PERGAMON

Deep-Sea Research I 49 (2002) 1609–1621

DEEP-SEA RESEARCH
PART I

www.elsevier.com/locate/dsr

Stable isotope constraints on the nitrogen cycle of the Mediterranean Sea water column

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Received 7 December 2001; received in revised form 14 May 2002

Abstract

We used the nitrogen isotope ratio of algae, suspended particles and nitrate in the water column to track spatial variations in the marine nitrogen cycle in the Mediterranean Sea. Surface PON (5–74 m) was more depleted in ^{15}N in the eastern basin (-0.3 ± 0.5‰) than in the western basin ($+2.4\pm1.4$‰), suggesting that nitrogen supplied by biological N_2 fixation may be an important source of new nitrogen in the eastern basin, where preformed nitrate from the Atlantic Ocean could have been depleted during its transit eastward. The $\delta^{15}N$ of nitrate in the deep Mediterranean ($\sim$3‰ in the western-most Mediterranean and decreasing toward the east) is significantly lower than nitrate at similar depths from the North Atlantic (4.8–5‰), also suggesting an important role for N_2 fixation. The eastward decrease in the $\delta^{15}N$ of surface PON is greater than the eastward decrease in the $\delta^{15}N$ of the subsurface nitrate, implying that the amount of N_2 fixation in the eastern Mediterranean is great enough to cause a major divergence in the $\delta^{15}N$ of phytoplankton biomass from the $\delta^{15}N$ of the nitrate upwelled from below. Variations in productivity associated with frontal processes, including shoaling of the nitracline, did not lead to detectable variations in the $\delta^{15}N$ of PON. This indicates that no differential fertilization or productivity gradient occurred in the Almerian/Oran area. Our results are consistent with a lack of gradient in chlorophyll-*a* (chl-*a*) and nitrate concentration in the Alboran Sea. ^{15}N enrichment in particles below 500 m depth was detected in the Alboran Sea with respect to surface PON, reaching an average value of $+7.4\pm0.7$‰. The $\delta^{15}N$ in sinking particles caught at 100 m depth (4.9–5.6‰) was intermediate between suspended surface and suspended deep particles. We found a consistent difference in the isotopic composition of nitrogen in PON compared with that of chlorophyll ($\Delta\delta^{15}N$[PON-chlorin] $= +6.4\pm1.4$‰) in the surface, similar to the offset reported earlier in cultures for cellular N and chl-*a*. This indicates that $\delta^{15}N$ of phytoplankton biomass was retained in surface PON, and that alteration of the isotopic signal of PON at depth was due to heterotrophic activity.

1. Introduction

The distribution of nitrogenous compounds in the sea is governed by physical, chemical, and biological factors. In the euphotic zone, the distribution of nitrogenous species is primarily determined by (a) the supply of nitrate from depth,

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0967-0637/02/$ - see front matter
PII: S0967-0637(02)00066-3

(b) uptake of dissolved inorganic nitrogen by phytoplankton, (c) mineralization of organic nitrogen by heterotrophic organisms, (d) loss of nitrogen associated with sinking of particulate organic nitrogen (PON) and vertical migration of organisms, (e) downward or lateral transport of dissolved organic nitrogen (DON), and (f) biological N_2 fixation. Depending on the nitrogen source for phytoplankton assimilation, we differentiate between new and regenerated production, concepts of great ecological value (Eppley and Peterson, 1979). Regenerated production is driven by ammonium and DON uptake, whereas nitrate uptake and N_2 fixation both contribute to new production. Estimates of N_2 fixation rate are particularly variable because cyanobacteria are difficult to sample in the ocean. However, the use of indirect approaches suggests that N_2 fixation is more important than traditionally thought, both as an immediate source of nitrogen for the surface ocean ecosystem (Karl et al., 1997) and as a contributor of new nitrate to the ocean interior (Gruber and Sarmiento, 1997).

The study of the nitrogen cycle in the Mediterranean Sea has received considerable attention because of its apparent complexity. One of the issues in debate is the nutrient budget for the basin as a whole. Béthoux and Copin-Montégut (1986) suggest that the net loss of nitrogen at the Strait of Gibraltar exceeds combined inputs from rivers and the atmosphere, and that this deficit is made up by N_2 fixation within the Mediterranean Sea. Alternatively, Coste et al. (1988) indicate that any small deficit in the input at the Gibraltar Strait could be balanced by continental inputs of nitrogen. Moreover, although presently oligotrophic and oxygenated, the Mediterranean Basin has undergone dramatic physical, chemical and biological changes in the past that resulted in the deposition of organic-rich sediment deposits (sapropels) (e.g. Calvert et al., 1992; Sachs and Repeta, 1999). Thus, this semi-enclosed basin with local sources of nutrients is a suitable setting to evaluate the importance of biological N_2 fixation using nitrogen stable isotopes as well as past changes in the nitrogen cycle.

The circulation of the Mediterranean Sea is driven by an excess of evaporation over precipitation. North Atlantic surface waters flow into the basin through the Strait of Gibraltar and move towards the eastern basin. Once in the eastern basin surface water density increases as intense evaporation increases surface salinity. A resultant outflow of nutrient-enriched subsurface water at the Strait of Sicily balances the inflow of Atlantic water (Miller, 1983). Photosynthetic algae extract nutrients from surface waters, causing extreme nutrient impoverishment of Mediterranean surface waters (Miller, 1983; Azov, 1991).

