

The effect of nutrient additions on the partitioning of nutrients in an experimental coastal Mediterranean system

CARLOS M. DUARTE^{1,*}, ANNA LUCEA^{1,3} and MORTEN SØNDERGAARD²

¹IMEDEA (CSIC-UIB), Instituto Mediterráneo de Estudios Avanzados CSIC – UIB, C/Miquel Marqués, 21, Esporles, 07190, Mallorca, Spain; ²Freshwater Biological Laboratory, University of Copenhagen, Helsingørsgade 51, Hillerød, 3400, Denmark; ³Current address: NYLSTAR S.A., Avda. Estació, 53, Blanes, 17300, Spain; *Author for correspondence (e-mail: cduarte@uib.es; phone: 34 971 611725; fax: 34 971 611761)

Received 5 February 2002; accepted in revised form 26 February 2003

Abstract. The hypothesis that the importance of dissolved organic matter (DOM) as a reservoir of C, N, and P declines, relative to that of the particulate pool, with increasing nutrient inputs was tested using mesocosms exposed to a gradient of nutrient inputs in the Spanish Mediterranean. The nutrient additions included a treatment equivalent to the loading in the coastal ecosystem studied (5 mmol N m⁻² d⁻¹), and mesocosms receiving half, 2-, 4-, 8-, and 16-fold this value, as well as a mesocosm to which no nutrients were added. Nutrients were added at ratios of 20 N (as ammonium): 7 Si: 1 P. The initial concentration of dissolved inorganic nutrients was very low (dissolved inorganic nitrogen $< 0.05 \mu$ M, phosphate < 0.01) and comprised, together with the particulate pool < 25% of the total N and P in the system, with the bulk N and P in the system present as DOM (> 75%). Particulate and dissolved organic matter was depleted in N (C/N ratio > 15) and, particularly, P (C/P ratio > 1000), indicative of a strongly nutrient, particularly phosphorus, deficient ecosystem. Experimental nutrient additions lead to a parabolic change in C/N and C/P ratios in the dissolved organic matter with increasing nutrient inputs, which approached the Redfield stoichiometry at nutrient inputs > 8 fold above the ambient loading. The relative size of the dissolved inorganic nutrient pools (about 20% of the N and P) did not vary, but there was a tendency towards an increase in the relative size of the particulate pool at the expense of a decrease in the relative importance of DOM as a reservoir of N, P and C, with increasing nutrient inputs. The production of nutrient-depleted organic matter at low nutrient inputs likely prevents efficient recycling, leading to the dominance of nutrients in DOM in the system.

Introduction

Dissolved organic matter (DOM) is a major reservoir of nutrients and carbon in the sea (Druffel et al. 1992; Hedges 1992), particularly in unproductive waters, where the pools of dissolved organic carbon (DOC) and nutrients are dominant (e.g., Hedges 1992, Wheeler et al. 1997, Doval et al. 1999, Vidal et al. 1999). The greater importance of DOM in unproductive compared to productive systems is paradoxical, for it suggests that the plankton communities in nutrient-poor environments should be less efficient in conserving the nutrients incorporated by phytoplankton than those in nutrient-rich waters. Yet, the apparent tendency towards a dominance

of DOM as a nutrient reservoir in oligotrohic systems relies on scattered information from the relatively few reports that include simultaneous data on dissolved and particulate nutrient pools. Moreover, this pattern may reflect a covariation between nutrient availability and other traits influencing the biota (e.g., temperature, cf. Li 1998, Agawin et al. 2000) rather than responses to a gradient of nutrient supply across an oligotrophic to eutrophic gradient. There is, therefore, a need to test the changes in the partitioning of nutrients among different pools (inorganic and dissolved and particulate organic) in response to a gradient of nutrient supply.

We test here the hypothesis that the importance of dissolved organic matter as a reservoir of C, N, and P declines, relative to that of the particulate pool, with increasing nutrient inputs. We do so on the basis of the examination of the changes in the concentration of nutrients (nitrogen and phosphorus) and carbon in different pools in response to experimental nutrient additions to experimental mesocosms moored in the Spanish Mediterranean (Blanes Bay, NE Spain).

