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Comparison between chemical and isotopic measurements of biological nitrate utilization: further evidence of low new-production levels in the equatorial Pacific

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Abstract New-production (nitrate uptake) rates in the equatorial Pacific were estimated by parallel measurements of nitrate disappearance from sea water using a colorimetric method and of 15N-labelled nitrate (15NO3−) incorporation into particulate organic nitrogen (PON) collected on GF/F filters (net nitrate uptake, conventional 15N-tracer method) and Anopore (0.2 μm) membranes. Regression analyses of 74 sample pairs gathered during 12 and 24 h productivity experiments revealed a significant positive relationship between decreasing nitrate level and 15NO3− accumulation into PON retained on GF/F filters, but the slopes of Model I and Model II regression lines were 1.18 and 1.29, respectively, suggesting that 15 to 22% of 15NO3− removed from the dissolved fraction were lost to another N-pool. Two possible avenues for the missing 15NO3− have been examined: uptake by submicron particles passed through the GF/F filters, and loss as dissolved organic nitrogen (DON). Nitrate uptake by small cells not recovered on GF/F filters, could be safely eliminated as a cause of loss, since 15NO3− uptake rates obtained from 15N entering PON collected on GF/F filters agreed well with those obtained from 15N entering PON collected on Anopore membranes (32 sample pairs). Inspection of the DON pool of 0.2 μm filtrates for excess 15N enrichment (20 samples) revealed that in nitrate-rich waters (equatorial upwelling between 1°N and 10°S), loss of 15NO3− as DON accounted for <5% of net nitrate uptake. In samples from subtropical oligotrophic waters (from 11°S southward), however, 15NO3− loss as DON represented up to 20% of net NO3− uptake. These results, as well as experimental considerations concerning the use of colorimetric and isotopic methods to measure new production show that: (1) earlier reported high discrepancies between nitrate decreases (ΔNO3−) and 15NO3− incorporation into filterable particles (ΔNO3−/15NO3− incorporation >2) were probably erroneous; (2) the use of GF/F filters does not result in an underestimation of new production, although it was found to underestimate PON concentrations by up to 60%; (3) in the equatorial upwelling area (1°N to 10°S), which has high ambient nitrate levels (>2000 nmol l−1) but only slight changes in concentration (0 to 80 nmol l−1 d−1), new production is more accurately estimated by the isotopic method than by the chemical method; (4) in subtropical oligotrophic waters (from 11°S southward) with low ambient nitrate levels (0 to 100 nmol l−1), both procedures are appropriate as long as nitrate removal per incubation period is >3 nmol l−1 (lower rates are only detectable with the isotopic method); (5) the traditional 15N-tracer technique does not substantially underestimate net new-production in the equatorial Pacific, and failure to account for the loss of 15NO3− as DON, i.e. to estimate gross nitrate uptake (gross uptake = net uptake + 15N loss) tends to underestimate new production on an average by only 10%. Overall, the apparent low level of new production in the nitrate-rich area of the central equatorial Pacific seems to be a fact, and may be ascribable to other nutrient (macro and micro) deficiencies and/or to intense in situ recycling of ammonium and nitrate (regenerated production) rather than to inaccurate nitrate uptake rates measured with the classical 15N-tracer technique.