The circulation in the upper 100–300 m of the western Mediterranean Sea is dominated by an eastward jet of Atlantic water entering through the Strait of Gibraltar that separates Mediterranean and frontal waters (Prieur and Sournia, 1994). Persistent density fronts resulting from the interaction of saline Mediterranean and fresher Atlantic waters are associated with higher primary production rates than in surrounding waters (Lohrenz et al., 1988) and in the eastern basin (Azov, 1991). Previous observations in the region (ALMOFRONT I program, Prieur and Sournia, 1994) showed anomalously high algal biomass and productivity associated with the front, with chlorophyll values up to $23\,\mu g\,l^{-1}$. This system gives rise to "the paradigm of frontal fertilization", in contrast to the notoriously oligotrophic adjacent water masses (Atlantic and Mediterranean) (Prieur and Sournia, 1994). The nitracline rises from ca. 60 m north and south of the front to 18–30 m in the front. Chlorophyll-*a* (chl-*a*) rises from $20\,mg\,m^{-2}$ north and south of the front to $80–100\,mg\,m^{-2}$ in the front. Diatoms contribute up to 76% of the biomass in the front. At the frontal boundaries, small flagellates and cyanobacteria contribute 80% of the phytoplankton biomass (Prieur and Sournia, 1994).

Isotopic signals of organic and inorganic nitrogenous material (nitrate, N_2, PON, etc.) suggest the possibility of tracking changes in the marine nitrogen cycle in the water column (e.g. Altabet and McCarthy, 1986) and in marine sediments (e.g. Altabet and François, 1994), including changes in denitrification and N_2 fixation (e.g. Altabet et al., 1995; Ganeshram et al., 1995; Haug et al., 1998; Sachs and Repeta, 1999). Natural abundances of the two stable isotopes of nitrogen,

^{14}N and ^{15}N, are affected by kinetic isotope effects during biologically mediated reactions. Slightly faster kinetics for reactions involving ^{14}N result in ^{15}N-depletion of reaction products relative to substrates. Thus, in a process roughly analogous to fractional distillation, nitrate (or ammonium) containing ^{14}N is preferentially taken up by phytoplankton (e.g. Montoya and McCarthy, 1995; Wasser et al., 1998), and the remaining dissolved inorganic nitrogen pool becomes progressively enriched in ^{15}N as consumption increases (e.g. Sigman et al., 1999a). In the case of biological N_2 fixation, which converts atmospheric N_2 into organic nitrogen, the conspicuous isotopic composition of atmospheric nitrogen ($\delta^{15}N_2 \approx 0‰$), which is lower than that of the nitrate pool within the ocean ($\delta^{15}N \approx 5‰$), could be indicative of its relative importance (Delwiche and Steyn, 1970; Wada and Hattori, 1976; Carpenter et al., 1997). Biological N_2 fixation fractionates by $-2.6 \pm 1.3‰$ relative to atmospheric N_2 (Hoering and Ford, 1960; Delwiche and Steyn, 1970; Macko et al., 1987; Minagawa and Wada, 1986; Carpenter et al., 1997).

Nitrogen recycling also lowers the $\delta^{15}N$ of surface particles, because of isotopic fractionation during heterotrophic processes. Zooplankton appear to release ammonium that has a lower $\delta^{15}N$ than their food source, making their tissues and solid wastes $\sim 3‰$ higher in $\delta^{15}N$ than their food source (Checkley and Miller, 1989; Altabet and Small, 1990). The low-$\delta^{15}N$ ammonium is consumed by phytoplankton and thus retained in the surface ocean N pool, while the ^{15}N-enriched particulate N is preferentially exported, leading to a lower $\delta^{15}N$ of surface particulate N where recycled N is an important component of the gross N supply to phytoplankton (Altabet, 1988). In the low-latitude, low-nutrient ocean surface, such as the Sargasso Sea and western tropical Pacific, the relative importance of N_2 fixation and N recycling in producing low-$\delta^{15}N$ surface particles is uncertain. Whereas N_2 fixation adds new low-$\delta^{15}N$ fixed N to the water column, N recycling does not. Thus, coupled measurements of surface PON and subsurface nitrate hold promise for distinguishing the isotopic effects of N_2 fixation from that of N recycling.

We report here results from three research programs in the Mediterranean Sea in which we studied the systematics of nitrogen isotopes: the ALMOFRONT II cruise in the Alboran Sea (western Mediterranean Sea), the Minos cruise from Toulon, France, to Heraklion, Greece, and one station in the eastern Mediterranean Sea during the PROSOPE cruise (Table 1). The observational programs gave us two different scales on which to study the nitrogen cycle in the Mediterranean Sea. In the western Mediterranean, the strong horizontal gradients marking the boundaries of the Almerian/Oran front create a geographically small system of nutrient fertilization and productivity set within a larger, oligotrophic sea (Prieur and Sournia, 1994). Our aim was to search for variations in the $\delta^{15}N$ of particles in the geostrophic Almeria-Oran front (western basin), associated with gradients in nutrient concentration and biological productivity. On a larger scale, the antiestuarine ("lagoonal") circulation of the Mediterranean Sea Basin results in depletion of nutrients supplied at the Gibraltar Strait, resulting in very low primary production levels in the eastern Mediterranean Sea (Azov, 1991). On this basin scale, we searched for N isotopic changes from the western to the more oligotrophic eastern basin. An additional goal was to assess the preservation of the isotopic signal of phytoplankton in bulk suspended PON. In order to fulfill those objectives we performed measurements of $^{15}N/^{14}N$ ratios in sinking and suspended PON, nitrate, and chl-*a*.