Methods

The experiment was conducted between June 18 and July 8, 1997 near the town of Blanes (NE Spain). The experimental design involved a gradient of nutrient additions to mesocosms following a geometric series of nutrient inputs centered at the nutrient loading calculated for the Bay of Blanes. The background loading is estimated to be about 5 mmol N m⁻² d⁻¹ in summer (Duarte et al. 2000a), and is hereafter referred to as a "business as usual" control. Phosphorus and silicon were added to maintain their average summer stoichiometry with nitrogen in the sedimentary flux (20 N: 7 Si: 1 P, Duarte et al. 2000a). Nitrogen was added as ammonium, the dominant form of summer nitrogen inputs to the Blanes Bay (Duarte et al. 2000a).

We used a series of 7 large (nominal and effective volume 50 and 33 m³, respectively) mesocosms, consisting of 14 m high bags and a 4.2 m² cross-sectional area. One of the units (that receiving twice the "business as usual" nutrient loading), was found to be damaged four days prior to the end of the experiment. Nutrients, as solutions of ClNH₄, KH₂PO₄, and Na₂SiF₆, were added to the mesocosms in alternate days, following the collection of samples. The nutrient additions included a treatment equivalent to the "business as usual" control, a treatment equivalent to half of that nutrient input, and enriched nutrient additions, we also examined the changes in nutrient pools in a mesocosm to which no nutrients were added. The nutrient input to this mesocosm unit, derived from wet and dry atmospheric deposition, was determined from short-term nutrient mass balances to be about 0.005 μ M N d⁻¹ (Duarte et al. 2000a). Additional details on the experimental design are reported by Duarte et al. (2000a).

Integrated water samples (0-13 m) to determine nutrient concentrations and phytoplankton biomass, as chlorophyll a concentration, were collected in alternate days at 7:00 am. Briefly, a washed and pre-rinsed hose (internal diameter 8 cm)

with a ballasted end connected to the surface by a piece of rope was slowly deployed down to 13 m depth, and the ballasted end subsequently retrieved to the surface while maintaining the opposite end higher up as to prevent backflow, delivering the contents to an acid-washed 30 L carboy. The first sampling event occurred prior to the first nutrient addition, as to allow identification of any possible initial difference not attributable to the treatment. The water samples were transported to the laboratory for analysis within 30 min. At four days interval, the sedimentary material accumulated in the cone-shaped bottom was pumped out of the mesocosm, using a hand pump connected to the bottom of the mesocosm by a hose.

The concentrations of dissolved inorganic phosphorus (DIP), ammonium and silicate were measured spectrophotometrically following standard methods (Hansen and Koroleff 1999), using a 10-cm cuvette cell when necessary to increase the detection limit. Samples for dissolved organic N and P determinations were immediately filtered through previously rinsed, 0.2 µm cellulose ester filters mounted on polyvinyl chloride Millex units (Millipore). Controls with distilled water showed that the filtration step yielded no measurable contamination of the samples. Filtered samples were recovered in polycarbonate bottles, which had been cleaned by filling them with distilled water and running the oxidation procedure several times, soaking in diluted HCl and rinsing with distilled water. Independent samples were analyzed for total dissolved nitrogen (TDN) and phosphorus (TDP). TDN and TDP concentrations were determined after oxidation at 120 °C for 30 min of the filtrate of 20 ml subsamples in alkaline and acidic persulfate, respectively, and subsequent analyses of dissolved nitrate and phosphate (Hansen and Koroleff 1999). EDTA standards revealed a recovery of DON of 97%. The analytical precision, estimated as the standard deviation of replicated samples, was 0.26 and 0.014 µM for TDN and TDP, respectively. DON and DOP were calculated as the difference between total and inorganic N and P. The detection limits of dissolved nutrient concentrations were 0.005, 0.05, 0.01 μ M for NO₃⁻ + NO₂⁻, NH₄⁺, and PO₄³⁻, respectively.

