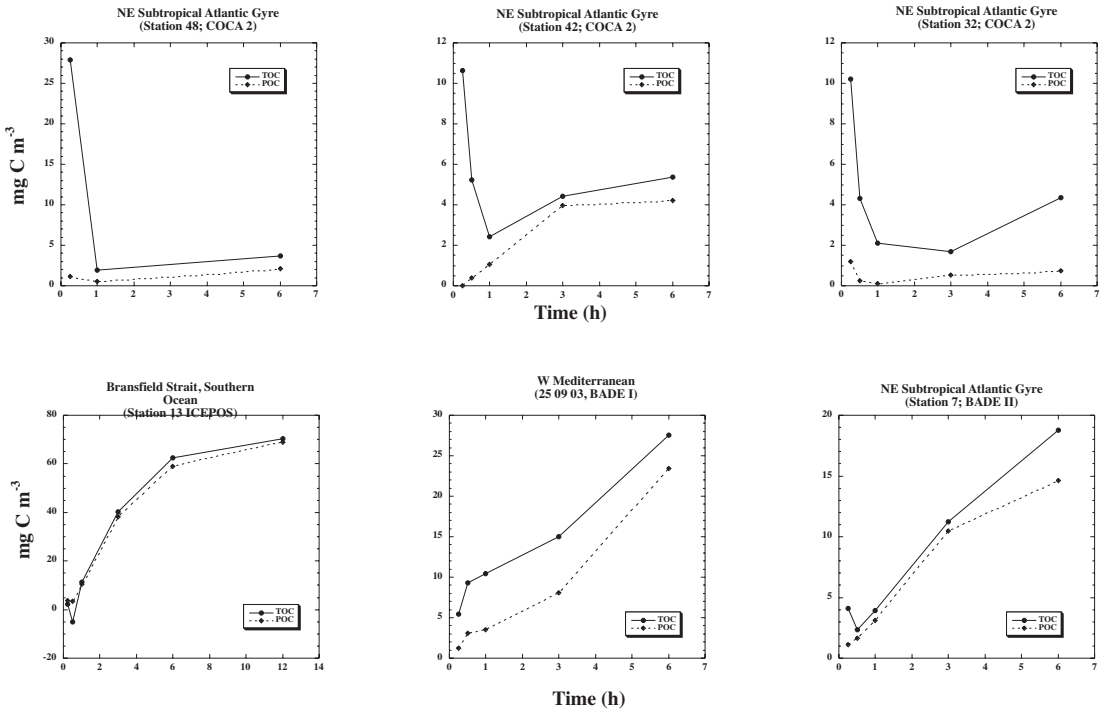

Fig. 2. Time series of total TOC and DOC produced in ^{14}C addition experiments in oligotrophic **(a–c)** and more productive **(d–f)** communities showing the diversity of time courses observed in the experiments.

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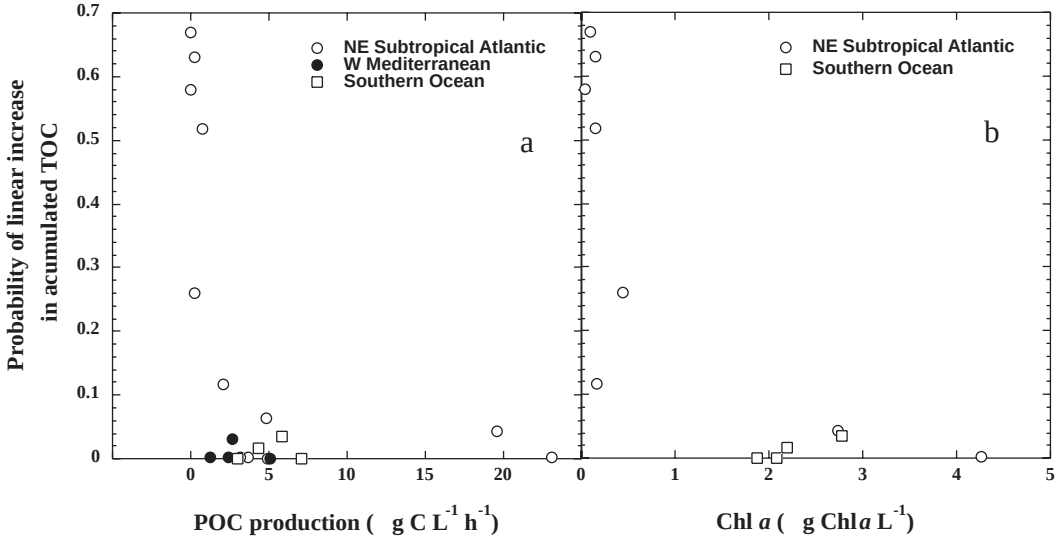

Fig. 3. The relationship between the probability that the slope of the accumulation of total organic carbon (TOC) over time in time series of ^{14}C addition experiments equals 0 (*t*-test) and **(a)** the production of particulate organic carbon and **(b)** Chlorophyll a concentration of the communities tested.