2. Methods

2.1. Sampling

Results reported here are representative of three seasons (spring, fall and winter, Table 1). The spring sampling cruise was carried out at stations located between Toulon (France) and Heraklion (Greece) aboard R/V *Le Suroit* (Fig. 1). Most of the data from this cruise was published in Sachs and Repeta (1999), Sachs et al. (1999) and Sachs and Repeta (2000). The winter sampling was done aboard R/V *L'Atalante* during Leg 2 of the French

Table 1
Location of sampling and samples in the Mediterranean Sea

Cruise/dates	R/V	Longitude	Samples	Analyses
Minos 05/22–06/5/1996	*Le Suroit*	Western basin 6°09′E Eastern basin 17°59′E 20°20′E 22°16′E	Suspended particles	δ^{15}N-chlorin, δ^{15}N-PON
ALMOFRONT II Leg 2 12/23/1997–01/16/1998	*L'Atalante*	Western basin 0°19′W 0°30′W 0°39′W 0°45′W 0°57′W 1°00′W 1°26′W 1°55′W	Suspended particles, sinking particles, water	δ^{15}N-NO_3^-, δ^{15}N-chlorin, δ^{15}N-PON
PROSOPE 09/4–10/14/1999	*Thalassa*	Eastern basin 21°55.8′E	Water	δ^{15}N-NO_3^-

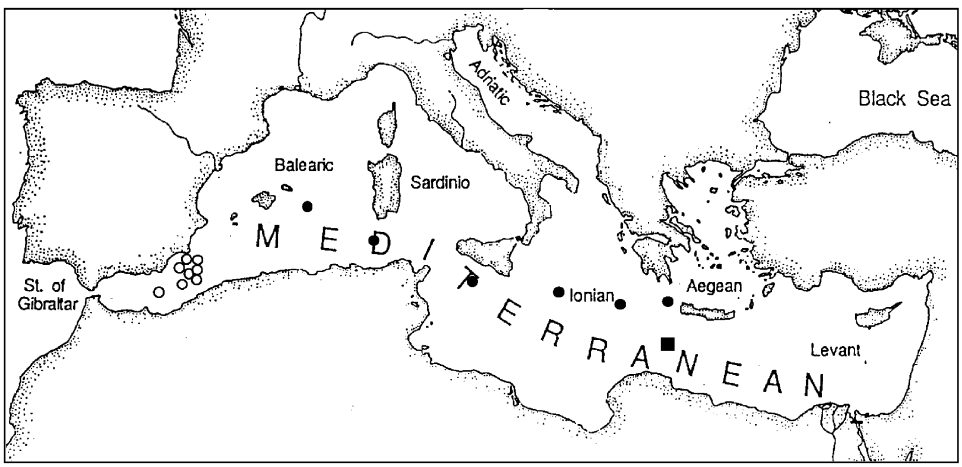

Fig. 1. Map of the Mediterranean Sea showing the sampling location. Open circles are sampling stations of Almofront 2 cruise (winter 1997–1998). Black dots are sampling stations of Minos cruise (Spring, 1996). Square denotes sampling of Prosope cruise (Fall, 1999).

research program ALMOFRONT II in the Almeria-Oran front (Fig. 1). Here, we occupied eight sampling sites, representative of the three characteristic water masses in the Alboran Sea (Mediterranean, Front and Atlantic, Prieur and Sournia, 1994). Sites within each water mass were treated as replicates, and isotope data were averaged for each site (see Results). We also

collected water samples in the eastern Mediterranean Sea during the PROSOPE cruise aboard R/V *Thalassa* (Table 1).

Surface samples (0–70 m) for $\delta^{15}N$ measurements of PON and chlorophyll were taken by filtering ca. 1000 l of seawater through a 293-mm diameter Gelman A/E filter (nominal pore size 1.0 μm) precombusted at 450°C for 8 h. Sampling was achieved with a pneumatic pump and hose (ca. 600 l h^{-1}), which was lowered to the sampling depth. Sediment traps (1 m^2 collection surface) were deployed at Sites 1 and 6 during the ALMOFRONT II cruise (Atlantic Gyre) at 100 m depth for 7–9 h (LeBlond, 2000). Sediment trap particulate matter was filtered onboard onto precombusted GF/F filters (0.7 μm).

Choice of sampling depths was based on real-time fluorescence depth profiles taken at each station, and typically samples above, in, and below the depth of the chlorophyll maximum were taken. Duplicate filters were sampled on three occasions. Seawater from greater than 100 m was sampled for $PO^{15}N$ by rosette mounted Niskin bottles. On board, 16–20 l of seawater, combined from several depths, was pressured-filtered (ca. 15 psi) through GF/F filters.

Samples for $\delta^{15}N$ analysis of nitrate were taken with pneumatic pump ($\leqslant 70$ m) or during a CTD cast ($\geqslant 100$ m). One liter was pressure-filtered through GFF filters. Filtrates were acidified with 50% reagent grade HCl (1 ml l^{-1}). All samples (filters and seawater) were frozen immediately after collection and stored frozen.

2.2. Analyses

2.2.1. $\delta^{15}N$-general

Isotopic values are expressed as permil (‰) relative to atmospheric N_2. Instruments were calibrated with laboratory standards. Precision of the analysis, determined with a caffeine standard, was 0.14‰ with samples containing 1 μmol N or more.