Samples (1 to 4 L, depending on seston concentration) for particulate organic carbon (POC) nitrogen (PON) and phosphorus (POP) determinations were filtered through precombusted (450 °C, 2 h) GF/F filters, kept frozen until analysis. POC and PON were analysed in a Carlo-Erba analyser after thawing the filters in an atmosphere of HCl fumes to remove carbonate and then drying at 60 °C. POP was determined following oxidation, at 120 °C for 1.5 h of the sample in acidic persulfate and subsequent analysis of the dissolved phosphate (Hansen and Koroleff 1999).

Samples for analysis of dissolved organic carbon (DOC) were immediately filtered (burned Whatman GF/F filters) and 10 ml was collected directly in burned glass vials, added 100 μ l 2 M HCl, sealed and stored in the dark for later measurement. DOC was measured by Pt-catalyzed high temperature combustion in a Shimadzu TOC-5000 with autosampler injection and after sparging with the carrier gas for 6 minutes at 75 ml/min (Sharp et al. 1993). A 4-point calibration curve between 50 and 200 μ M was prepared for each series of measurement. A mean blank area (n = 10) was subtracted before the slope of the calibration curve was used to calculate sample concentrations. Blanks were prepared with the water used for the standard solutions and evenly distributed within each series of measurements. Measurements of the DOC reference materials (blank and deep ocean water) prepared and supplied by Jonathan Sharp (personal communication, 1997) were included in each series. A statistically significant deviation (10% level, t-test) in concentration of deep ocean water of more than 6 μ M (n = 6) from the stated 44 μ M led to a second analysis of the samples. Our blank samples (MQ-water) were on the average 3 ± 1 μ M (SD, n = 25) higher than the reference blank, which is the reason to accept a deviation of 6 μ M. In consequence the presented DOC concentrations might be slightly biased to the low side.

A variable water volume (50 to 500 ml, depending on phytoplankton biomass) was filtered through Whatman GF/F filters for fluorometric analysis of chlorophyll a concentration (Parsons et al. 1984). The filters were homogenised and kept refrigerated in the dark while pigments were extracted in 90% acetone for ca. 6 h. Fluorescence was measured in a Turner Designs fluorometer calibrated with pure chlorophyll a (Sigma Co.).

Results

The biomass of the phytoplankton community increased with increasing nutrient inputs, following a time lag of about 4 to 12 days, reaching a maximum chlorophyll a concentration of 40.8 µg/L at the greatest nutrient inputs (Figure 1). The initial concentration of dissolved inorganic nutrients was very low (dissolved inorganic nitrogen $< 0.05 \,\mu$ M, phosphate = 0.01, Figure 2), and among the lowest values recorded in a 7-year monitoring program in Blanes Bay (Duarte, unpubl. data). Initial particulate and dissolved organic nitrogen concentrations at 1.52 and 2.5 µM N, respectively, were substantially higher than inorganic N (Figures 3 and 4), and DON comprised 88% of the total nitrogen. This was also the case for phosphorus, for which the initial POP and DOP concentrations (0.022 and 0.15 μ M P) exceeded that of dissolved inorganic P by two and ten fold, respectively (Figures 3 and 4). Hence, the dissolved inorganic and the particulate pools comprised < 10% of the total N and P (4.5% and 8.1% respectively) and about 15% of the total N and P (17.5% and 14.2% respectively) in the system at the onset of the experiment. About 78% of the N and P in the system was, therefore, in DOM, and DOC also comprised most (84%) of the organic carbon present in the system at the onset of the experiment.

The added nutrients were rapidly assimilated by the community, so that accumulation of the added dissolved inorganic nutrients was only observed after increasing nutrient inputs 4 (for ammonium and silicate) to 8 (for phosphate) times the "business as usual" value (Figure 3). After an initial period (4 to 8 days), the communities present in the mesocosms built a biomass sufficient to lower the accumulated nutrient pools in the mesocosms receiving nutrient inputs elevated > 4 fold above the ambient levels. As a result, the dissolved inorganic nutrient concentrations were low (< 0.5 μ M for ammonium, < 1 μ M for dissolved inorganic nitro-