Introduction

Understanding biogeochemical fluxes in the equatorial Pacific is one of the major goals of the Joint Global Ocean Flux Study (JGOFS), since this part of the ocean is a strong source of CO2 for the atmosphere (Broecker et al. 1986; Lefevre and Dandonneau 1992). Warming of
upwelled waters with a subsequent increase in CO₂ partial pressure and expected intense new (nitrate-supported) production (Dugdale and Goering 1967) are the main processes responsible for exchanges of CO₂ with the atmosphere. Precise measurements of nitrate utilization are crucial to correctly relating new production to exports of particulate (Eppley and Peterson 1979) and dissolved (Toggweiler 1989) organic matter to the deep ocean, and to quantifying the biological impact of the equatorial Pacific on the global carbon cycle. The potentially large role of the equatorial Pacific in global new production was discussed by Chavez and Barber (1987), who estimated new production by calculating the advective supply of nitrate to the euphotic zone arising from upwelling. Given the immensity of the area, this estimate implies that new production in the equatorial Pacific accounts for a significant proportion (18 to 56%) of global new production. This result has been recently confirmed by modelling the turbulent flux of nitrate into the photic zone (Carr et al. 1995). However, other models (Bacastow and Maier-Reimer 1991; Fiedler et al. 1991) and direct measurements of new production which have included observations of particle flux into sediment traps (Murray et al. 1989) and uptake of 15N-labelled nitrate ([15NO₃⁻]) (Peña et al. 1991; Dugdale et al. 1992; Wilkerson and Dugdale 1992; McCarthy et al. 1996), yielded estimates of new production much lower than expected. Some authors (Eppley and Renger 1992; Carr et al. 1995) ascribed these discrepancies to inaccurate new-production rates estimated with the classical 15N-tracer technique. During comparative studies between chemical and isotopic methods used to estimate nitrate-nitrogen uptake by phytoplankton, a deficit in 15N in the final budget has been repeatedly observed; i.e., except in three cases (Harrison and Davis 1977; Eppley and Renger 1986; Boyd et al. 1995), nitrate decrease (ΔNO₃⁻) measured by chemical means was greater than incorporation of 15NO₃⁻ into the particulate fraction (Price et al. 1985; Dugdale and Wilkerson 1986; Slawyk et al. 1990). While in the latter studies removal of nitrate substrate from solution exceeded 15NO₃⁻ incorporation by a factor of >1 to 2, Eppley and Renger (1992) reported from the equatorial Pacific the most extreme case of disagreement between chemical and isotopic measurements of planktonic nitrate utilization (ΔNO₃⁻/15NO₃⁻ incorporation = 7.2), leading to a mean underestimation of new production by the 15N-isotopic procedure of up to 72%. Possible mechanisms suggested to explain the latter discrepancies (often named “missing 15N”: Laws 1984) usually include two unmeasured nitrogen fluxes: (1) Passage of 15N labelled particles through GF/F glass-fiber filters routinely used for productivity measurements (Altabet 1990; Slawyk and Raimbault 1995; Libby and Wheeler 1997); it has been suggested that 0.2 µm filters would yield better recovery of small plankton biomass (Li et al. 1983) and consequently better estimates of primary production for picoplankton. (2) Release of dissolved organic nitrogen (DON) (Chan and Campbell 1978; Laws 1984). Estimates of nitrate vertical flux in the equatorial Pacific were consistently much higher than net nitrate uptake rates, and more in line with gross nitrate uptake, i.e. if DON release had been taken into account (Carr et al. 1995). Field studies of nitrate uptake taking into account DON release are extremely rare because of the difficulty of identifying organic compounds and measuring their 15N enrichment. Bronk and Glibert (1991) and Slawyk and Raimbault (1995) have measured significant release rates of DO15N during short-term experiments, and Bronk et al. (1994) have shown that failure to account for DO15N release in classical tracer experiments would lead to underestimating gross nitrate uptake rates by up to 74%.

Nitrate-disappearance rates represent net fluxes of nitrate, since changes in concentration may encompass uptake, regeneration (nitrification) and fluxes to another inorganic pool (ammonium in the case of nitrate reduction: denitrification). Thus, depending on the relative importance of the latter processes, net uptake rates measured with the colorimetric method, would be underestimated or overestimated. If multiple nitrogen transfers occur in an experimental system, then the most correct approach for measuring new production would be 15N incorporation into particles. If however, particles leak significant amounts of 15N during incubation, then new production rates and f-ratios based on these rates would be underestimated by the 15N-tracer technique.

Methods are now available to investigate the potential importance of both submicron particle losses (Altabet 1990) and DO15N release (Bronk and Glibert 1991; Slawyk and Raimbault 1995) as well as to precisely measure nitrate concentration changes (Garside 1982; Oudot and Montel 1988; Raimbault et al. 1990). The present paper reports on new production rates in the equatorial Pacific obtained from the simultaneous use of chemical and isotopic procedures and, bearing in mind the methodological problems peculiar to each analytical procedure, whether new production is more accurately estimated from nitrate disappearance or from 15NO₃⁻ incorporation.

Materials and methods

This work was carried out during the OLIPAC cruise on the R.V. “L’Atalante” from 3 November to 1 December 1994. New production measurements were performed at 21 productivity stations occupied on a latitudinal cross-section between 1°N and 16°S at Longitude 150°W (Fig. 1). Samples were collected from several depths in the euphotic zone before sunrise (04:00 hrs local time) using a CTD (conductivity–temperature–depth) rosette system comprising 12-litre Niskin bottles with silicone rubber closures that were carefully checked and regularly changed to avoid introduction of toxic metals. Water was drawn into 1.2 or 2.4-litre polycarbonate incubation bottles [acid-cleaned (10% HCl) and rinsed with deionised water (MilliQ system)] for immediate nutrient analysis and subsequent initiation of the 15N-tracer experiment. Samples for nitrate and nitrite were pumped directly from the incubation bottles, using a capillary glass-tub, into two separate channels of a Technicon AutoAnalyser® equipped with manifolds, according to