2.2.2. $\delta^{15}N$-PON analysis

Subsamples (2-cm diameter) were removed from the 293-mm filters, dried at 60°C and combusted on a Europa Roboprep elemental analyzer on-line with a Europa 20/20 isotope ratio mass spectrometer at the Marine Biological Laboratory (Woods Hole, MA). Average precision for subsamples taken from a 293-mm filter and analyzed for $\delta^{15}N$ was 13% (CV). Average precision for samples taken at the same site on different days and analyzed for $\delta^{15}N$ was 17% (CV). Deep particulate material caught on 47-mm filters was treated similarly for this analysis. Here, filters were split in two and run as duplicates.

2.2.3. Nitrate isotopic analysis

Natural abundance-level measurements of nitrate $\delta^{15}N$ were made by two methods. The first is the "passive ammonia diffusion" method, in which nitrate is quantitatively converted to ammonia under basic conditions, and the ammonia is collected by gas-phase diffusion of ammonia out of the seawater sample, through a porous Teflon membrane, and onto an acidified glass fiber disk (Sigman et al., 1997). The acidified disks were combusted by elemental analyzer and the $\delta^{15}N$ of the resultant N_2 gas analyzed by mass spectrometer, as for the particulate N samples. The second method, the "denitrifier method", is the quantitative conversion of nitrate to N_2O by bacterial denitrification, followed by isotopic analysis of the product N_2O by continuous flow isotope ratio mass spectrometry (Sigman et al., 2001). For most samples, the two methods gave indistinguishable results. Nitrate concentration was determined in each sample by reduction of nitrate to NO followed by chemiluminescence detection of NO (Braman and Hendrix, 1989).

2.2.4. $\delta^{15}N$-chlorin in surface particles

Chlorins (chl-*a* or macrocycle derivative) were purified from 293-mm filters according to the procedure described by Sachs and Repeta (2000), with minor modifications. Briefly, filters were shredded into small pieces and extracted with methanol buffered with $NaHCO_3$ (8 g l^{-1}) in a sonicator bath (200 ml, 3×10 min). The extract was partitioned between water and hexane ($2\times$) and the hexane fraction evaporated to dryness. The extract was redissolved in acetone and applied onto a Kromasil Kr100-5-C-18 preparative column (10×250 mm), with a 10 mm $\times$ 50 mm guard

column. A 35-min program gradient of methanol and acetone at a variable flow rate of 6–7 ml min^{-1} was used to separate chl-*a*, pheophytin, and pheophorbide. They were detected with a photodiode array detector set up at 440 and 666 nm, and collected as they eluted from the column. Chlorins were further purified on a 4.6 mm × 150 mm SiO_2 analytical column (Supelco LC-Si, 3 μm), with acetone/hexane (10/90) in an isocratic mode at 2 ml min^{-1}. For each sample, an average isotopic composition was calculated as weighted average of the isotopic composition of each component.

Throughout the purification procedure, chlorin recoveries were monitored by spectrophotometry. Yields for 75% of the samples were higher than 80%, and for the other 25% of the samples were between 50% and 79%. Isolated chlorins were analyzed for nitrogen isotopic composition on a Finnigan-MAT DeltaPlus isotope ratio mass spectrometer, after combustion to N_2 in a Carlo Erba/Fisons elemental analyzer. Coefficient of variation for chlorin $\delta^{15}N$ was 0.35‰ when 100 nmol N was analyzed. A plot of $\delta^{15}N$ against yields resulted in zero slope, indicating that the yields we obtained did not affect chlorin isotopic values.

2.2.5. Sampling of suspended material

In order to collect enough chlorin-N for isotopic analysis, we used 293-mm A/E filters of nominal pore size of 1 μm, which allowed us to filter about 1 m^3 of seawater. The pore size of these filters is slightly larger than the more commonly used GF/F filters (nominal pore size 0.7 μm); small phytoplankton and other particles may not be retained as efficiently. Discrepancies in chlorophyll concentrations were also found when the two filters were compared (Fig. 2). In most cases, more chl-*a* was measured with GF/F filters, suggesting that A/E filters did not retain some smaller particles that were retained on GF/F filters. Another possibility is that transport and storage of samples on A/E filters for up to 9 months resulted in some degradation of pigments. Samples collected with GF/F filters were analyzed within days. Total organic carbon and nitrogen values were up to 50% higher on samples collected with

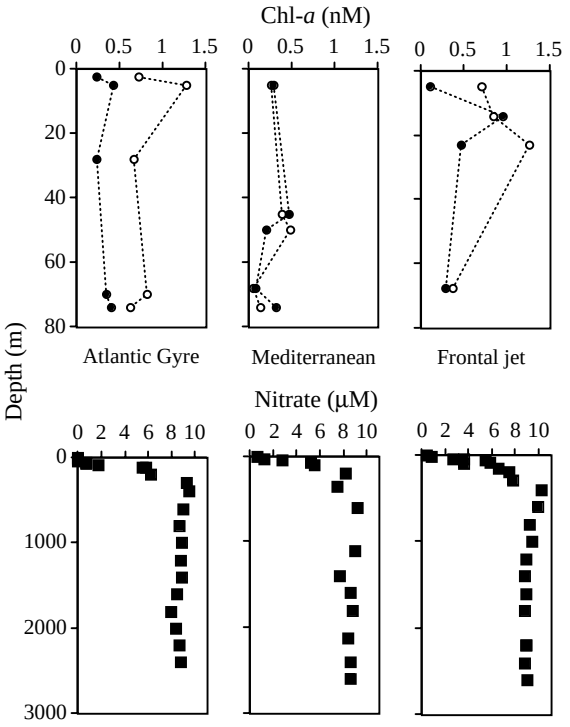

Fig. 2. Chl-*a* and nitrate concentration profiles in the Alboran Sea. Chlorophyll profiles were obtained with 25-mm GF/F filters (open circles) and subsamples from the 293-mm A/E filter (closed circles). Inventories of chl-*a* from the 25-mm filters were 52 mg m^{-2} at the Front sites, 21 mg m^{-2} in the Mediterranean sites, and 55 mg m^{-2} in the Atlantic Gyre sites. Chl-*a* profiles from GF/F filters were provided by H. Claustre. Nitrate data from are from P. Morin (unpublished data).