Figure 1. The response of phytoplankton biomass, as the chlorophyll a concentration, in mesocosms enclosing receiving increasing nutrient inputs.

gen, < 0.2 μ M for phosphate) in all mesocosms by the end of the experiment (Figure 2). DON concentrations increased from the initial concentrations of 2.5 μ M shortly (4 days) after the initiation of nutrient inputs but decreased subsequently to reach levels below the initial ones (Figure 4). DOP concentrations also showed a declining trend, following an initial increase at high nutrient dosage, along the experiment, except in the mesocosm receiving the highest nutrient dose, where DOP concentrations increased over the last week of the experiment to reach values four-fold greater than those encountered at the onset of the experiment (Figure 4).

DIC comprised most of the carbon present in the mesocosm, but was reduced as nutrient inputs increased, indicating an important draw-down by photosynthetic uptake (Figure 2). As a result, the POC pool increased greatly with increasing nutrient inputs (Figure 3). Yet, DOC, which comprised about 6 times more carbon than that present in particulate organic form at the onset of the experiment, remained relatively constant, except for an increase at a rate of $17.8 \pm 1.7 \mu$ mol C L⁻¹ d⁻¹ over the last 2 weeks of the experiment in the mesocosm receiving the highest nutrient load (Figure 4).

The very low concentration of dissolved inorganic nutrients at the onset of the experiment were mirrored by the nutrient depleted organic pools, with average C/N ratios of 18 and 15.8 and average C/P ratios of 1101 and 1031 in the dissolved and particulate organic pools, respectively. Phosphorus was in deficit relative to nitro-



Figure 2. The response of dissolved inorganic nutrient and carbon concentrations in mesocosms enclosing receiving increasing nutrient inputs. Dissolved inorganic phosphorus concentration from Duarte et al. (2000a).



Figure 3. The response of particulate organic nutrient and carbon concentrations in mesocosms enclosing receiving increasing nutrient inputs.



Figure 4. The response of dissolved organic nutrient and carbon concentrations in mesocosms enclosing receiving increasing nutrient inputs.

gen in all pools at the onset of the experiment, with atomic N/P ratios of 31, 104 and 69 in the dissolved inorganic and organic, and the particulate organic pools, respectively. Hence, the nutrient pools at the onset of the experiment deviated greatly from the general Redfield stoichiometry, indicative of a strongly nutrient deficient ecosystem, particularly with respect to phosphorus. Experimental nutrient additions altered this situation, leading to a parabolic change in C/N and C/P ratios in the dissolved organic matter with increasing nutrient inputs (Figure 5). The nutrient ratios in the particulate organic fraction substantially exceeded the Redfield stoichiometry until nutrient inputs were increased > 8 fold above the "business as usual scenario", and reached values closer to Redfield stoichiometry at higher nutrient inputs (Figure 5). The deviation of nutrient ratios from the Redfield stoichiometry was often highest at nutrient inputs ranging between the "business as usual" and 4 fold higher loadings (Figure 5). The C/N and N/P ratios in the sedimentary material removed from the mesocosms averaged 17.7 and 8.3, respectively, and tended to decrease with increasing nutrient inputs to reach average C/N ratios of 13.5 However, the average N/P ratio in the material did not change with increasing nutrient inputs.

Increased nutrient inputs led to a shift in the partitioning of nutrients between the dissolved inorganic and organic, and the particulate organic pools by the end of the experiment (Figure 6). While the relative size of the dissolved inorganic nutrient pools (about 20% of the N and P) did not differ substantially with increasing nutrient inputs, there was a tendency towards an increase in the relative size of the particulate pool at the expense of DOM as a reservoir of N, P and C (Figure 6). These tendencies were significant (P < 0.05) in all cases except for the decrease in the relative size of the DOP pool with increasing nutrients, which was not statistically significant (P = 0.24). Despite these tendencies, the DOC pool remained the dominant pool of organic carbon, whereas most of the N and P were contained in the particulate organic matter at the highest nutrient inputs tested (Figure 6).