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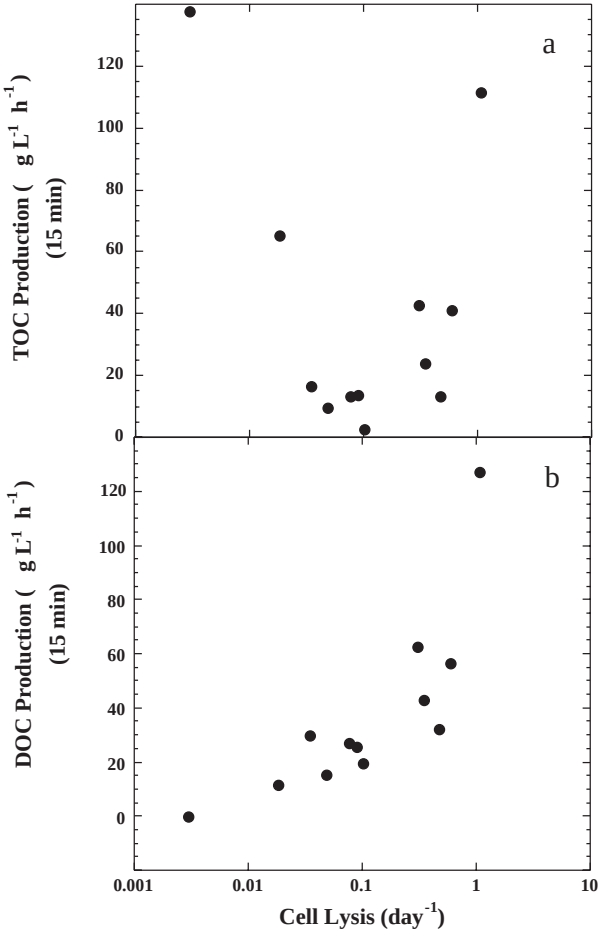

Fig. 4. The relationship between short-term rates of total organic carbon (TOC) and dissolved organic carbon (DOC) production and cell lysis rates of the plankton communities tested.

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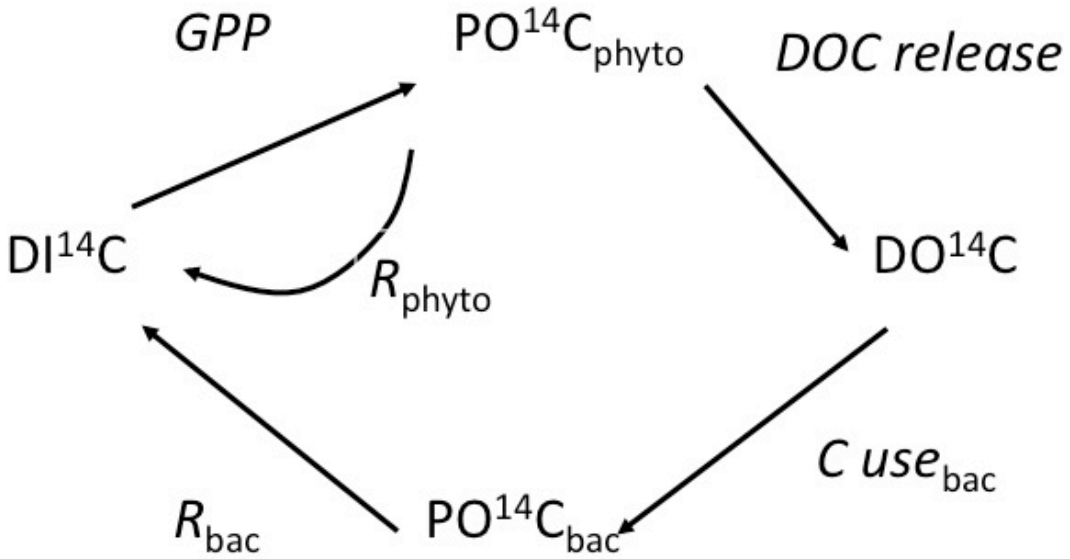

Fig. 5. Conceptual model of the cycling of carbon in the microbial food web following additions of labelled dissolved inorganic carbon ($DI^{14}C$). GPP = gross primary production; R_{phyto} = phytoplankton respiration; $PO^{14}C_{phyto}$ = labelled particulate phytoplankton organic carbon; $DOC_{release}$ = dissolved organic carbon release by phytoplankton; $Cuse_{bac}$ = carbon use by bacteria; $PO^{14}C_{bac}$ = labelled particulate phytoplankton bacteria carbon (and that of bacteria grazers); R_{bac} = Bacterial respiration (and that of bacteria grazers).

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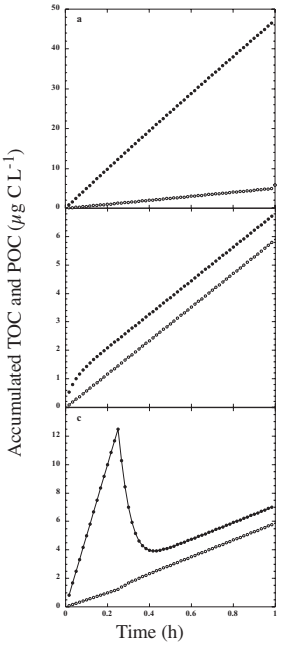

Fig. 6. Time course of total and particulate organic carbon accumulation showing contrasting trajectories derived by different model parameters. Most combinations of parameters resulted in a linear increase in the total TOC and POC produced through time with different slopes **(a)**; parallel TOC and POC trajectories were obtained when high bacteria V_{max} and relatively low K_m for phytoplankton-derived DOC were used **(b)**; and the fast initial increase in TOC, but not POC, and subsequent decline to follow a parallel increase over time to that of POC was reproduced if, in addition to high V_{max} and relatively low K_m for phytoplankton-derived DOC, a percent extracellular release in excess of 40 % and a time lag of about 15 min for bacterial use of phytoplankton-derived DOC were introduced **(c)**. The model parameters yielding the trajectories displayed here are shown in Table 2.

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