GF/F filters, confirming this sampling bias. Most relevant to the results reported here is the nitrogen isotopic difference between A/E and GF/F samples. For bulk PON this offset was insignificant ($\delta^{15}N\text{-}PON_{GF/F} = 3.04 \pm 0.45‰$, $n = 4$; $\delta^{15}N\text{-}PON_{A/E} = 2.64 \pm 1.26‰$, $n = 4$). This correspondence may indicate that if small particles were retained on GF/F filters but passed through A/E filters, they had the same isotopic ratio as the A/E-retained larger particles. Another possibility is that the differences in chl-*a* concentration on A/E and GF/F filters resulted from the loss of PON on the former during sample collection. Indeed small tears in the center of the A/E filters were often observed during the filtration of ca. 1 m^3 of water.

3. Results

3.1. Nitrate and chlorophyll concentration

Nitrate concentrations in the Alboran Sea were similar to previously reported values during the ALMOFRONT-1 research program in the same region (Prieur and Sournia, 1994). Nitrate increased from 0 μM at the surface to ca. 10 μM at 400 m depth and remained approximately constant to 2500 m (Fig. 2).

Subsurface maxima in chlorophyll concentration were detected in all profiles but at different depths (Fig. 2). Integrated chl-*a* over 70-m depth at the Front sites was 52 mg m^{-2}, similar to the inventory at the Mediterranean sites, and twice as high as at the Atlantic Gyre sites in the Alboran Sea (Fig. 2). Prieur and Sournia (1994) observed higher chl-*a* inventories over 150-m depth during the ALMOFRONT-1 cruise in April–May 1991, with larger differences among sampling sites. In their study, the inventory was ca. 100 mg chl-*a* m^{-2} at the Front sampling sites, 30% and 40% higher than at the Atlantic Gyre and Mediterranean sites, respectively.

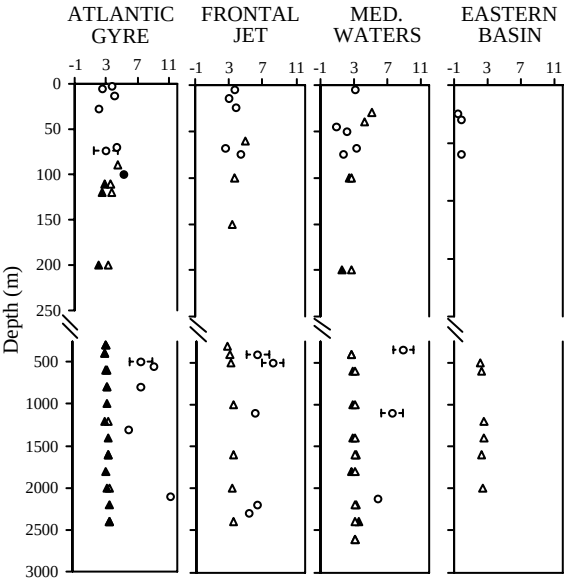

Fig. 3. Depth profiles of δ^{15}N of PON (open circles), nitrate (triangles), and sinking particles (closed circle) in the Alboran Sea. Closed triangles are δ^{15}N-nitrate by the diffusion method, open triangles are δ^{15}N-nitrate by the denitrifier method (see text for explanation). For δ^{15}N-PON, bars are 2SD of independent samples taken on different days. For δ^{15}N-nitrate, replicate analyses indicate a 1SD of ≈0.2‰.

3.2. Nitrogen isotope composition

A uniform distribution of suspended particulate (PON) δ^{15}N was detected in the three water masses of the Alboran Sea across all depths, with no differences among sample sites (Atlantic, Frontal, Mediterranean waters) (Fig. 3). δ^{15}N-PON was $+3.0\pm0.5$‰ at depths <100 m. Below 100 m, enrichment of ^{15}N was observed at all sites, averaging 7.4 ± 0.7‰ (Fig. 3). The δ^{15}N value of sinking particles sampled at 100 m depth at the Atlantic Gyre sites was 5.2 ± 0.5‰ (Fig. 3). For reference, suspended PON collected from the euphotic zone of the oligotrophic subtropical Sargasso Sea off Bermuda averaged −0.2‰, and sinking PON at 100 m averaged 3.7‰ (Altabet and McCarthy, 1986; Altabet, 1988).

We observed a significant west-to-east decrease (slope -0.17 ± 0.02, $P<0.001$) in the isotopic composition of PON from the upper 200 m (Fig. 4A). In the western basin, δ^{15}N-PON averaged 2.7 ± 1.2‰, compared to -0.2 ± 0.7‰ in the eastern basin. Samples deeper than 200 m are not available from the eastern basin, precluding a west-to-east comparison.