Discussion

The Mediterranean waters investigated were acutely oligotrophic at the onset of the experiment, representative of the nutrient-poor conditions that characterise Mediterranean waters in the summer season. In agreement with previous reports from oligotrophic waters (e.g., Doval et al. 1999, Vidal et al. 1999), dissolved organic matter was the dominant pool of nutrients and organic carbon in the nutrient-poor area investigated. The concentrations of nutrients, particularly that of N, in the dissolved organic matter was sufficiently high as to be able to fuel the planktonic community well above their normal biomass if this nutrient pool was available. That this was not the case, and that the planktonic community was strongly nutrient-limited (Duarte et al. 2000a) suggest that the dissolved organic nutrient pool was unavailable to the community, thereby accumulating in the system to comprise > 75% of the N, P and organic C in this oligotrophic ecosystem. The nutrient defi-



N input (μ M d⁻¹)

Figure 5. The average C/N, C/P and N/P ratios of the dissolved and particulate organic pools in the mesocosms between days 14 to 20 after the initiation of the experiment. Error bars represent the standard error of the average values.

ciency was alleviated by the increased nutrient inputs, which accummulated as dissolved inorganic nutrients until the community developed sufficient biomass as to



Figure 6. The average percentage of N, P and C in the dissolved (inorganic and organic) and particulate pools in the mesocosms between days 14 to 20 after the initiation of the experiment. Dissolved inorganic carbon is not represented, for its very large size compared to the other pools would obscure any patterns present there. Error bars represent the standard error of the average values.

be able to remove them. Dissolved inorganic nutrients were efficiently removed after this initial response occurred, which reduced the nutrient input per unit biomass, eventually driving the community back to a situation of nutrient limitation. The bulk of the nutrients added (> 70%) were lost through sedimentation of particulate organic matter, which was pumped out from the mesocosms at four-day intervals.

The distribution of nutrients at the onset of the experiment was indicative of a strongly nutrient-limited system, where both dissolved inorganic N and P, but particularly the latter, were deficient compared with the standard Redfield stoichiometry. This situations is consistent with reports of a general tendency for phosphorus limitation in the Mediterranean planktonic ecosystem (Krom et al. 1991; Thingstad and Rassoulzadegan 1995; Thingstad et al. 1998; Zohary and Robarts 1998). The particulate organic pool, which comprised a modest fraction of the nutrients (<

20%) was also characterised by exceedingly high C/P and C/N ratios, as a result of the dominance of detrital material (Duarte et al. 2000b). Nutrient-depleted organic matter pools are highly resistant and can only be degraded significantly if an external nutrient supply is available (e.g., Zweifel et al. 1993). The phytoplankton communities present in the oligotrophic waters examined have been reported to be major sources of DOM, derived from high cell lysis rates in summer (Agustí and Duarte 2000). Provided the very low nutrient concentration at the onset of the experiment, it is clear that organic matter would accumulate, albeit slowly given the low primary production, as DOM, thereby accounting for the dominance of this pool relative to the POM pool at low nutrient supply.

The nutrient ratios in the different pools, particularly the particulate pool, only approached the Redfield ratio at the highest nutrient additions (> 20 mmol N m⁻² d⁻¹), despite the "business as usual" nutrient loading being already considerable (5 mmol N m⁻² d⁻¹). The apparent refractory nature of the dissolved organic matter pool is associated with very high C/nutrient ratios (C/N = 18, C/P =1101) in the DOM, which are likely to result in a very ineffective use and growth yield of bacteria (Zweifel et al. 1993; Cherrier et al. 1996). The DOM was particularly poor in phosphorus, consistent with the general phosphorus deficiency in Mediterranean waters (Krom et al. 1991; Thingstad and Rassoulzadegan 1995; Thingstad et al. 1998; Zohary and Robarts 1998). The accumulation of dissolved organic matter in oligotrophic waters suggests that recycling processes are limited by nutrient availability, particularly that of P (e.g., Zweifel et al. 1993; Rivkin and Anderson 1997), which has been found to limit bacteria in oligotrophic marine waters (Rivkin and Anderson 1997; Cotner et al. 1997), including the Mediterranean Sea (cf. Thingstad and Rassoulzadegan 1995, Thingstad et al. 1998).

The high initial increase in DON concentrations at the highest nutrient additions is puzzling, since it would require that most of the added inorganic nitrogen be cycled to organic form by a comparatively small autotrophic community, and be rapidly released to the water. Phytoplankton mortality rates were high (> 1 day⁻¹, Agustí and Duarte 2000) at the onset of the experiment, providing a mechanism for the loss of the primary production to DON, and the maintenance of a modest biomass. Dissolved organic nitrogen was rapidly consumed following an initial increase. In contrast, DOC concentrations remained relatively uniform and only increased in the mesocosm receiving the highest nutrient loading. This increased occurred at a rate of $17.8 \pm 1.7 \ \mu mol \ L^{-1} \ d^{-1}$, corresponding to about 10% of the primary production. These results are in contrast with those of mesocosm experiments in Atlantic waters (Søndergaard et al. 2000), where very high DOC accumulation rates were observed even at low nutrient inputs compared to those applied here.

The experimental results do not necessarily represent steady-state conditions, since some nutrient pools were clearly increasing (e.g., DOC and PON) at the time the experiment was terminated. Continuation of the experiment over longer time scales, as to assess responses at the seasonal scales that largely drive the shifts between nutrient pools in temperate coastal waters, such as the seasonal increase in DOC in coastal waters (Williams 1995), would have introduced confounding fac-

tors from wall effects. This limitation in the time scale over which the experiment was run, a common limitation of mesocosm experiments (Duarte et al. 1997), implies that the results observed are not readily extrapollatable over longer time scales.

The results presented clearly support the hypothesis of a shift from a dominance of nutrients in the DOM pool to an increasing importance of the POM pool with increasing nutrient inputs. The percentage of nutrients in dissolved organic form, as well as the absolute concentration of dissolved organic nitrogen, tends to decline with increasing nutrient inputs, consistent with the observed contrast in the importance of particulate and dissolved pools in oligotrophic vs. eutrophic waters (e.g., Slawyk and Raimbault, 1995; Karl et al. 1997, Wheeler et al. 1997, Doval et al. 1999, Vidal et al. 1999, Packard et al. 2000). Oligotrophic waters are characterised by a dominance of nutrients, particularly N, in dissolved organic form, likely because the dissolved organic matter there is still so poor in nutrients that bacteria require external nutrient sources to recycle it. In contrast, high nutrient inputs result in a dominance of particulate nutrients. The shift in the relative partitioning of organic nutrients between the dissolved and the particulate pools with increasing nutrient inputs implies a shift in the form of carbon export from DOM accumulation and subsequent gradient-driven export to sinking fluxes with increasing nutrient inputs. Moreover, a dominance of nutrients in DOM form allows a long-range horizontal export of carbon and nutrients. In addition, the dominance of particulate organic nutrients in waters receiving high nutrient inputs implies that these should be available to be transferred to protists and metazoans. In contrast, the high fraction of the nutrients and carbon contained in DOM in oligotrophic waters can only be transferred up the food web through bacterial use, thereby accounting for differences between the importance of the microbial loop in oligotrophic waters and the dominance of the linear food web in more productive waters (Legendre and Rassoulzadegan 1995). The important consequences of the shift in the partitioning of carbon and nutrients between dissolved and particulate organic pools indicates that further progress in our understanding of the processes responsible for this pattern will substantially enhance our capacity to predict the fate of carbon and nutrients in pelagic waters.

Acknowledgements

This is a contribution to the project COMWEB, funded by the ELOISE programme of the European Commission (contract number MAS3-CT96-0045), and a grant from the Spanish Commission for Science and Technology. We thank O. Moreno for chlorophyll a analyses, J. Seppala, T. Tamminen, and the staff at Tallers FERM (Blanes, Spain) for advice in the design and construction of the mesocosms, A. Juán Sr. and Jr. for assistance with the operation of the mesocosms, the Blanes Peix company for permission to deploy the platform in their concession, and two anonymous reviewers for comments. M.S. was supported by a grant from the Danish Natural Sciences Research Council.

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