Nitrate δ^{15}N averaged 3.4 ± 0.5‰ ($n=61$) in the western basin and 2.5 ± 0.1‰ ($n=4$) in the eastern basin (Fig. 3), both substantially lower than the average value for deep nitrate in the world ocean (~5‰, Liu and Kaplan, 1989; Sigman et al., 2000). No isotopic differences were observed in deep nitrate from the three sites in the Alboran Sea (Fig. 3). δ^{15}N of chlorins averaged -2.6 ± 2.3‰ ($n=13$) in the western basin and -7.1 ± 1.3‰ ($n=3$) in the eastern basin (Sachs et al., 1999). Thus, chlorins were isotopically depleted relative to suspended particles in the euphotic zone of both basins by 6.0 ± 1.6‰ (Fig. 5). This depletion is consistent with the 5.1 ± 2.0‰ difference between cellular nitrogen and chl-*a* observed in cultures of eight algal species (Sachs et al., 1999).

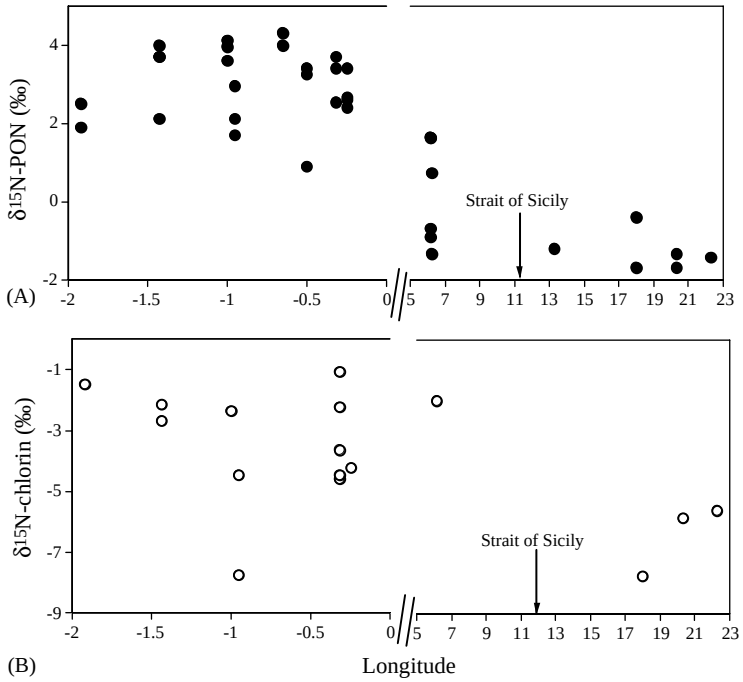

Fig. 4. (A) Longitudinal distribution of δ^{15}N-PON in the top 200 m in the Mediterranean Sea. (B) Longitudinal distribution of δ^{15}N-chlorin in the top 200 m in the Mediterranean Sea. Horizontal scale is a decimal transformation of geographical degrees. At 35°N (Mediterranean Sea), a degree of longitude is about 89 km.

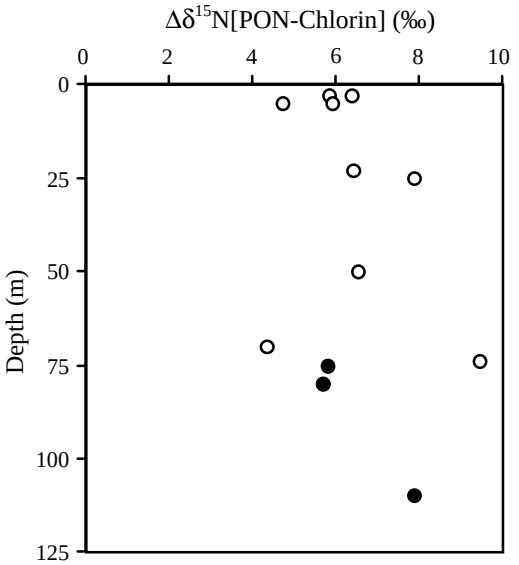

Fig. 5. Depth profile of the difference in nitrogen isotope composition between PON and chl-*a* in the Mediterranean Sea. Average = 6.4 ± 1.4‰. Dark and open circles are values from the eastern and western basin, respectively.

4. Discussion

4.1. Generation of the isotopic signal of PON and nitrate

Suspended particles in the marine environment contain algal and detrital nitrogen. The δ^{15}N value of surface PON reflects the isotopic composition of nitrogenous nutrients (Montoya, 1994), the extent to which those nutrients are consumed (Altabet et al., 1991), and the decomposition processes resulting from heterotrophic activity (Saino and Hattori, 1980). The diagenetic overprint of algal δ^{15}N can be circumvented by measuring the δ^{15}N value of chlorophyll from PON and applying an empirically determined offset between cellular and chlorophyll δ^{15}N in marine phytoplankton (calculated as δ^{15}N algal N = δ^{15}N chl-*a* + 5.1, Sachs et al., 1999). Calculated in this fashion, the mean isotopic ratio of Alboran Sea phytoplankton from the upper 200 m was 2.1 ± 1.8‰ (Fig. 4B). This

value is only slightly lower than the $\delta^{15}N$ of subsurface nitrate being supplied to the surface of the Alboran Sea ($\sim 3‰$, Fig. 3). This suggests that neither the rates of N recycling nor N_2 fixation are sufficiently large in the western Mediterranean to cause a substantial divergence in the $\delta^{15}N$ of new phytoplankton biomass from the $\delta^{15}N$ of the nitrate being supplied from below.

The mean isotopic depletion of chl-*a* relative to PON ($6.4 \pm 1.4‰$, $n = 16$) was ca. 1‰ higher in our Mediterranean surface samples than that reported by Sachs et al. (1999) for cultured phytoplankton ($5.1 \pm 1.1‰$, Fig. 5). Although this difference is not significant, it is in the direction expected for the diagenetic overprint of algal nitrogen, suggesting only a modest ($\sim 1‰$) diagenetic alteration of the $\delta^{15}N$ of PON by degradation in the surface layer.

However, we detected significant enrichment in ^{15}N in PON with depth in the Alboran Sea (Fig. 3): PON below 500 m was ca. 8‰ higher in $\delta^{15}N$ than surface particles. This increase in the $\delta^{15}N$ of PON with depth has been observed previously and was attributed to isotopic fractionation during bacterial degradation or other heterotrophic activity (Saino and Hattori, 1980; Altabet and McCarthy, 1986; Montoya et al., 1990). This is consistent with other evidence for ^{15}N enrichment during early diagenesis in the presence of oxygen, such as the ^{15}N enrichment of surficial sediment relative to abyssal sinking particles (Altabet and François, 1994; Sachs and Repeta, 1999) and of diagenetically vulnerable sedimentary organic N relative to protected microfossil-bound N (Sigman et al., 1999b).

Sinking particles trapped at 100 m had a $\delta^{15}N$ value of $5.2 \pm 0.5‰$ ($n = 2$), intermediate between suspended particles at the surface and at depth (Fig. 3). This trend could be consistent with isotopic enrichment during remineralization of particles in the ocean interior or derived from zooplankton fecal pellet production. Previous results from depth arrays of sediment traps in open ocean settings typically do not show an increase in sinking particle $\delta^{15}N$ with depth (Altabet et al., 1991; Voss et al., 1996), with at least one study showing the opposite trend of decreasing $\delta^{15}N$ values in sinking particles with depth (Altabet et al., 1991). Moreover, the $\delta^{15}N$ value of sinking particles reported here is substantially higher than that of nitrate supplied from below ($\sim 3‰$), so that it does not fit the expected isotope balance between upwelled nitrate and sinking nitrogen. It may be that the $\delta^{15}N$ of the material caught in the sediment trap is not representative of the annually integrated $\delta^{15}N$ of the sinking flux at that site in the Mediterranean, which is possible given the seasonal variation in the $\delta^{15}N$ of sinking N observed in the open subtropical Atlantic and elsewhere (Altabet et al., 1991; Schäfer and Ittekot, 1993; Voss et al., 1996). Alternatively, it may be that a significant fraction of the nitrate consumed by phytoplankton in the surface layer is exported, either laterally or vertically, as dissolved organic N or some other form of N that does not accumulate in the sediment trap, and that this missing N flux has a characteristically low $\delta^{15}N$.

While it is surprising that the measured sinking particulate $\delta^{15}N$ is substantially higher than that of the subsurface nitrate, its isotopic enrichment relative to surface PON is expected. For instance, Altabet (1988) reported an average value of 3.7‰ in sinking particles and $-0.2‰$ in suspended particles from the Sargasso Sea. As discussed earlier, this difference has been explained as a result of N recycling, during which zooplankton recycle ^{15}N-depleted ammonium, preferentially removing ^{15}N from the upper ocean as sinking particles and causing phytoplankton and surface PON in general to migrate toward lower $\delta^{15}N$ values (Checkley and Miller, 1989; Altabet and Small, 1990).

4.2. East–west trends in the $\delta^{15}N$ of surface PON and deep water nitrate

The observed east–west trend in $\delta^{15}N$-PON in the upper 200 m of the Mediterranean is a conspicuous feature (Fig. 4A). The $\delta^{15}N$ of PON is higher in surface waters of the western basin ($2.4 \pm 1.4‰$) than in the eastern basin ($-0.3 \pm 0.5‰$). Similarly, the $\delta^{15}N$ of chlorin in surface particles decreases from the west to the east, from $-3.3 \pm 1.8‰$ to $-7.1 \pm 1.3‰$ (Fig. 4B). The $\delta^{15}N$ of algal biomass can be estimated from

the chlorin $\delta^{15}N$ data and the relationship observed by Sachs and Repeta (1999) for phytoplankton in culture: $\delta^{15}N_{cellular-N} = \delta^{15}N_{chlorin-N} + 5.1$, yielding $\delta^{15}N$ for phytoplankton biomass of 1.8 ± 1.8‰ in the western basin and -2.0 ± 1.3‰ in the eastern basin. As discussed above, the $\delta^{15}N$ of phytoplankton and surface PON can be lowered by N recycling; however, we know of no reason that this recycling would be so much more prevalent in the east than in the west. This suggests that PON in the eastern basin is produced from the addition of isotopically depleted nitrogen, either from terrestrial sources or from atmospheric nitrogen (through biological N_2 fixation). The $\delta^{15}N$ of groundwater nitrate in the lower Nile region varies between 2.9‰ and 14.5‰ (Aly et al., 1982), much higher than surface water PON in the eastern basin. Although other forms of terrestrial fixed N inputs (e.g., ammonium and dissolved organic N) also need to be characterized isotopically, existing measurements of global riverine and estuarine $\delta^{15}N$ values argue against these sources providing adequate quantities of isotopically depleted N to lower $\delta^{15}N$ values of surface PON in the eastern Mediterranean. Global freshwater and estuarine nitrogen have mean $\delta^{15}N$ values of 4.3 ± 2.7‰ ($n = 64$) and 4.6 ± 2.0‰ ($n = 199$), respectively (Owens, 1987). By elimination, this leaves the fixation of molecular dinitrogen, which produces organic nitrogen (and subsequently nitrate) that is roughly −3‰ to 0‰ relative to atmospheric N_2 (Hoering and Ford, 1960; Delwiche and Steyn, 1970; Macko et al., 1987; Minagawa and Wada, 1986; Carpenter et al., 1997).

Cellular nitrogen with low $\delta^{15}N$ can also be produced in the Mediterranean Sea by assimilation of isotopically depleted DON advected through the Strait of Gibraltar. Unfortunately no isotopic data is available for this nitrogen source. The only published $\delta^{15}N$ values of DON are for the high molecular weight (>1000 Da) fraction from Pacific, Atlantic, and Gulf of Mexico samples (Benner et al., 1997). $\delta^{15}N$ values for that material, which comprises ca. 30% of the total DON, range between 6.6‰ and 10.2‰. If similarly high isotopic ratios are assumed for Mediterranean Sea DON, then DON cannot be a source of isotopically depleted nitrogen to phytoplankton and suspended particles in the Mediterranean Sea surface waters.

Both N_2 fixation and N recycling can cause a decrease in the $\delta^{15}N$ of surface phytoplankton and PON, but only N_2 fixation represents a source of low $\delta^{15}N$ fixed N to the ocean. Thus, the $\delta^{15}N$ of deep nitrate in the Mediterranean provides a more rigorous test of the role of N_2 fixation in the N budget of the basin. The $\delta^{15}N$ of deep nitrate (>1000 m) in the Mediterranean is significantly lower than average values for deep ocean nitrate in the world ocean (2.4 ± 0.1‰ in the eastern basin, 3.2 ± 0.2‰ in the western basin, compared with 4.8–5‰ in the deep Atlantic). This requires an input of low-$\delta^{15}N$ fixed N to the Mediterranean, such as would be provided by N_2 fixation.

The $\delta^{15}N$ of both surface PON and subsurface nitrate decrease from west to east (Fig. 3), but the isotopic decrease of deep nitrate is not sufficiently large to account for the ~4‰ decrease observed in surface PON. This is consistent with the inference that N_2 fixation is an important fraction of the fixed N budget in the surface waters of the eastern Mediterranean. The chlorin and bulk PON $\delta^{15}N$ data can be used to estimate the fractional input of N from N_2 fixation. If we assume that the observed $\delta^{15}N$ of algae in the western and eastern basins (1.8‰ and −2‰, respectively) derives from two sources, nitrate (with a $\delta^{15}N$ of 3‰ in the west and 2.4‰ in the east) and diazotrophy (−2.6‰, Sachs and Repeta, 1999), we can estimate the relative contribution of N_2 fixation to nitrate uptake by isotopic balance (Shearer and Kohl, 1993). Results from this calculation indicate that up to 20% of nitrogen in the western basin and up to 90% in the eastern basin may derive from biological N_2 fixation.

Although direct measurements of N_2 fixation rates are scarce, the observed isotopic trends are consistent with the suggestion of Béthoux and Copin-Montégut (1986) on the role of the seagrass *Posidonia* and its epiphytes in fixing atmospheric nitrogen. Moreover, pigment analyses of suspended particles in the western basin have revealed that chl-*a* from cyanobacteria accounts for up to 53% of total chl-*a*, with an average of 19 ± 13% (Barlow et al., 1997). Additionally, concentrations

of zeaxantin (a carotenoid marker for cyanobacteria) were always higher than those of fucoxanthin (a carotenoid marker for diatoms) in the upper 70 m of the eastern basin (Claustre, unpublished data from PROSOPE Cruise). The lack of direct evidence for substantial populations of N_2-fixing algae in the Mediterranean Sea is perhaps not surprising in the light of the very recent discovery of large populations of previosuly undetected unicellular cyanobacteria in surface waters of the tropical Pacific Ocean (Zehr et al., 2001).

Béthoux and Copin-Montégut (1986) suggested that biological N_2 fixation could maintain a nitrogen imbalance in the Mediterranean Sea by supplying between 7% and 41% of the nitrogen presumably lost at the Strait of Gibraltar. Sachs and Repeta (1999) reported low nitrate $\delta^{15}N$ in the eastern-most Mediterranean (−0.7‰) and estimated that N_2 fixation could supply between 46% and 70% of eastern basin new nitrogen. The observed decrease in $\delta^{15}N$ of PON from west to east in the Mediterranean basin (Fig. 4A) indicates a larger contribution of depleted nitrogen to the inventory of the eastern than of the western basin. Although the isotope data might be interpreted to indicate higher rates of N_2 fixation in the eastern basin than in the western basin, the nitrate concentration in the eastern basin water column is half that in the western basin (Azov, 1991). Thus, even with similar absolute rates of N_2 fixation, the relative impact of N_2 fixation (on fluxes, nitrate concentration, and N isotopes) would be expected to be twice as great as in the eastern basin as in the western basin. These questions aside, our data add to the growing evidence that N_2 fixation represents a significant fraction of the annual fixed N supply to subtropical surface waters (Karl et al., 1997; Zehr et al., 2001) and that a significant fraction of subsurface nitrate is generated from the oxidation of newly fixed N (Gruber and Sarmiento, 1997).

Acknowledgements

We thank Hervé Claustre and Louis Prieur for the invitation to participate in the cruises Minos, ALMOFRONT-2, and Prosope in the Mediterranean Sea. H. Claustre and P. Morin graciously provided access to unpublished ambient chlorophyll and nitrate concentrations measured during the ALMOFRONT-2 cruise, respectively. N. Leblond provided us subsamples from sediment traps. This research was supported by NSF OCE-9711702 to D. Repeta and by NSF OCE-9981479 to D. Sigman. S. Pantoja was supported at WHOI by funds provided by the Johnson Foundation.

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